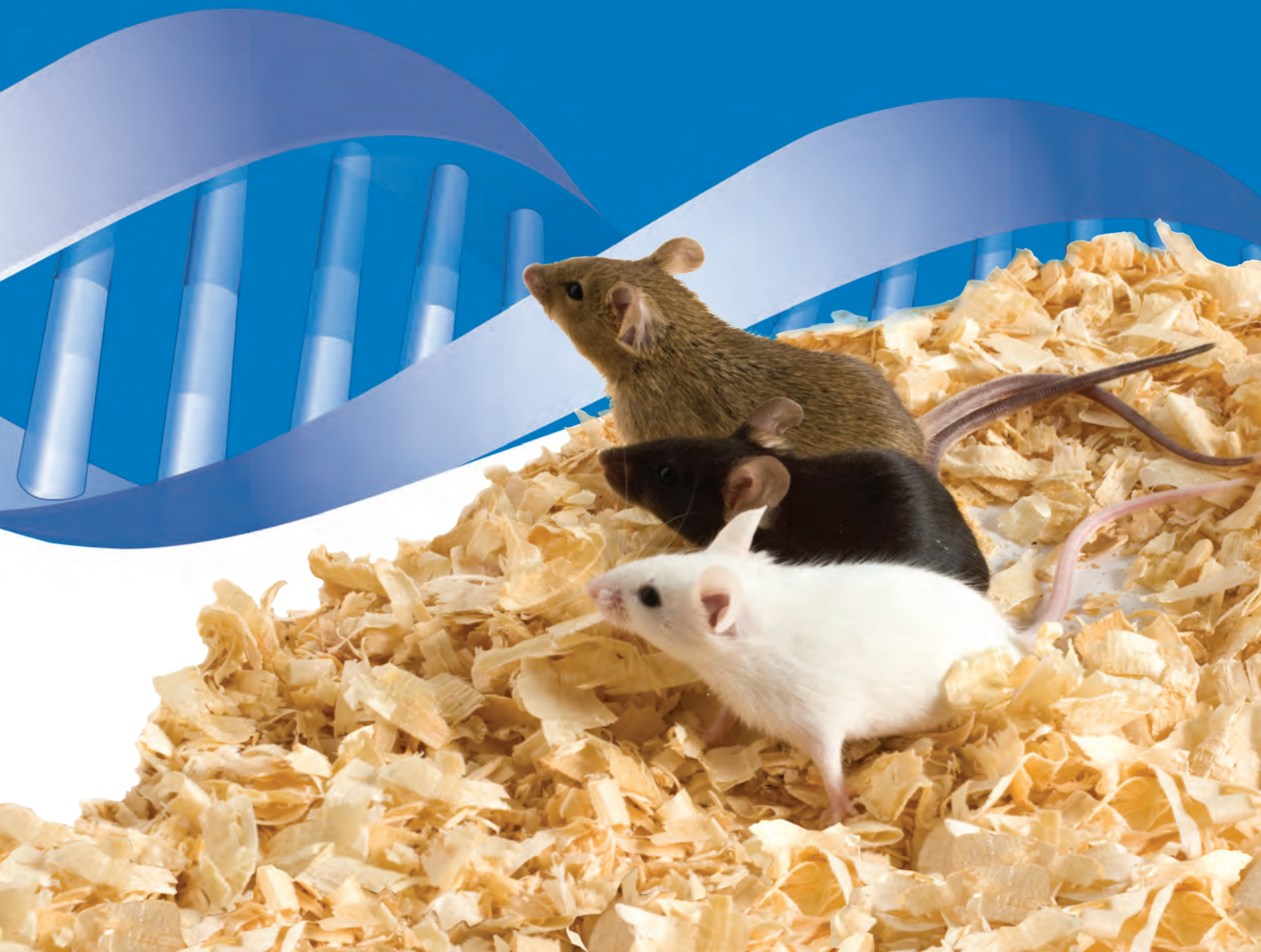
The Jackson Laboratory Handbook on Genetically Standardized Mice



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A note to our readers from the editors…
For optimal viewing of page layout in Adobe Acrobat, we recommend the following: Under the View menu, Page Display, select Two-Up and Show Cover Page During Two-Up. If using Preview on a Mac, under the View menu, PDF Display, select Two Pages.

Because we want this Handbook to be as accurate as possible, we would greatly appreciate it if you would email kevin.flurkey@jax.org with any suggested changes or corrections.

About The Jackson Laboratory

Founded in 1929, The Jackson Laboratory is a non-profit biomedical research institution dedicated to leading the search for tomorrow's cures.

Our mission: We discover precise genomic solutions for disease and empower the global biomedical community in our shared quest to improve human health.

The Jackson Laboratory Handbook on Genetically Standardized Mice

Sixth Edition

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We also want to acknowledge the help we received from other reviewers. Specific contributions are noted in individual chapters throughout the book.

And we want to recognize employees of The Jackson Laboratory not directly involved in the Handbook. It is because of the contributions of each employee of The Jackson Laboratory that JAX® Mice are so widely used—and so well respected—throughout the global research community; that our animal care, technical support, and other services are so highly regarded; and that our reputation as a research institution remains as exceptional as when the first Handbook was published in 1962. It was a privilege to be involved in this project.

Thanks to all.

Kevin Flurkey
Joanne Currer

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Preface

This is the 6th edition of *The Jackson Laboratory Handbook on Genetically Standardized Mice*, which at The Jackson Laboratory, we often refer to simply as the Handbook.

The first edition was published in 1962 as a small booklet. The editor was Earl Green, the Director of The Jackson Laboratory. At that time The Jackson Laboratory employed approximately 350 people; 68 were researchers. We offered "over 60 strains of mice," half of which were maintained by researchers. The objective of that edition of the Handbook was to provide assistance "in planning experiments, in choosing the best types of mice, and placing orders for mice with our Production Department."

In the 46 years since then, The Jackson Laboratory has undergone tremendous change—as have the ways by which we record and access information. We now employ about 1,400 people, with a research staff close to 500. We offer more than 4,000 strains of mice, which are used by approximately 16,000 investigators in 53 countries. Ninety-seven percent of these strains are available only at The Jackson Laboratory. Our website provides access to information about mice in a variety of databases, several of which are updated daily. But the objectives of the Handbook remain consistent: to support our mission by enabling research and education for the global biomedical community, and by providing information about laboratory mice and about choosing and ordering mice.

With this edition of the Handbook, we were faced with two major challenges: how to balance the benefits of paper-based vs. web-based documentation, and how to avoid unnecessary redundancy both within the Handbook and with other sources of information about JAX® Mice. Our strategy is to include information that readers may want to browse or keep handy by their desks. For information that is updated frequently, we refer the reader to the resource with the most current information.

The organization of The Handbook

The Handbook is organized as follows:

Section I, Introduction (Chapters 1 and 2): background information about the history of the laboratory mouse and its value as a mammalian genetics research tool; also, overview information related to the genetics of laboratory mice.

Section II, Using Mice in Research (Chapters 3–5): reference information about laboratory mice and JAX® Mice and about selecting a strain and controls for research.

Section III, Bioinformatics (Chapter 6): information about bioinformatics resources available through our and other websites.

Section IV, Colony Management (Chapters 7–16): information about managing a mouse colony, including animal health, genetic quality control, breeding strategies, day-to-day care, emergency planning, and vivarium staff development.

Section V, Ordering JAX® Mice and JAX® Services (Chapters 17–19): information about placing orders, getting technical help, and frequently asked questions; also, an overview of JAX® Services and The Jackson Laboratory—West.

Back matter: Appendixes and an index.

Conventions used in the Handbook

Throughout the handbook, we include the JAX® Mice stock number in parentheses after the full strain name, as in B6.129P2-*Apoe*^{tm1Unc}/J (002052). We define abbreviations the first time they are used within each chapter. If we think a good portion of readers might not understand a term, we define it within the text.

Chapter 1: Why the Mouse?

Kevin Flurkey, Joanne M. Currer

It is a very exciting time for biomedical research. Over the past century, advances in science, medicine, and public health have led to preventions and cures for many of the most devastating infectious diseases. Polio has been virtually eliminated; smallpox has been completely eliminated. Lifespan in developed countries has nearly doubled.

During the last half of the 20th century, research efforts also led to a greater understanding of a different category of major killers generally unrelated to infectious disease—chronic diseases such as cancer, diabetes, and heart disease—which are heavily influenced by genetic factors. The discovery, in the late 1970s, of the means to rapidly sequence DNA led to powerful techniques for the identification of the very genes that determine the risk factors for developing these diseases. As researchers learn more about the genetic bases for such diseases, they develop more options for intervention, such as drug therapies that alter the way specific genes work. Ultimately, today's research sets the stage for the introduction of novel gene therapies that will directly alter the functions of defective genes or even introduce entirely new genes.

From the beginning of mammalian genetics research, the mouse, especially the inbred mouse, has been a critical tool in the endeavor to understand the genetics of human disease. Subsequent developments in technology have led to a substantial increase in the versatility and value of the inbred mouse. Today, the inbred mouse is universally accepted as the primary model for inherited human disease (Davisson and Linder, 2006).

We anticipate that the great success of science and medicine over the past century will extend into the current century to continue the remarkable progress in alleviating human suffering and improving human health. Much of this progress will be a result of the revolution in genetic technology and its application to research using models based on the inbred mouse.

1.A. From inbred sweet peas to mice

1.A.1. Mendel's work with peas

For most of us, our first formal exposure to the genetics of inheritance was probably when we learned about Gregor Mendel's research on sweet peas, conducted in the 1860s in a monastery in the Austrian Empire (now the Czech Republic). Ironically, Mendel had wanted to study mice, but due to restrictions in the monastery, he instead worked with a species of peas that he bred for generations so that characteristics that differed between plants were maintained constant within a line. It is through Mendel's work with these inbred peas that we learned about simple, dominant vs. recessive, inherited traits: When he crossed purple-flowered plants with white-flowered plants, he got more plants with purple flowers (dominant) than white flowers (recessive). No flowers were pink. Even today, we call such "simple" traits, i.e., traits that are determined primarily by a single gene, Mendelian traits.

1.A.2. Galton's work with peas

Later in the 19th century, Englishman Sir Francis Galton, who had a broad range of scientific interests, studied inheritance in sweet peas from a different perspective: A pioneer in statistical analysis, Galton's research indicated that inheritance of some traits, such as the size of the peas, was not quite as straightforward as Mendel's conclusions regarding flower color. These traits were complex, and often additive, and they were just as heritable as the traits Mendel studied. Today, we call these types of traits "quantitative." They are determined by multiple genes rather than a single gene.

1.A.3. Application of Mendel's and Galton's theories to mammals

Toward the end of the 19th century and the beginning of the 20th century, scientists began to ask if the rules of inheritance formulated by Mendel and Galton applied to other organisms. Mammals were an obvious choice as models to test these ideas because of the potential for applications to normal human biology and to human disease. These researchers became interested in the "fancy" mice that hobbyists had been breeding in China, Japan, Europe, and the United States to create pets with specific coat styles and colors. These fancy mice were appealing as research models because they were already domesticated, readily available, and easy to breed and maintain. Importantly, variants of a simple phenotype, coat color, were well known. In fact, the first paper describing the application of Mendelian genetics to mammals was on the coat colors of mice (Cuénot, 1902).

How do simple and quantitative traits relate to human physiology and disease?

Inheritance of some human traits and disease is simple—based on a single gene. One example related to normal anatomy is ear lobe structure. A single gene determines whether ear lobes are attached or free. Another example relates to blood type. Combinations of the three versions of the same gene result in one of four blood types, commonly known as A, B, AB, or O. Two examples related to disease are cystic fibrosis and Huntington's disease, both of which are the result of a mutation in just one gene.

However, most human traits and genetically-based diseases are quantitative—related to multiple genes. Several examples of normal traits under complex genetic regulation are eye color, size and shape of the nose, and height. Several examples related to disease are type 2 diabetes and most types of cancer, which involve multiple genes that interact with each other to establish an individual's risk of developing the disease.

1.B. From mice to inbred mice

1.B.1. Little's early research and ideas on inbreeding

Clarence Cook Little was one of the early researchers interested in using fancy mice. His first two papers, published when he was still an undergraduate in William Castle's laboratory at Harvard University, were on the coat colors of mice (Castle and Little, 1909, 1910), but his interests also included the possibility that the "new" field of genetics might provide solutions to the problem of cancer. The discovery, in the late 19th century, that tumors could survive transplantation in mice was exciting for researchers because it gave them a way to experimentally control the incidence of cancer. Disappointment followed, however, as success rates proved to be highly variable (Strong, 1978). Little believed that one way to eliminate some of that variability would be to study animals as genetically similar as possible (Staats, 1966). The only known strategy to achieve this was genetic fixation by inbreeding—producing stocks that would "breed true" for any genetically determined characteristic. Little recognized three distinct advantages with this strategy:

- By removing genetic variance within a strain, researchers could more directly relate disease expression to a specific genotype.
- By developing multiple inbred strains, each with unique characteristics, researchers could compare one inbred strain with another. Any difference between the strains could be defined as genetically based, even though the genes involved were not known. By selectively intercrossing inbred strains, researchers could begin to understand the heritability of disease .
- By using the same inbred strains in multiple laboratories and from program to program, researchers could expect experimental results to be reliable and replicable.

1.B.2. Little's and Strong's development of inbred mice

Castle, among many others, recognized the advantages of inbreeding, but he was dubious that inbred lines of mice would survive the severe impairments in reproductive performance resulting from inbreeding depression. For some young scientists such as Little and Leonell Strong, however, the potential payoff was worth the considerable effort and years of work required in attempts to inbreed mice. Thus, in 1909, at the newly founded Bussey Institute (Harvard), Little began developing the first inbred mouse lines for his study of coat color genetics. His work succeeded, and eventually led to the first inbred strain—DBA. By 1918, at the Cold Spring Harbor Laboratory (New York), Little and Strong were instrumental in developing several of the most common inbred strains still in use today. One of these strains was C57BL/6, the progenitor of the C57BL/6J (000664) strain of JAX® Mice—the first inbred strain chosen (in fact, the first animal after humans) for complete DNA sequencing (Mouse Genome Sequencing Consortium, 2002).

1.C. From inbred mice to JAX® Mice

1.C.1. Little's founding of The Jackson Laboratory

Little continued his research using mice to explore the genetic basis of cancer. He further developed his academic career as president of first, the University of Maine, and then the University of Michigan. When he left the presidency of the University of Michigan in 1929 to return to full-time research, it was funding by philanthropists that enabled him to found The Jackson Laboratory.

1.C.2. Little's vision for The Jackson Laboratory

Little's goal was to continue the development of inbred strains of mice at The Jackson Laboratory and to use these strains to study cancer and other genetically-based human diseases. With the inclusion of Leonell Strong on the original Jackson Laboratory staff, a number of inbred strains—including A, C3H, and CBA—were added to the unique and growing collection maintained at The Jackson Laboratory. As recognition of the value of inbred mice grew, it became difficult for The Jackson Laboratory staff to keep pace with requests from other researchers for breeding stocks (Strong, 1978).

The stock market crash of 1929 decimated research funding opportunities, which were primarily from private sources at the time. Little was determined to continue his research, however. To defray some of the costs of rearing the inbred strains at The Jackson Laboratory, in the early 1930s he began a formal program to distribute mice to other researchers. And thus he solidified the mission of The Jackson Laboratory that exists to this day: to conduct critical mammalian genetic research *and* to supply high-quality inbred mice—JAX® Mice—to researchers throughout the world (Rader, 2004).

Why geneticists love mice…

The numerous advantages of mice as mammalian research models include size (among the smallest mammals, they are inexpensive to maintain); robustness (they thrive and breed under a wide range of environmental conditions); and fecundity (they have a short gestation, produce large litters, and develop rapidly, which allows for rapid expansion of a colony). And, most laboratory mice are quite tame.

As research with mice has progressed, a surprising, yet frequent, observation has been how closely mice and humans are related biologically, despite the size difference. Mice have almost all the same organs as humans, and they share a 95% DNA coding sequence identity with humans. Because of their close metabolic and anatomical similarities to humans, mice have many syndromes that are similar to human inherited diseases. In fact, mice are at least as closely related biologically to humans as any of the familiar agricultural and domestic mammalian species. Only primates are closer evolutionarily. This results in the comparable ordering of genes for long stretches on mouse and human chromosomes (synteny), which makes the mouse extremely useful for comparative genetics.

Almost certainly, the single most valuable characteristic of the mouse as a research tool, however, is the ability of some lineages to survive inbreeding depression, permitting the development of inbred strains.

1.D. JAX® Mice and The Jackson Laboratory

Many important scientific discoveries related to genetics have involved research using inbred mice at The Jackson Laboratory. Several examples are worth noting here because they advanced the field of mammalian genetics and resulted in broad application to human health.

1.D.1. George Snell and congenic strain development

1.D.1.a. Snell's research on tumor rejection among inbred strains

One of the most significant advances in the use of inbred mice as research tools was George Snell's development of congenic strains. In a congenic strain, a small segment of DNA containing a genetic variant of interest is transferred from one strain into another through directed breeding. In the 1940s, a few years after Snell joined The Jackson Laboratory, he began looking for a research project that offered "the prospect of yielding some really clear-cut and basic information." (Snell, 1978.) Snell learned that Little had identified inbred mouse strains that had genetic differences in tumor rejection and that these differences involved numerous loci. Snell recognized that this work was relevant to the genetics of tissue rejection and that understanding this issue had important biomedical implications. To progress, however, he needed a way to isolate the loci that determined rejection (tumor resistance) so that he could study each locus individually.

Snell began the laborious process of creating his congenic lines by crossing a strain that accepted a specific transplanted tumor (a susceptible strain) with a strain that rejected the transplanted tumor (a resistant strain). He then tested the hybrid offspring using tumor transplantation. Resistant mice were then backcrossed again to the susceptible strain. With repeated backcrossing, the alleles unrelated to resistance eventually became extinct in the line. This isolated the allele of the gene (or genes) in the selected congenic segment responsible for resistance on a defined, inbred, genetic background.

An interesting problem was that, because tumor resistance is usually recessive, every backcross to the susceptible recipient strain (producing offspring heterozygous for the susceptibility alleles) produced only susceptible offspring. How was Snell to identify carriers of the resistant alleles? He solved this problem with a creative breeding strategy (for the solution see Appendix H, "Transfer of a Mutant or Variant Allele to a New Genetic Background by Phenotypic Selection"), and after years of work, produced a series of "congenic resistance lines" that identified a number of histocompatibility loci. One locus, the *H2* locus, appeared far more frequently in Snell's lines than any of the other loci because it had a more powerful effect on resistance than the other loci. This led to the discovery of a comparable locus in humans, the major histocompatibility locus (MHC), which governs immune self-recognition and thus, rejection of transplanted tissue in humans, as the *H2* locus does in mice.

1.D.1.b. Snell's legacy: organ transplantation, the congenic mouse…and the Nobel Prize

Snell's work is well known because it paved the way to successful organ transplantation in humans. But this same research also resulted in two remarkable achievements specifically related to the use of the inbred mouse in research. First, by creating a set of inbred lines that differed at only at a defined genetic segment, he enabled future researchers to characterize the molecular mechanisms of tissue rejection, as well as identify the mechanism of antigen presentation, which helps determine the specificity of the acquired immune response. These discoveries have implications for the treatment of virtually all infectious diseases, as well as all autoimmune diseases such as lupus and type I diabetes. Second, Snell's work demonstrated that effects of a single gene could be isolated for study, even for a complex phenotype, by creating a congenic strain—a "living tool." Snell showed how a gene could be converted from one allele type to another in a living animal, and thus, long before the development of modern genetics, established a strategy that still is used extensively today, more than 50 years later. Indeed, the use of congenic strains in conjunction with the tools of genetic engineering makes it possible for researchers to isolate and archive, in living repositories, both natural and engineered genetic variants that enable the study of virtually any gene in its vital setting. None of this could have been accomplished without the inbred mouse.

Snell's work, conducted in the 1940s and 1950s at The Jackson Laboratory, secured the value of the congenic strain in biomedical research. Today, in fact, the congenic strain is the predominant type of inbred strain populating The Jackson Laboratory's national repository for mutant mice, the Induced Mutant Resource (IMR). For his work using inbred mice, which laid the foundation for tissue and organ transplantation, Snell was awarded the Nobel Prize in Physiology or Medicine in 1980.

1.D.2. Donald Bailey, the recombinant inbred strain panel, and the Collaborative Cross

1.D.2.a. The concepts of linked traits and linkage groups

Shortly after the rediscovery of Mendel's laws at the end of the 19th century, geneticists recognized that Mendel's law of independent assortment applied to *groups* of phenotypes, later called linkage groups, rather than to all individual phenotypes. The assignment of phenotypes to linkage groups was a major challenge to geneticists throughout the mid 20th century. The results of this effort built the foundation for gene mapping and gene discovery in mice, which is essential for today's application of the genetics revolution to human health.

Before 1985, mapping a genetic locus usually took years, and in many cases, was impossible. Success depended on determining whether a phenotype of interest assorted independently of, or was linked to, a previously mapped genetic marker (such as coat color) for each linkage group. This was accomplished by testing for the co-expression of the phenotype and the marker in progeny from crosses between a strain that expressed the phenotype and a strain that expressed the marker. Such crosses are called mapping crosses. Mapping a locus for a new phenotype required multiple mapping crosses and many hundreds of mice.

1.D.2.b. Bailey's insight into preserving "recombinations" in a panel of inbred strains

In the 1960s Don Bailey, explored ways to simplify mapping. Bailey's insight (1971) was that the unique, random recombinations of the genomes of two parental strains that are necessary for mapping could actually be preserved for future research by creating inbred strains from the progeny of a mapping cross. These inbred strains would make up a recombinant inbred (RI) panel. Because, for each RI strain, the recombinant genotype would be stable across generations, it would be necessary to genetically type the mice only once, no matter how many mapping studies the RI panel was used for. To map a new phenotype, it would be necessary only to evaluate that new phenotype in the RI strains of the panel.

Bailey's work was pivotal in accelerating the pace of gene mapping in mice through the 1970s and 1980s. The results provided the scaffolding necessary for later detailed mapping that eventually led to the complete sequencing of the mouse genome in 2002 (Mouse Genome Sequencing Consortium, 2002).

1.D.2.c. The reinvigoration of the recombinant inbred strain

Today, the powerful tools of molecular genetics have diminished the use of RI strains for mapping fully penetrant phenotypes, i.e., phenotypes that are expressed in all individuals that have the appropriate genotype. RI strains are still useful, however, for phenotypes with low heritability or low penetrance because multiple individuals with the identical recombinant genotype (i.e., individuals of the same RI strain) can be evaluated.

One of the drawbacks of the current RI strain panels is that they are primarily useful for mapping monogenic traits, whereas the majority of disease traits are determined by complex combinations of genes. This problem can be overcome by increasing the number of RI strains in a panel and by increasing the diversity of founder strains. These tasks will soon be accomplished through the efforts of the Complex Trait Consortium (Churchill *et al*., 2004; Chesler *et al*., 2008), by creating a huge RI strain panel, called The Collaborative Cross, in which hundreds of strains will be generated from a highly diverse 8-way cross. The genetic diversity of the founder strains, which includes three subspecies, was devised to more accurately simulate the genetic diversity of human populations, and specifically designed for complex trait analysis. By providing a common set of genetically defined inbred mice in a very large RI panel, the Collaborative Cross is intended to become a focal point for cumulative and integrated data collection across institutions and time. These data will greatly expand the use the inbred mouse by enabling a more effective analysis of the complex genetics of normal and pathological functioning of the mammalian organism.

1.D.3. The inbred mouse and further seminal research at The Jackson Laboratory

Throughout the history of The Jackson Laboratory, many investigators have been involved in pioneering research. Their scientific contributions, made possible by the genetic consistency of inbred mice and related models, have influenced the course of biomedical research to improve human health. Several examples follow.

1.D.3.a. A few of the biomedical research breakthroughs at The Jackson Laboratory

Leroy Stevens, in the 1950s, conducted research on pluripotency and differentiation. Stevens had observed that mice of the 129 strain were more susceptible than mice of other strains to teratocarcinoma, a rare form of cancer in which tumor cells of one tissue spontaneously differentiate into cell types of other tissues (for example, a teratocarcinoma cell in ovarian tissue might differentiate into neuronal cells). Using directed breeding strategies, he created a 129 substrain with enhanced expression of the cancer, and was thus able to isolate and study the pluripontency of the teratocarcinoma cells. This work led to the demonstration that embryonic stem cells could be similarly isolated and studied, which enabled two revolutionary technologies in genetics: First, with embryonic stem (ES) cells in culture, researchers gained access to the cellular substratum required to create living knock-out and knock-in mouse models from genomes genetically engineered *in vitro*. Second, with the knowledge that ES cells could produce various differentiated cells, researchers recognized the real prospect of human stem cell therapy.

Other researchers at The Jackson Laboratory used inbred mice in their pioneering work in the field of physiological genetics. Elizabeth "Tibby" Russell's work on hematopoiesis, using a number of inbred strains, helped define the field of red blood cell biology and led to the first bone marrow transplant to cure a disease—in this case, anemia.

Douglas Coleman discovered a recessive mutation that causes obesity and diabetes on one inbred strain background, but only obesity on another. He observed the same background effect when another recessive, obesity-producing mutation—originally discovered by Snell—was studied on the same two strain backgrounds (the diabetes-susceptible background was C57BLKS/J [000662]; the diabetes-resistant background was C57BL/6J [000664]). Both mutations produced identical phenotypes when studied on the same inbred strain background, but clearly were on different chromosomes. Coleman intuited that one might be a gene that encodes a receptor in a satiety center, and the other its ligand. Indeed, the obesity-producing mutation originally discovered by Snell was later found to encode leptin, the first hormone shown to be secreted by fat tissue (and inform the brain as to nutrient homeostasis). The mutation discovered by Coleman was in the leptin receptor gene. Coleman's work not only established the relationship between the two mutations, but also was the first demonstration of the presence of modifier genes that differed in the various inbred strains of mice. This was a seminal discovery in the field of biochemical genetics.

1.D.3.b. Bioinformatics

Groundbreaking work at The Jackson Laboratory also enhanced the way researchers collected, organized, and disseminated information about inbred strains of mice. In 1958, Margaret Green established an "index card" file of genetic data on inbred strains. In 1990, Muriel Davisson and Thomas Roderick used these data as a foundation for one of the first computer-based mouse databases (Gbase). And in 1992, Janan Eppig began the process of integrating the access to a series of key databases related to mouse genetics. This work grew into the Mouse Genome Informatics (MGI) database collaboration, which today provides integrated retrieval and analysis of data on the genetics of the laboratory mouse to researchers throughout the world at the click of a button. It is due to the genetic consistency of the inbred mouse that so much reliable data has been collected throughout the years and that these data are still relevant today. It is now possible for a researcher almost anywhere in the world to use the power of the computer to expand the power of the inbred mouse—to analyze data and even develop novel hypotheses—even before setting foot in a mouse room.

1.D.3.c. JAX® Mice

Many researchers at The Jackson Laboratory have developed new inbred strains and new mouse models based on the inbred strain. The Jackson Laboratory also has continued to import new strains from other investigators, while maintaining even the oldest of the original inbred strains, such as the C57BL/6J (000664) strain, which has now been inbred for more than 226 generations. The number of available strains of JAX® Mice is now around 4,000.

1.E. It is still about the inbred mouse…

It is not clear why mice can be successfully inbred. Perhaps it is because they evolved living in small, closely knit family groups (demes). Whatever the reason, we are fortunate that a mammal that physically is so ideally suited for research—and genetically so similar to humans—is also so ideally suited for inbreeding.

Because mice could be inbred, a foundation of genetic information was established upon which ever more sophisticated "living" research tools could be built. And because knowledge of the mouse genome became more advanced than that of any other experimental mammal, the inbred mouse became the ideal vehicle for translation of the recent genetics revolution to mammalian biology. Today, we can add, subtract, or selectively alter virtually any gene in the mouse genome, and we can even use the mouse as a host for genes from other species ranging from algae to human. The present capability to manipulate the mouse genome was almost unimaginable just 35 years ago, and the potential advancement of our understanding of mammalian genetics, and its relationship to human disease, now seems virtually unlimited.

Little's legacy—the inbred strains he developed and The Jackson Laboratory that he founded—remain at the forefront of biomedical research today. As we search for tomorrow's cures in the 21st century, we still turn to the inbred mouse—and models based on the inbred mouse—that Little pioneered almost 100 years ago.

1.F. For more information…

To learn more about the history of the mouse in research or The Jackson Laboratory, see the following resources:

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Chapter 2: Some Basic Genetics of the Mouse

Kevin Flurkey, Joanne M. Currer

Throughout this handbook, we presume that readers have a familiarity with basic genetics. For readers who might be unfamiliar with some of the concepts, especially as they apply to the mouse, this chapter provides background and perspective.

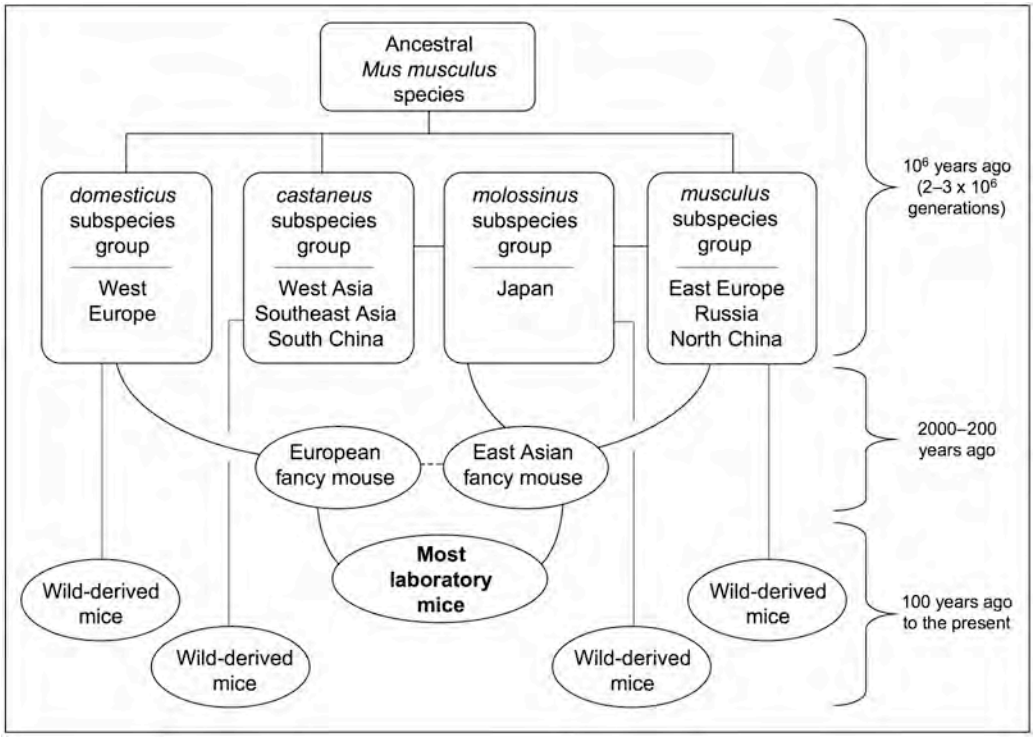
The chapter is organized as follows:

***Acknowledgments**: We would like to thank Janice Pendola and James Yeadon for their valuable review of this chapter.*

2.A. Origins and basic genetic characteristics of the laboratory mouse

Most conventional strains of laboratory mice are genetic mixtures of *Mus musculus domesticus* (about 85–90%), *M. m. musculus* (about 5–15%) and *M. m. castaneus* (less than 1%) (Yang *et al*., 2007). Most wild-derived lines are pure *M. m. domesticus*, *M. m. musculus*, or *M. m. castaneus*; however, some are *M. m. molossinus*, which is an ancient mixture of *M. m. musculus* and *M. m. castaneus*. Other *M. musculus* subspecies, such as *M. m. bactrianus, M. m. praetextus* and *M. m. wagneri*, remain greatly underrepresented among inbred laboratory strains. Figure 2.1 provides an overview of the origins of mice used in research.

Figure 2.1. Origins of mice used in research.



Drawing adapted from Yoshiki and Moriwaki (2006).

Table 2.1. Basic genetic information about the laboratory mouse; comparisons with humans.

Characteristic	Value for mice	Value for humans
Number of chromosomes	Haploid number = 20 (19 autosomes; 1 sex chromosome) Diploid number = 40	Haploid number = 23 (22 autosomes; 1 sex chromosome) Diploid number = 46
Number of genes	23,000 identified; potentially 25,000	20,000–25,000 (Human Genome Project Information, 2008)
Number of centimorgans (cM)*	1,500 (average chromosome: 75 cM)	3,000 (average chromosome: 130 cM)
Number of base pairs	2,700,000,000	3,200,000,000

*The centimorgan (cM) refers to genetic distance based on recombination frequency.

2.B. The vocabulary of genetic architecture

The genetic architecture of a phenotype refers to the specific genes and alleles—and their interactions—that influence the expression of the phenotype. Much of biomedical genetics is concerned with elucidating the genetic architecture of heritable traits. Following are definitions and comparisons of terms used when defining the genetic architecture of a phenotype.

2.B.1. Terminology

Basic terms used throughout the book include the following:

genome	The genetic makeup of an organism as a whole, represented by a full set of chromosomes.
genotype	Depending on context, the allelic composition of • a single gene in an individual, • multiple genes that affect a single trait in an individual, or • all the genes of an organism.
phenotype	A physical characteristic determined by a genotype and its interaction with the environment. The term phenotype is often used interchangeably with "trait" and "character."

Genetic diversity within a species occasionally is due to deletion or duplication of genes, but usually is due to genes that are polymorphic.

polymorphic	Refers to • a trait that occurs in multiple versions, or • a locus or gene with multiple alleles. By convention, a trait or a locus is considered polymorphic when the most common version or allele occurs at a frequency of less than 95%. This definition contrasts with "monomorphic," which refers to a trait or a locus with little or no variation within a defined population.

Terms used to describe specific types of alleles include the following:

wild-type allele	The most common allele of a gene within a population.
mutant allele	An allele of a gene that appears at less than 1% frequency in the population.
variant allele	An allele of a gene that appears less frequently than the wild-type allele, but at greater than 1% frequency in the population. All alleles arise through mutation. If the frequency of a mutant allele increases in a population, it may be called a variant. The distinction, 1% frequency in the population, is only approximate.

The relationship between the alleles at a specific locus is described by the following:

heterozygous	Having two different alleles at a locus.
homozygous	Having two identical alleles at a locus.
hemizygous	Having only one allele at a locus, such as an allele on the unpaired Chr X of a male or an unpaired transgene. When, for a designated population, all alleles for a gene or a locus are identical, the gene or locus is said to be "fixed."

The terms used to describe the functional relationship of two different alleles at a specific locus are the same terms used to describe the "mode of inheritance" of a phenotype:

Recessive	For the phenotype to be expressed, the alleles must be homozygous.
Dominant	For the phenotype to be expressed, the allele can be homozygous or heterozygous.
Semi-dominant or additive	The expression level of the phenotype in heterozygotes is intermediate between the expression level of the phenotype in homozygotes for one allele and homozygotes for the alternate allele.

Most of the terms defined on the previous page refer to single genes or loci, or to traits (phenotypes) that are determined primarily by a single gene. Many individual traits, however, are influenced by multiple genes. The description of the additive and interactive effects of polygenic regulation of a trait completes the description of the trait's genetic architecture.

Simple trait	A trait for which the genetic variance is due primarily to allelic variation at a single locus.
Complex trait	A trait for which the genetic variance can be apportioned among multiple loci.
Quantitative trait	A complex trait—such as body weight—that is measured on a continuous scale. Polygenic influences can be additive (the effect of the genotype at each locus is independent of the genotypes at other loci) or interactive (the influence of the genotype at one locus is altered by the genotype at another locus) or both.
Epistasis	The result of an interaction of two or more loci on the expression of a phenotype (e.g., see 2.B.2.c).

2.B.2. Selected topics

2.B.2.a. When is a "gene" not a gene?

"How many of you have the cystic fibrosis gene?" Eric Lander would ask as he began his lecture on basic genetics at The Jackson Laboratory's annual Short Course on Biomedical Genetics. Typically, about half the class would raise their hands. Of course, everyone in the class had the gene, as virtually all of us do. In fact, if you did not have the gene, you would *have* the disease. The "cystic fibrosis gene" is a chloride ion channel gene formally named "cystic fibrosis transmembrane conductance regulator" or CFTR. When both copies of the gene in a person are null alleles (i.e., the alleles produce either nonfunctional CFTR proteins or no protein), the person is completely missing the CFTR chloride ion channel. The disease, cystic fibrosis, results. In other words, people with two cystic fibrosis alleles at their CFTR gene have the disease; those with at least one wild-type allele at their CFTR gene are normal. But we all have the "cystic fibrosis" gene, whether or not we have the disease.

A gene is a heritable unit that influences a phenotype. An allele is a version of a gene that produces a specific phenotype. A given gene may have only one known allele, in which case the terms are effectively interchangeable. Or, a given gene may be polymorphic, with two or more known alleles, in which case the terms gene and allele refer to different things—for example, the gene "leptin" (*Lep*); the alleles "leptin; obese 2 Jackson" ($Lep^{ob\text{-}2J}$), "leptin; obese" (Lep^{ob}), and the wild-type allele "leptin; wild-type" (Lep^{+}).

The widespread practice of informally naming a gene after the deviant phenotype produced by a mutation of the gene leads to occasional misunderstandings, even among geneticists. To say that an inbred strain does not have the "gene" for some disease phenotype because it does not express the disease is almost always incorrect (except where deletions are involved). The practice of calling a mutant allele a "gene" has become entrenched because of its convenience, and to simplify public communication, but it should be avoided in all scientific communications.

Scientific communication is also not well served when investigators continue to use purely phenotypic descriptors (originally used to name mutations) after the gene itself is identified. For example, once a missense mutation in the carboxypeptidase E (*Cpe*) gene was shown to be the molecular basis for the "fat" mutation, the correct nomenclature for the mutation became Cpe^{fat} rather than "fat."

2.B.2.b. How can an allele be dominant and recessive at the same time?

Formally, dominance is a phenotypic, *not* a genotypic, relationship. Mendel was unaware of our concept of genotypes when he introduced the terms "dominant" and "recessive" to describe the relationship between the phenotypes of traits he studied. Yet, for the sake of convenience, we commonly refer to a "dominant" or "recessive" allele as the allele that produces a dominant or recessive phenotype. This shorthand is generally clear, except in cases where the gene is pleiotropic, i.e., it influences multiple phenotypes.

The 2008 Wikipedia provides an example of how multiple modes of inheritance can apply to a single allele. The allele that produces the sickle cell trait in red blood cells is caused by a base pair substitution in the beta-globin gene that replaces a glutamine with a valine. When only one of the two copies of the beta-globin gene is the mutant allele, resistance to malaria is conferred. This indicates that the allele is dominant. But both copies of the same mutant allele are needed to produce anemia. This indicates that the allele is recessive. Furthermore, the phenotype of blood cell sickling is co-dominant—it occurs when only one copy of the mutant allele is present, but it is more severe when both copies are the mutant allele. Thus, the same allele can display different modes of inheritance if the gene is pleiotropic (has multiple effects), even though each of the associated phenotypes has only one mode of inheritance.

2.B.2.c. When is "dominance" not dominance?

Dominance refers to a relationship of two traits that are controlled by the same locus. "Masking epistasis" refers to a relationship of two genes, in which a particular genotype at one gene prevents the expression of the other gene. For many phenotypes that involve complex inheritance, dominance often gets confused with "masking epistatis."

Coat color in mice provides a classical example. Among laboratory strains, at least five genes have common variants that alter coat color. One of these, *Tyr* (tyrosinase), governs the influence of the other genes on coat color because its activity is required to produce melanin, the pigment responsible for coat and skin color. When *Tyr* is inactive, melanin is not produced, and albinism results, no matter which alleles are at the other coat color genes. The albino trait "masks" the expression of the other coat color genes. The albino trait is sometimes, incorrectly, said to be dominant over other coat color phenotypes. But because both copies of the *Tyr* gene must be inactive for albinism to result, the albino trait is *recessive*. Thus, albinism is a recessive trait that masks the expression of other coat color genes; it is *not* dominant over other coat color phenotypes. (For more details about coat color genes, see 2.F., "Coat color genetics.")

2.B.2.d. Is a locus also a gene?

Often the terms "locus" and "gene" are used interchangeably; however, they do have different meanings. A locus is a segment of a chromosome that is demonstrated, by mapping, to contain at least one genetic modification that influences a trait. That chromosomal segment may contain hundreds of genes or only one gene. Although naming a locus for a specific trait (for example, a hyperlipidemia locus) does not mean the gene is known, once a locus is named, often the putative gene that influences the trait is referred to by the same name. Thus, as of 2008, a hypertriglyceridemia locus (e.g., *Tgl1*) may be said to contain the "*Tgl* gene," even though a specific gene within that locus has not been unequivocally specified.

On the other hand, numerous genes that were historically identified as loci before the specific gene was known may still be referred to as "loci." The albino locus is one common example. We now know that the albino phenotype results when the gene tyrosinase (*Tyr*) is inactivated. There is no need to continue to use the less precise term "albino locus" to refer to the gene; however, the practice continues due to traditional usage. Thus, although "gene" and "locus" have precise, and different, definitions, common usage often mixes the terms.

2.B.2.e. What do geneticists mean by "segregation"?

Segregation refers to the separation, in the parent, of two heterozygous alleles into different gametes (and therefore, into different offspring) during meiosis. When geneticists refer to segregation of a phenotype in a cross, they mean that the phenotype appears in some, but not all, offspring because the genotype is not fixed. When a population is said to be segregating, it means that the line is not fully inbred and that one or more loci are not fixed. It is worth noting that variable expression of a phenotype is not sufficient evidence to claim that the phenotype is segregating. For example, all genes affecting the *phenotype* may be fixed, but the phenotype may display incomplete penetrance; i.e., environmental or random factors may prevent expression of the phenotype in some individuals.

2.B.2.f. Complex genetic regulation and the myth of the Mendelian trait

When Mendel started his studies of inheritance, he specifically chose to study only binary traits—traits with only two alternatives, such as purple or white flowers. He also limited his study population to plants that bred true, i.e., plants that did not express other flower colors, seed coat varieties, or any alternate variations of the other binary traits. As a result, Mendel studied traits that were each controlled by a single gene. Today, we use the term "Mendelian trait" when referring to binary traits that appear to be controlled by a single locus.

However, there may be no true Mendelian traits. The idea that some phenotypes may be determined by a single gene is an oversimplification. It is more likely that all phenotypes are determined by networks of genes. But to simplify the study of a trait, researchers often create experimental conditions so that a single locus accounts for almost all of the genetically determined variance of a phenotype within their study population. (For example, researchers will study genetic regulation of a trait using only two parental genotypes rather than genotypes representing the entire population of mice.) Under such conditions, we still use the term "Mendelian" as shorthand to indicate that much of the genetic variance can be explained by a single locus.

An example of how this shorthand can create confusion is when a variant or mutant allele for a "Mendelian" trait is transferred to a different genetic background, as when creating a congenic strain. Often, the expression of the phenotype disappears or is altered by interactions of the gene of interest with other genes that differ between the two backgrounds and act epistatically as "modifier" genes. The altered phenotype can be surprising to those who considered the trait truly Mendelian. Thus, it is wise to keep in mind that, although we may use the term Mendelian as shorthand to describe the inheritance of a trait under limited conditions, the genetic regulation of the trait is unlikely to be that simple.

2.C. The basic inbred strain experiment—strain differences capture genetic differences

The basic inbred strain experiment compares the expression of a phenotype between two inbred strains. Any strain difference indicates the existence a *genetic* difference that governs the phenotype. This difference could be due to a variant allele, a mutation, or a deletion or addition of a gene.

This experimental strategy is possible because of the nature of inbred strains. Mice were originally inbred to make expression of a trait as consistent as possible by removing genetic variance. Inbreeding achieves this by 1) fixing an allele that produces the trait, so that alternate alleles are excluded from the lineage, and 2) removing variation in the background genotype, so that epistatic interactions do not vary. The result is a strain of mice in which *all* members are genetically uniform. A profound consequence of this genetic uniformity is that, when comparing two inbred strains under controlled conditions, any phenotypic difference between the strains must result from a genotypic difference. For example, if researchers discover that mice from one inbred strain have a different level of high density lipoproteins than mice from another inbred strain, they have demonstrated that a gene or genes regulating blood lipids differs between the two strains.

An additional advantage of inbreeding is that, by preserving exact genotypes, it facilitates replication and greatly simplifies further study of the genetic regulation. Initial characterization involves three steps. The first question is to resolve the degree of penetrance of the phenotype. If the phenotype is expressed in some, but not all, individuals of a homogenic population (a population in which all individuals have the same genotype, such as an inbred strain or an F1 population), it is considered incompletely penetrant. Incomplete penetrance must be taken into account in further analysis.

The next question concerns the mode of inheritance. Typically, this is determined by crossing the strains that differentially express the trait, i.e., by making F1 hybrids. Additional information about maternal and paternal effects can be obtained by analyzing reciprocal F1 hybrids—hybrids produced by reversing the strains of the mothers and fathers.

The final question to resolve in a preliminary genetic analysis is whether the phenotype is monogenic or polygenic, i.e., whether the trait is determined by one locus or more than one. To address this question, typically, F1 mice are crossed to produce a population of F2 mice. The distribution of values for the trait among the F2 mice is used to determine whether the trait is monogenic or polygenic. The F2 mice can also be used to map the trait using linkage analysis, thus identifying the approximate location of the gene (or genes) that controls expression of the phenotypic difference.

2.D. Linkage analysis

Before it was possible to map a gene to a chromosome, researchers knew that not all traits assorted independently. Two traits were considered "linked" when they appeared together in the same individuals more often than expected by chance. Groups of linked traits were called "linkage groups."

If two traits distributed independently of each other within a population, they were defined as being from different linkage groups. The more frequently two linked traits appeared together, the "closer" they were considered to be. In fact, the genetic distance between the "linked" loci (that determined the linked traits) was defined by the degree of association between the linked traits. Thus, even before researchers could determine the physical positions of loci on chromosomes, they could calculate genetic distances.

How to get from a strain difference to a locus: mapping a gene.

1. Cross 2 inbred strains that differ in a trait of interest.
2. Phenotype the offspring in the first generation that produces genetically unique individuals (usually an F2 or N2 generation).
3. Genotype the offspring using about 6–10 DNA markers (that distinguish the 2 strains) per chromosome, as evenly spaced as possible.
4. Correlate the trait variation (phenotype) with the allelic variation (genotype) at each DNA marker.
5. Where this correlation is very strong, express the results as statistical scores and chromosomal positions (loci) or confidence intervals that are likely to contain a gene that regulates the trait.
6. Using statistical procedures on the mapping data, test for epistatic interactions among the mapped loci.

(Adapted from a presentation given by Ken Paigen [2007].)

Once researchers assigned conveniently scored genetic markers to each linkage group, they could map a newly discovered phenotype by evaluating the association of the new phenotype with those established genetic markers. Because researchers could calculate the genetic distance of the new trait from other traits in the linkage group, they could identify a locus for the trait.

In 1971 Eva Eicher reported the first assignment of a linkage group (linkage group XII) to a chromosome (Chr 19). Within a few years, assignment of the other linkage groups was completed. By the mid 1980s, technical advances enabled the use of the numerous DNA polymorphisms as markers for mapping. This method, which soon replaced the classical physical and biochemical markers, allowed much more rapid and precise mapping of loci. (Mapping is discussed further in 2.F.) Before the mid 1980s, it took years to map a simple trait; today, multiple loci for complex traits can be mapped within months. Typically, the slowest steps are the generation of the mapping cross (typically an F2 or N2) and the development of the phenotype. The genotyping for hundreds of DNA markers, and association of the genotypic variation with phenotypic variation among individuals in the cross, takes only days. The steps involved in mapping the genes that govern a phenotypic variant are summarized in the sidebar, "How to get from a strain difference to a locus: mapping a gene."

2.E. Genotyping: what it is and how it is used

As a noun, genotype refers to the genetic makeup of a locus, a genomic region, or an entire organism. As a verb, genotype is a way to determine or identify a genetic composition. Genotyping has multiple purposes, which include the following:

For single loci:

- To select appropriate subjects for an experiment when the phenotype does not clearly distinguish mice with differential genotypes, as occurs with many quantitative traits or when the phenotype is not directly observable.
- To identify carriers, for example, when selecting appropriate breeders.

For portions of genomes or for entire genomes involving multiple loci:

- To map genes.
- To test for genetic contamination due to inadvertent outcrossing.
- To determine lineage relationships among heterogenic mice.

Sometimes we can genotype mice by sight—a specific coat color or an observable phenotype such as a kinky tail or a behavior. But sometimes, we need sophisticated techniques such as a biological assay or a variation in DNA sequence such as a single nucleotide polymorphism (SNP). Table 3.1 provides an overview of some of the most frequently used methods of genotyping. For information on genotyping strains of JAX® Mice, refer to the sidebar, "If you need to genotype your JAX® Mice or have questions about genotyping…," in 13.F.2, "Confirming phenotypes and genotypes."

Table 2.3. Commonly used methods of genotyping.

Markers	Comments
Single nucleotide polymorphisms (SNPs): Allelic distinction based on a single nucleotide difference in a very short DNA sequence. Typically, any 2 strains differ by thousands of SNPs.	• Uses polymerase chain reaction (PCR): reliable, simple, quick, inexpensive; amenable to high throughput. • Suitable for large- and small-scale production. • Useful for typing mice before they are bred.
Simple sequence length polymorphisms (SSLPs), sometimes called microsatellite markers or "MIT markers": Allelic distinction based on DNA sequence differences in base pair repeats (usually CA or CG). Typically, any 2 strains differ by hundreds of SSLPs.	Same comments as for SNPs (above).
Primers based on sequence variation of a vector or engineered gene, for example, sequence variation of a transgenic vector.	• Uses polymerase chain reaction (PCR): reliable, simple, quick, inexpensive; amenable to high throughput. • Used frequently to genotype transgenics, knockouts, and knockins.
Biochemical markers (isoenzymes) and immunological markers: Comparison of proteins that exhibit different physical characteristics, such as electrophoretic mobility or enzymatic activity. Typically, any 2 strains differ by from 3–50 biochemical and immunological markers.	• Quick, technically simple, readily reproducible, inexpensive. • Determinations can often be made from plasma or red blood cell lysates.
Coat color and other observable phenotypes such as body size, skeletal structure, behavior, reproductive performance, tumor susceptibility, transplanted tissue rejection.	• Readily observable indicators of possible mutations or breeding errors. • Can include disease onset and necropsy.

2.F. Mapping: definition and tools

Genetic mapping locates a region of a specific chromosome that contains one or more genes that influences a trait. Mapping is of particular interest for genes that control diseases and is usually the first step in identifying the gene itself, which can lead to new approaches for treatment of the disease.

2.F.1. What is mapping? What is a centimorgan (cM)?

The process of genetic mapping usually involves a mapping cross—a cross between individuals of two different inbred strains that differentially express the trait of interest and that possess numerous differences in genetic markers. (Markers are useful only when they distinguish maternal- vs. paternal-derived alleles in the offspring.) The first generation of the cross (F1) is homogenic (i.e., all individuals are identical to each other), with each pair of chromosomes comprising one from the mother and one from the father. Gametes from a single F1, however, will differ from each other because chromosomes assort independently during meiosis; each gamete will carry a unique a mix of parental chromosomes.

By scoring the offspring in the F2 generation (the first segregating generation) for the genetic markers and the trait, the researcher can identify which marker the trait associates with, thus designating the chromosomal location of a gene or genes controlling the trait.

An additional feature of genetic mapping that permits resolution to a sub-region of a chromosome involves recombination. When homologous chromosomes recombine during meiosis, a genetic marker on one chromosome will cross over to the other chromosome. The further apart two different markers are on one chromosome, the more crossovers will occur between them. Thus, the frequency of crossovers between markers provides a genetic "distance" between the markers that is approximately related to the physical distance between them. This genetic distance is quantified in centimorgans (cM), a unit of measure of recombination frequency that is equal to a 1% chance that a marker at one locus will be separated from a marker at a second locus due to crossing over in a single generation. For example if 20% of the offspring are the result of a recombination between two markers, the markers are said to be 20 cM apart. Binary traits, which have only two values (Mendel's pea plant flowers that were either purple or white, for example) can be treated exactly the same as genetic markers, and so the genetic distance between a known genetic marker and a gene that controls a binary trait can be determined directly in a segregating cross. For quantitative traits such as body weight, which are measured on a continuous scale, the analytical procedure is somewhat different, but the principle to determine the genetic distance between a marker and a gene is the same.

In mammals, recombinations do not occur entirely at random across the chromosome, but tend to cluster in recombinational "hotspots." As a result, centimorgans do not correlate precisely with physical distances. Hundreds of hotspots are distributed across each chromosome. The specific locations of hotspots along each chromosome differ among inbred strains.

2.F.2. Why map a trait?

Why do geneticists go to all this trouble just to determine the approximate chromosomal location and number of genes that regulate a trait? As stated above, it is the first step in identifying the *specific* genes that regulate a trait. In mice, this is relatively straightforward when studying a trait for which a single gene accounts for most of the variance in a phenotype—as with a mutant mouse—because researchers can create a segregating population in which the phenotypic signal-to-noise ratio is very high. As a result, a large number of genes have been identified by analyzing mutants.

However, for quantitative trait loci (QTLs), each of which accounts for only a portion of the phenotypic variance of a trait, mapping has resulted in identification of far fewer genes. Yet, researchers devote considerable effort to map quantitative traits because knowledge of map locations enables further study of the gene, even before its identity is known. In particular, researchers may construct congenic strains to isolate and highlight effects of the variant allele

on a defined genetic background and to study genetic interactions with other (modifier) genes. Also, researchers continue to develop more advanced mapping techniques that improve the success rate of gene identification using QTLs (e.g., see 2.F.5, "How does bioinformatics enhance mapping?").

2.F.3. What strategies are used for mapping?

Generally, initial mapping is performed using an F2 (segregating) cross with four to ten polymorphic markers per chromosome. An N2 population (a backcross to one of the parental strains) may be used if the mode of inheritance is known. However, an N2 population will reveal only recessive, not dominant, traits contributed by the parental strain to which the F1 generation is backcrossed, and only dominant, not recessive, traits of the other parental strain. Additive traits can also be detected in backcrosses, but the power to do so is diminished because the full range of the trait will not be expressed. In addition, because the background genotype in a backcross is less diverse than in an F2 cross, the potential to discover epistatic interactions is diminished. Thus, generally an F2 population is used for mapping an uncharacterized trait, and an N2 population is used to test specific hypotheses about map locations.

Panels of inbred strains that were constructed specifically for mapping may also be used (see 3.D, "Recombinant strain panels"). Such panels "freeze" the recombinations of a cross by creating inbred strains from the progeny of a cross. Because mice of these mapping panels are inbred, the identical chromosomal recombination, represented in a given strain of the panel, can be reproduced in any quantity (for example, see Figure 3.8, "Creation of recombinant inbred [RI] lines). Use of a strain panel provides an opportunity to study traits with low penetrance, where it may be necessary to generate multiple individuals before the trait can be observed in a few individuals, and to study traits with high environmental variance, because effects of this variance can be minimized by testing multiple individuals with the same recombinations. An additional advantage of mapping panels is that information on the strains is cumulative; once the strains are genotyped, studies of new traits do not require additional genotyping.

Initial mapping studies typically identify a locus that is 10–30 cM long, usually containing more than 100 genes. Often, congenic strains are then created to study the mapped mutant or variant allele on a standard genetic background or to investigate interactions with other genes. If the locus is too large to identify a few good candidates for the specific gene that accounts for the phenotype of interest, the next step in this process is to fine map—reduce the size of the locus, usually to less than 1 cM, to a region containing just a few candidates. Fine mapping was traditionally accomplished using crosses of up to 1,000 mice, often with F3 or F4 generations, to produce a large number of crossovers and enhance the mapping precision. However, strategies using the continually growing databases of phenotypic and genotypic information—bioinformatics databases—are increasingly supplanting the large, advanced cross for fine mapping.

2.F.4. What tools are used for mapping?

Two of the common genotyping methods (Table 2.3) are well suited for mapping studies: single nucleotide polymorphisms (SNPs) and simple sequence length polymorphisms (SSLPs), sometimes called microsatellite (or MIT) markers. The chromosomal density of these markers for any strain comparison is much higher than the density for morphologic and biochemical markers. Typically, hundreds to thousands of markers are polymorphic for most pairs of strains, which permits the detailed mapping of any differential locus between all but the most closely related strains.

2.F.5. How can bioinformatics be used to enhance mapping?

Bioinformatics refers to the computer access, integration, and analysis of collections of biological data. The high-density genotyping information now available through multiple databases can be employed to enhance mapping strategies. Four strategies using comparative genomics and bioinformatics approaches were described by DiPetrillo *et al*. (2005); their application was illustrated by Burgess-Herbert *et al*., (2008):

1) *Comparative genomics* uses synteny among species to narrow a locus for a phenotype. Synteny refers to the comparable linear organization, between two species, of genes on a chromosomal segment. Because of evolutionary relationships, long segments of chromosomes from different related species contain homologous genes in the same order. Comparing mice to humans, about 340 syntenic segments are conserved (Pennacchio, 2003).
2) *Combined cross analysis* compares results from different mapping crosses for the same phenotype. Most of the phenotypic differences among inbred strains result from the different mixes of the ancestral substrain genotypes (primarily *M. m. domesticus* and *M. m. musculus*), rather than fixed mutations. Thus, when trait loci for the same phenotype are found at the same chromosomal location in multiple crosses using different inbred strains, all strains displaying the one phenotype are assumed to share the same ancestral allele of the same gene. In contrast, strains displaying the alternate phenotype are assumed to share the alternative ancestral allele. This assumption justifies the application of statistical methods to combine data from multiple crosses of different inbred strains to increase the power to map a given trait.
3) *Interval-specific haplotype analysis*, also called haplotype block analysis, uses SNP-defined haplotypes as markers to identify a chromosomal interval that could contain a causal polymorphism. A haplotype, which is usually defined by three–five SNPs, is a combination of alleles at loci that are so closely linked that they rarely recombine. Thus, a given haplotype generally derives from an ancestral source and is unlikely to contain a DNA sequence variation. Among different strains, haplotype polymorphisms at the same locus are inherited from different ancestral sources, and thus, potentially, contain a causal polymorphism. Compared to the typical 10 cM trait locus identified by standard mapping crosses, haplotypes are very small. Thus, when haplotypes within a QTL are compared between strains in a cross, it is often possible to considerably narrow the region that is proposed to contain the causal polymorphism without additional genotyping of the hundreds of mice traditionally required for fine mapping.
4) *Genome-wide haplotype association*, unlike interval-specific haplotype analysis, predicts the location of trait loci without prior mapping studies. This analysis compares phenotypic expression among multiple strains (a strain panel) with haplotype differences across the genome, using a sliding window of analysis. As this window moves through the marker map, strains with shared haplotypes are grouped, the mean phenotype values of the haplotype groups are computed, and permutations are performed to establish thresholds of significance. The statistical power of the analysis is influenced by the composition of the strain panel, the density and distribution of SNPs used, and the sliding window size. Genetic loci where haplotype differences correlate with phenotypic differences can then be identified. Because such loci often overlap, but are not identical to, loci for the same trait that were identified by other means, comparison of the results often can narrow a trait locus to less than 1 cM. Although genome-wide haplotype association is controversial because of the potential for false positives (Chesler *et al*., 2001) and other concerns with the precision of the technique (Cervino *et al*., 2007), Burgess-Herbert *et al*. (2008) state that, under specific conditions, the technique has proven useful.

Application of bioinformatics techniques: from quantitative trait locus to candidate genes.

Burgess-Herbert *et al*. (2008) illustrate the use of combined bioinformatics strategies to systematically specify candidate genes for quantitative trait loci (QTL) for plasma levels of high-density lipoprotein (HDL). Using combined cross analysis, the researchers first identified a QTL for HDL on Chr 12 that they could limit to a 26.3 megabase (Mb) interval containing 135 genes. Next, by comparison to human HDL mapping data, using comparative genomics, they further reduced the locus to 12.0 Mb, containing 49 genes.

The researchers then used haplotype block analysis, employing data from a combination of SNP resources (Wellcome Trust, Broad Institute, and Perlegen) and the Mouse Phenome Database (MPD; www.jax.org/phenome) to further reduce the locus to 2.9 Mb and the number of genes in the interval from 49 to 11.

Finally, using haplotype association mapping with data from the MPD, from published studies, and from their own laboratory, the researchers further reduced the QTL from 2.9 Mb to 0.6 Mb, corresponding to a final reduction to 7 candidate genes.

The researchers then searched publicly available sequence databases and gene expression databases to specify, from among the 7 possible genes, the 3 most likely candidates based on their altered DNA sequences or altered expression profiles that were consistent with altered plasma HDL levels in the appropriate strains. Using these criteria, the researchers designated the aryl hydrocarbon receptor (*Ahr*) as the most likely candidate.

Using these bioinformatics strategies, Burgess-Herbert *et al*. (2008) suggest specific hypothesis-driven studies that could confirm *Ahr* as the gene responsible for the effect of the Chr 12 QTL on circulating HDL levels.

2.F.6. Once a locus is established, how is the gene found?

With increasing frequency, mapping strategies such as those mentioned on previous pages can narrow a trait locus to just a few candidate genes—those genes potentially responsible for the phenotypic difference. Once the locus is narrowed, testing each candidate gene can begin. Such studies include sequence analysis (determination of genetic polymorphisms in the candidate genes themselves that could explain predicted functional differences) and expression analysis (determination of whether differences in expression level of the product of the candidate genes correlates with phenotypic differences across strains). While positive results from such studies can enhance the "candidacy" of a gene, they do not constitute definitive proof that a candidate gene is the responsible gene. However, if a new mutation has arisen on an inbred genetic background and it is kept on that strain (making it coisogenic to the original strain), then identification of the mutant-specific nucleotide change that maps within the critical interval is often considered sufficient proof.

If the mutation or variant is not on a coisogenic strain, definitive proof requires complementation testing or gene conversion. Complementation testing can be carried out when the variant being tested is recessive and when another recessive allele exists that is known to produce the same phenotype. Mice that carry the known allele (either heterozygously or homozygously) are mated with mice that carry the allele being tested. If the phenotype does not appear in any offspring, the allele being tested is said to be "complemented" by a wild-type allele from the other parent. Complementation is taken as proof that the tested allele and the known allele exist at different loci. If, on the other hand, the variant phenotype does appear in the offspring, the alleles are called "non-complementary," which means that the tested allele and the known allele are at the same locus.

An alternative proof is gene conversion by homologous recombination. This may entail "knock-out" or "knock-down" of a wild-type allele, or replacement of a critical sequence in a wild-type allele with a putative mutant sequence. If such procedures convert a strain that does not express the mutant phenotype to a strain that does (or vice versa, thus "rescuing" the phenotype), it is taken as proof that the candidate gene is responsible for the mutant phenotype.

For QTL candidate gene analysis, complementation or homologous recombination studies are difficult to perform because each locus typically has a relatively small impact on the phenotype. However, the continued development of bioinformatics resources and techniques such as interval-specific and genome-wide haplotype mapping, when combined with the development of new animal resources such as the Collaborative Cross (see 3.D.2.d), should achieve sufficient precision in gene mapping to permit scientists to routinely narrow the search to a limited number of candidate genes for each QTL, and eventually, to unequivocally identify quantitative trait genes through mapping studies alone.

2.G. Coat color genetics

The coat color of mice is under complex genetic regulation; at least 50 genes are known to influence coat color. The coat color genes participate in a hierarchy of epistatic relationships and include one of the most polymorphic genes known in mice, the nonagouti gene. Because of the availability of coat color varieties and the simplicity of scoring the phenotypes, coat color has been studied as a model genetic system from the beginning of modern mammalian genetics (Cuénot, 1902).

The complex genetics of coat color as a model system.

The coat color system in mice is one of the best-characterized genetic systems in mammals. If the polymorphisms, the allelic hierarchies, the epistatic interactions, and the pleiotropic effects of the coat color genes provides a typical example of how genes function in a system to regulate phenotypic expression, we can expect that sorting out the genetic regulation of most traits will continue to provide mammalian geneticists with interesting challenges well into the future.

Coat color was an important marker in early mapping studies that established genetic linkage groups. Coat color is used today as a powerful quality control marker for genetic contamination, and as a marker for the segregation of linked genes in balanced stocks. (For an example of the practical use of coat color to maintain a balanced stock—the BKS.Cg-*m* +/+ *Lepr*^{db}/J [000642] strain of JAX® Mice—see Appendix I, "Using a Balanced Stock to Carry a Recessive Mutation That Is Sterile or Lethal, Including Embryonic Lethal.")

Here, we present an overview of coat color genetics in mice that illustrates how genes can interact in a system to regulate the expression of a phenotype. Other genes for common coat color mutants are listed in Appendix E, "Coat Color Alleles for Popular Strains of JAX® Mice." For full detail on all the coat color genes, visit the MGI website, www.informatics.jax.org.

Coat color was one of the earliest phenotypes studied by researchers.

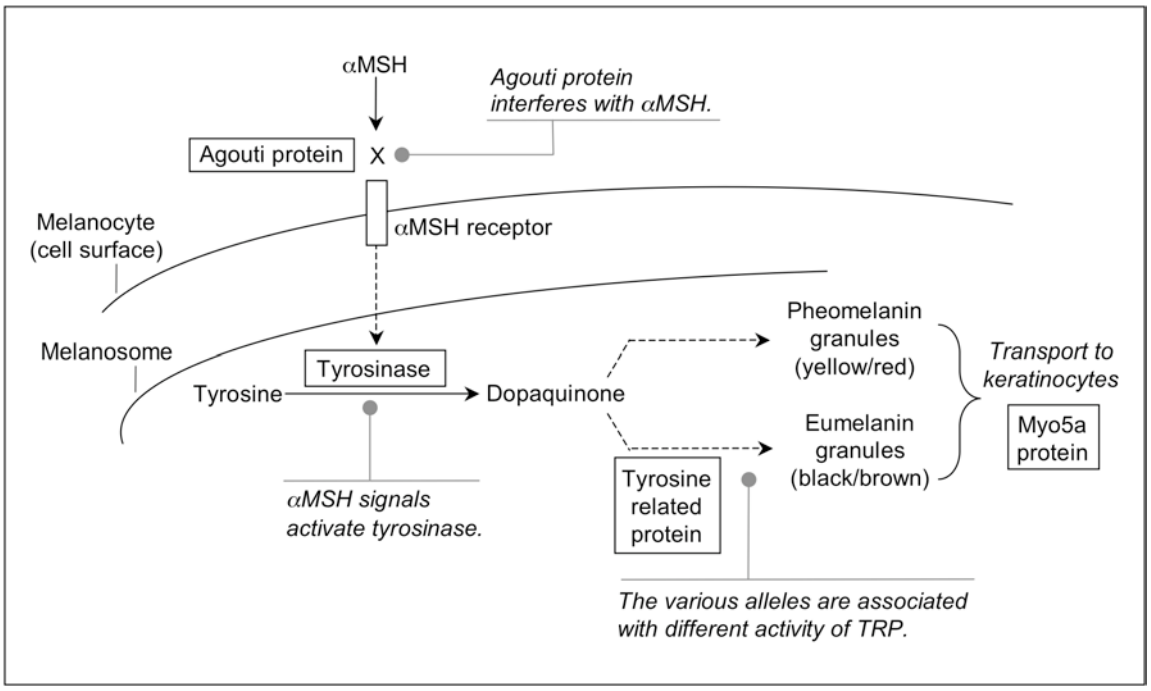
In the 19th century, mouse fanciers bred mice on the basis of coat color. Scientists studied coat color because it was a heritable trait they could identify easily, even if they did not understand how it worked or how many genes were involved (Silvers, 1979).

C. C. Little, the founder of The Jackson Laboratory, studied coat color at Harvard beginning in 1907. It was this work that led him to inbreed sister–brother pairs of mice carrying recessive genes for the dilution, brown, and nonagouti genes, which resulted in the dbr strain of inbred mice. (Later, the name of this strain was changed to dba, after the three recessive genes. In 1950 it was renamed DBA.)

2.G.1. The interactions of coat color genes

Coat color is determined directly by the amount and type of melanin in the pigment granules and by the shape of the granules. Melanin pigment granules are produced in the melanosomes, organelles of the melanocyte (the pigment-producing cell), and transported to skin and hair follicle keratinocytes to provide skin and hair with color. Melanin production is controlled by alpha melanocyte stimulating hormone (αMSH), a hormone that binds receptors on the melanocyte cell surface to initiate a signal transduction process that activates tyrosinase. Tyrosinase is the enzyme that converts tyrosine to dopaquinone in the melanosome and begins the synthesis of melanin. A series of reactions follow that ultimately produce pheomelanins (yellow/red pigment) and eumelanins (black/brown pigment). The melanin pigments are organized into granules, which can vary in shape and surface texture. Granules are then transported out of the melanocyte to the keratinocytes.

Figure 2.2. The regulation of pigment synthesis in mice.



2.G.2. Five genes responsible for the most common coat color variations in laboratory mice

Following is a brief discussion of five genes responsible for the most common varieties of coat color in laboratory mice.

The nonagouti locus: *a* (Chr 2)

The nonagouti locus is named after the recessive allele that prevents expression of the agouti protein. The agouti protein produced by the wild-type allele binds the αMSH receptor, preventing αMSH-initiated signaling and providing different signals of its own that regulate a eumelanin/pheomelanin switch. The result is an alternating pattern of pheomelanin (yellow/red) and eumelanin (black/brown) bands on the hair shaft—yellow on the distal portion and black on the proximal portion. The overall appearance of the mouse is brown, but separation of the fur on the skin surface reveals the black band of fur near the skin. The agouti allele (*A*) is dominant over the nonagouti allele (*a*). The recessive nonagouti allele *a* permits unrestricted activation by αMSH, resulting in fur that is entirely black. The nonagouti locus is highly polymorphic; at least 15 additional alleles of the nonagouti locus exist.

The albino locus: *Tyr* (Chr 7)

The wild-type allele of the albino locus, Tyr^{+}, produces tyrosinase, which converts tyrosine to dopaquinone, initiating the series of reactions that produce melanin. The recessive allele Tyr^{c}, for which the albino locus is named, produces an inactive form of tyrosinase, and therefore produces no melanin, resulting in albinism. Homozygosity for Tyr^{c} results in masking epistasis of all other coat color genes.

The brown locus: *Tyrp1* (Chr 4)

The wild-type allele of the brown locus, $Tyrp1^{+}$, produces tyrosinase-related protein 1 (TYRP1), an enzyme that complexes with, and may stabilize, tyrosinase in the melanosome membrane. TYRP1 catalyzes the last step in eumelanin production and promotes its polymerization into melanin granules. The wild-type $Tyrp1^{+}$ allele produces black eumelanin, and a recessive mutant $Tyrp1^{b}$ allele produces brown eumelanin. The melanin granules produced in homozygous $Tyrp1^{b}$ mice are more rounded, compared to the normal ovoid shape, and they contain less eumelanin.

The dilute locus: *Myo5a* (Chr 9)

The wild-type allele of the dilute locus, $Myo5a^{+}$, produces myosin Va protein, which is involved with transport of organelles in cells. In mice homozygous for the recessive $Myo5a^{d}$ (dilute) allele, melanosome trafficking defects result in uneven release of the melanin granules to keratinocytes. As a result, the overall coat color of the mouse takes on a lighter ("dilute") appearance, whatever the color itself is. Thus, the dilute locus is considered a modifier of other coat color loci.

The pink-eyed dilution locus: *Oca2* (Chr 7)

The pink-eyed dilution locus in the mouse is named after the mutation *p* that results in hypopigmentation of the eyes, skin, and fur. The gene in the mouse, *Oca2*, is named for the deficiency in the homologous human protein that is associated with oculocutaneous albinism type II. The function of the *Oca2* gene product is not known, but it localizes to the melanosome (and other organelle) membranes, and it may regulate the pH of the melanosome. The eumelanin content of melanin granules in mice that are homozygous for $Oca2^{p}$ (the pink-eyed dilute mutants) is greatly diminished, resulting in pink eyes and diluted coat color. The *Oca2* gene is linked to the *Tyr* gene by < 20 cM.

2.H. For more information

Books accessible online from the Mouse Genome Informatics (MGI) website at www.informatics.jax.org/resources.shtml:

- *The Anatomy of the Laboratory Mouse*. Cook M. 1965. Academic Press. M.R.C. Laboratory Animals Centre, Carshalton, Surrey, England.
- *Biology of the Laboratory Mouse*. 1968. Green EL (ed). Dover Publications, Inc., NY.
- *The Coat Colors of Mice, A Model for Mammalian Gene Action and Interaction*. 1979. Silvers WK. Springer Verlag.
- *Mouse Genetics: Concepts and Applications*. 1995. Silver L. Oxford University Press.
- *Origins of Inbred Mice*. 1978. Morse HC III (ed). Academic Press. National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

Other resources:

- Festing's characteristics of inbred mice: www.informatics.jax.org/external/festing/search_form.cgi.
- *The Laboratory Mouse*. Hedrich HJ (ed.) Elsevier, London.
- *The Mouse in Biomedical Research, 2nd edition*. Fox JG *et al*. (eds). American College Laboratory Animal Medicine.

2.I. References

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Chapter 3: Categories of Laboratory Mice—Definitions, Uses, Nomenclature

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About 100 years ago, C. C. Little began inbreeding mice for his studies on the genetics of cancer. Since then, advancements in genetics and technology have allowed scientists to develop derivitive inbred strains and strain panels that are tailored to specific research needs. Because of the wide variety of these derivative strains and the ability to construct precise allelic mixes—combined with the inherent advantages of the inbred strain—the laboratory mouse continues to be the most versatile and valuable tool in mammalian genetics a century after C. C. Little first inbred his mice.

The objective of this chapter is to provide an overview of the various categories of laboratory mice, including information about why and how they were developed and how they are used in research. The chapter is organized as follows:

For details about these categories of mice, see

- Silver (1995),
- Berry and Linder (2007),
- the Festing inbred strain database, available at www.informatics.jax.org/external/festing/mouse/STRAINS.

For lists of JAX® Mice organized by these categories, visit www.jax.org/jaxmice/type.

3.A. A few words about nomenclature and terminology

3.A.1. Nomenclature

Mouse strain nomenclature provides two types of technical information: the background or parental strains upon which the strain is based, and details about relevant genes and alleles. The strain name can also include such information as who developed the strain, where it was developed, and where it is currently maintained.

When publishing results, it is important that investigators use the full strain name at least once so that readers can correctly interpret research results and precisely reproduce research.

Table 3.1 shows where to find information related to nomenclature throughout this handbook and elsewhere.

Table 3.1. Sources of information about nomenclature.

Type of information	Location of information
Nomenclature guidelines	• In this handbook – Discussions by category in this chapter – Summary in Appendix A, "Strain Nomenclature Quick Reference" • On the web – Guidelines on interpreting nomenclature: www.jax.org/jaxmice/faq/nomenclature_hints – Online nomenclature tutorial www.jax.org/jaxmice/nomenclature • Other – "Mouse strain and genetic nomenclature: an abbreviated guide" (Eppig, 2007)
Official nomenclature rules (International Committee on Standardized Genetic Nomenclature for Mice)	• Mouse Nomenclature Home Page: www.informatics.jax.org/nomen
Official nomenclature for each strain of JAX® Mice	• Individual strain datasheets www.jax.org/jaxmice/query
Lab and investigator codes used within strain names to denote developers and holders of strains.	• Institute for Laboratory Animal Research (ILAR), U.S. National Academy of Sciences, Washington, DC: http://dels.nas.edu/ilar
How to register the name of a new mouse strain	• Web form: www.informatics.jax.org/mgihome/submissions/strains • Email assistance: strains@informatics.jax.org
How to submit a proposed locus symbol	• Web form: www.informatics.jax.org/nomen/nomen_submit_form.shtml • Email assistance: nomen@informatics.jax.org
How to submit the name of a new mutation	• Web form: www.informatics.jax.org/nomen/allmut_form.shtml

3.A.2. Strain definition and breeding terminology

Throughout this handbook, we use very specific terminology related to strain definition and breeding. Tables 3.2 and 3.3 provide definitions and, where necessary, examples.

Table 3.2. Strain definition terminology.

Term	Definition and comments
Stock	An isolated, interrelated breeding population.
Line	A pedigreed stock with a known lineage to a single breeding pair.
Inbred strain*	A line of mice that has been propagated by a single lineage of sister–brother mating for at least 20 generations. All mice of an inbred strain share the same 2 founders.
Subline	A stock of inbred mice that has been reproductively separated from the parental strain.
Inbred substrain*	A subline of mice that has been separated from the parental inbred strain for 20 generations or that has any known, fixed genetic difference, even if that difference is at one gene.
Coisogenic strain	A variant inbred strain of mice that differs from an established inbred strain by a mutation at only a single gene.
STOCK	Unique nomenclature for lines of JAX® Mice that have founders of 3 or more strains, founders of unknown genetic background, or outbred founders. A line designated with "STOCK" in the name may—or may not—be inbred. For example, as of 2006, STOCK Tg(TIE2GFP)287Sato/J (003658) could not be called an inbred strain because only 15 generations of sister-brother mating could be confirmed.

*Definitions condensed from Silver (1995).

Note: Sometimes the term "strain" is used as shorthand for "inbred strain," and sometimes the term "strain" is used to refer to an isolated breeding population that does not fulfill all criteria for an inbred strain. A reader should not assume that "strain" refers to an inbred strain unless it is stated explicitly.

Table 3.3. Breeding terminology.

Term	Definition	Examples
Incross	A cross of 2 animals of the same homozygous genotype.	• Breeding mice of the same inbred strain.
Outcross	A cross of 2 animals of unrelated genotypes. Outcrossed stocks are commonly referred to as "outbred."	• Breeding mice of 2 different inbred strains. • Breeding genetically different F1 hybrid mice. • Breeding any unrelated mice.
Intercross	A cross of any 2 animals that are identically heterozygous at a particular locus. A cross between any 2 siblings that are genetically different (that are not from the same inbred strain).	• Breeding mice of the same F1 hybrid genotype. • Breeding siblings when creating an inbred strain.
Backcross	A cross of 2 animals, one that is heterozygous at a locus under investigation, and one that is homozygous for one of those heterozygous alleles.	• Breeding an F1 offspring back to a parental genotype. • Breeding progeny back to the parental background genotype when developing a congenic strain.

Definitions condensed from Silver (1995).

3.B. Inbred strains and crosses

Inbred mouse strains and the crosses created from them are the "workhorses" of mammalian genetics. They have been used by researchers since their creation early in the 20th century. Many have been maintained for more than 200 generations. The inbred strain is such a powerful research tool that, today, it is the foundation for most mouse models used in biomedical research.

3.B.1. Inbred strains and substrains

Mice were originally inbred to make expression of a trait as consistent as possible by removing genetic variance. The result is a strain of mice in which all members are genetically uniform. A profound consequence of this genetic uniformity is that, when comparing two inbred strains under controlled conditions—the basic inbred strain experiment—any phenotypic difference between the strains must be due to a genotypic difference. Researchers have exploited the basic inbred strain experiment to greatly accelerate the development of our understanding of mammalian genetics. The homogeneity of the inbred strain also has enabled the derivation of powerful genetic "tools" such as genetic crosses and inbred strain panels. This genetic uniformity has become even more important with the advancement of genetic engineering. Because "noise" from the genetic background is minimized, the expression of engineered genes can be studied most effectively in inbred strains.

3.B.1.a. Definitions, characteristics, and value

3.B.1.a.1. Inbred strains

An *inbred strain* is a line of mice that has been inbred by intercross sibling (filial) mating for at least 20 generations (F20). Although any generation might include a large number of mice, each generation in the *lineage* of the strain comprises only two mice (one sister–brother pair). With a mean generation time of about three months, it generally takes about five years to create a fully inbred strain by sibling matings. An acceptable alternative breeding scheme is offspring–parent mating, used infrequently because inbreeding progresses more slowly than with sibling mating.

3.B.1.a.2. Substrains

A *substrain* refers to an inbred strain that is reproductively isolated from its founder inbred strain and that has any fixed genetic difference from that strain. This difference could be as small as an allelic variant at a single gene. Any heritable change that has been identified by either genetic or phenotypic analysis is sufficient to define a substrain. Whenever researchers identify a substrain, they should use appropriately updated substrain nomenclature.

Even if a genetic difference from the founder strain is not explicitly identified, a reproductively isolated population is considered a substrain when one of two conditions occur:

- If, between the 20th and 40th generation of an inbred strain, a breeding pair is removed to establish a new line (International Committee on Standardized Genetic Nomenclature for Mice, 2007). Typically, there is a greater than 50% likelihood that residual heterozygosity is still present in the strain up to the 36th generation (Bailey, 1978), and about a 20% likelihood by the 40th generation. Thus, mice in the newly separated line are likely to be different enough to create a new substrain even after just one generation of separation. (Beginning with the first separated generation, the new line will differ permanently from the founder at, on the average, 12.5% of the segregating loci in the founder strain at the time of separation [Figure 3.2]).
- If, after the 40th generation of an inbred strain, a subline is reproductively isolated for an additional 20 generations (International Committee on Standardized Genetic Nomenclature for Mice, 2007). At this point, genetic drift will have resulted in the fixation of, on the average, two–three new alleles in the substrain and two–three *different* new alleles in the parental strain (see sidebar, "The rate of genetic drift in mice," in 3.B.1.d.2).

Festing (1979) points out that once a substrain is created, in theory, the parental strain should also be considered a substrain, because both strains will continue to diverge from the generative genotype at about the same rate due to genetic drift.

Besides the general advantages given on the previous page, inbred strains of mice share the following characteristics and advantages:

- Each inbred strain has a unique set of phenotypes that distinguishes it from other inbred strains. Some phenotypes, such as coat color, are invariant. Other phenotypes, such as circulating levels of glucose, are highly dependent on environmental interactions.
- Phenotyping data for individual inbred strains and substrains are cumulative and comparable within and between laboratories (Festing, 1999).
- The phenotypic and genetic consistency of inbred strains enhances the potential to identify
  - genes that modify expression of specific mutations, and
  - genetic interactions in diet, drug, and gene therapy studies.
- With fixed major and minor histocompatibility loci, each member of a given inbred strain is a perfect recipient for tissue from any other member of the same sex. However, the Y antigen, which is expressed only in males, can influence whether or not transplanted tissue is accepted.

The use of inbred strains has enabled accumulation of knowledge of the mouse genome over decades. This cumulative knowledge provides synergies for new discoveries, making the inbred mouse the preferable choice for current research.

What are inbred strain panels? How are they used?

An inbred strain panel is a collection of inbred strains that is evaluated for one or more phenotypes under controlled conditions (e.g., in the same laboratory, at the same time, and at the same age). Strain panels are of 2 types: 1) a collection of independently-derived strains, or 2) a collection of derivative strains developed from a cross of 2 or more founder strains (see 3.D, "Recombinant strain panels"). Both types of strain panels are used for comprehensive analysis of phenotypic variation and interrelationships, and as tools for genetic mapping.

Strain panels enhance the advantages of inbred strains. Once a new phenotype is characterized in a number of well-chosen strains, its interrelationship with other phenotypes can be evaluated using previously published data for those strains. A shared strain distribution pattern among phenotypes suggests that the phenotypes may be causally linked, and critical hypothesis-testing experiments can be specified. In addition, because extensive genetic and phenotypic data for many strains are available—and are continually accumulating—well-chosen strain panels provide the opportunity to investigate phenotype–genotype relationships without the need for additional DNA sequencing. Especially useful is the potential for fine mapping of a phenotype using established genetic information, sometimes called "*in silico*" mapping.

For more information on inbred strain panels…

The Mouse Phenome Database (MPD) provides a compilation of strain panel studies. See www.jax.org/phenome.

Strain panels and their applications are described in the "Macroarray Resource Manual", available at www.jax.org/jaxmice/support.

3.B.1.b. Considerations

Considerations for the use of inbred strains and substrains include the following:

- An inbred strain or substrain is representative of only a single genotype—not laboratory mice in general. Thus, results of research using individual strains may be idiosyncratic and should not automatically be applied to all laboratory mice.
- Although descended from the same inbred strain, substrains may have very different phenotypes and genotypes. The greater the number of generations that separate the substrains, the further apart they are genetically. Following are two examples that highlight the genetic differences that can occur between even closely related substrains:
  - C57BL/6J (000664) mice carry a null mutation for the nicotinamide nucleotide transhydrogenase gene (*Nnt*). Although pancreatic β-cell glucose sensing is affected (Freeman *et al*., 2006), glucose clamp studies demonstrate that glucose clearance is normal (Berglund *et al.*, 2008) (For details see sidebar in 8.A.3.a.). C57BL/6JEiJ (000924) and C57BL/6NJ (005304) mice carry the wild-type allele for *Nnt*.
  - The CBA/J (000656) strain is the only CBA substrain to carry the *Pde6b*^{rd1} retinal degeneration allele (Sidman and Green, 1965), which causes blindness by weaning age. Also, CBA/J mice are not histocompatible with CBA/CaJ (000654) mice (Green and Kaufer, 1965), although they share the same *H2* haplotype. Either genetic drift or genetic contamination (see 3.B.1.d for a discussion) could have caused these differences.

  For substrain lineage charts of the most common inbred strains, see Appendix C, "Origins and Relationships among Common Strains and Substrains of Laboratory Mice."
- During the process of domestication of the mice that were used to develop the classical strains of laboratory mice, selection for early, high fecundity was almost inevitable, as was selection for docility. Thus, even a range of inbred strains or substrains represents a biased representation of genetic variance for mice (e.g., Yang *et al.,* 2007).

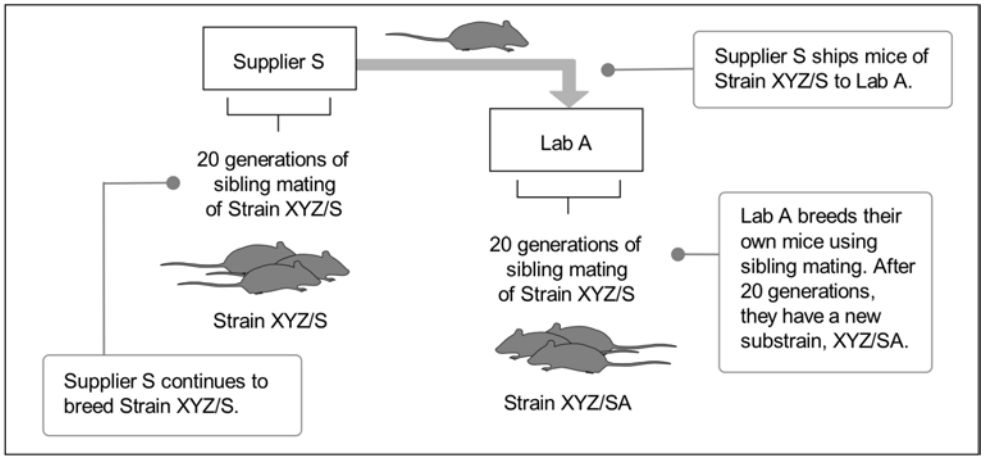
- The process of inbreeding diminishes individual genetic diversity and fixes deleterious alleles, so that, in general, inbred strains and substrains suffer from inbreeding depression. A dramatic example of this is the consistently poor breeding performance of inbred strains compared to genetically mixed stocks.

3.B.1.c. Maintenance breeding strategies

Generally, inbred strains and substrains are maintained by continued sibling mating, even after the 21st generation. But genetic drift, which will continue to affect the genome, must be minimized (see discussion in 3.B.1.d.). Therefore, in production facilities such as The Jackson Laboratory, inbred strains are maintained and expanded using foundation stocks, which tightly controls the numbers and genetic quality of progenitors used to expand the inbred strain. An additional strategy is to freeze embryos or gametes of a specific generation and refresh the strain periodically using the frozen material. (For details on how we minimize genetic drift using both strategies at The Jackson Laboratory, see Chapter 8, "Genetic Quality Control—Preventing Genetic Contamination and Minimizing Genetic Drift.")

In research facilities where investigators breed their own mice, it is surprising how easy it is to lose the genetic homogeneity that distinguishes inbred mice. To produce enough mice for a research project, investigators often expand an inbred strain by multiple matings. If these matings are random, after a few generations, the genetic reliability of the inbred strain is lost. The result is a stock of mice, not a substrain. If these matings are sibling matings, any single lineage could be used to propagate the strain. However, if the parental strain exists elsewhere, this single lineage would lead to a new substrain. To avoid these problems, we recommend replacing the breeding line with new founders from the supplier (sometimes called "refreshing" a strain) every five generations at a minimum. Figure 3.1 illustrates how on-site breeding without replacement can inadvertently lead to new substrains.

Figure 3.1. How isolated breeding can inadvertently create substrains.



Assuming that the XYZ/S strain has no residual heterozygosity from the original founders (see 3.B.1.d), mice shipped to Lab A are genetically virtually identical to all other XYZ/S mice of the same generation. However, after 20 filial generations at Lab A, a new substrain will be created. At the rate of one newly fixed mutation approximately every 7 generations (see sidebar, "The rate of genetic drift in mice," in 3.B.1.d.2), mice of this new substrain, XYZ/SA will have, on the average, 5–6 fixed differences from their XYZ/S contemporaries.

Certain inbred strains—segregating inbred strains—are maintained with forced heterozygosity. This is usually done to keep both a mutant and wild-type allele on a consistent genetic background. (See Section 3.C.1.c.3, "Breeding strategies," for details.) Two examples:

- 129P3/J (000690), which is maintained by mating a light chinchilla ($Tyr^{c}/Tyr^{c\text{-}ch}$) with an albino sibling (Tyr^{c}/Tyr^{c}) at each generation.
- C57BL/6J-$Ghrhr^{lit}$/J (000533), which is maintained by mating a heterozygote by homozygote mutant at each generation.

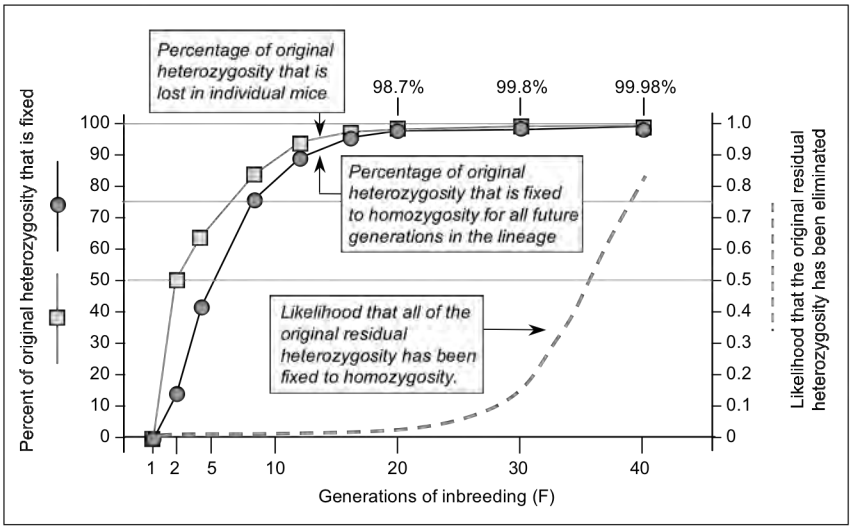
3.B.1.d. Individuals in inbred strains are not *completely* genetically identical—the influence of residual heterozygosity, genetic drift, genetic contamination, and copy number variation (CNV)

Except for the sex difference, mice of an inbred strain or substrain can be regarded as genetically and phenotypically uniform. They are almost completely homozygous; that is, both alleles at each locus are identical at virtually all loci. However, some real, and potentially changing, genetic variation exists for all inbred strains due to residual heterozygosity, genetic drift, copy number variation (CNV), and the possibility of genetic contamination.

3.B.1.d.1. Residual heterozygosity

Residual heterozygosity can be defined as the proportion of heterozygous loci in the original cross that is not yet genetically fixed at a given generation of inbreeding (Bailey, 1978). In an inbred line at F20, residual heterozygosity is almost certainly still present unless the founders were closely related. The likelihood that residual heterozygosity has been eliminated in a cross of two unrelated founders does not reach even 0.5 until about F36 (dotted line in Figure 3.2). And, it is not until F60 that this likelihood reaches 0.99, when we can be confident that none of the heterozygous loci in the founders are still segregating in the strain. As Bailey (1978) states, "Purity is not easily attained."

Figure 3.2. Effects of inbreeding on homozygosity in individuals and in a stock.



The solid lines show the difference in the percentage of original heterozygosity in the F1 that is lost at each generation for individuals (gray squares) vs. the lineage (gray circles). The dashed line shows the likelihood of *all* original residual heterozygosity being eliminated from the line at any generation. Redrawn from Silver (1995).

The effect of inbreeding on homozygosity in individuals and in a lineage of a strain is shown in Figure 3.2. The solid gray line (squares) indicates the percentage, on the average, of heterozygous loci that existed in the original cross that are fixed in each individual of a generation. At F20, typically 98.5–99.0% of the originally heterozygous loci are homozygous in each individual mouse. Furthermore, there will be a slightly different set, and a slightly different total number, of heterozygous genes in each parent. The residual heterozygosity that is permanently fixed to homozygosity at each generation is represented by the solid black line (gray circles). Note that if expansion of the inbred line occurs at F21, the parents used for this expansion could easily still be heterozygous at 99 genes (see sidebar).

One of the main points of inbreeding is to eliminate residual heterozygosity within a line. The actual differences in levels of initial heterozygosity combined with the random transmission of alleles at each generation will determine when this is accomplished for any given line (whether fewer or more than 20 generations of inbreeding is required). Figure 3.2 shows that, for a single locus that is heterozygous in both parents, the likelihood that this locus will be fixed to homozygosity for the lineage in one generation is 0.125.

Residual heterozygosity in inbred mice at generation F21: How did we compute the number of genes to be 99?

Number of structural genes	~25,500
Residual heterozygosity in the ancestral founders for many inbred strains (Bailey, 1978)	x 33%
Number of segregating genes in the ancestral founders	7,650
Genes still segregating at the 20th generation of sister–brother mating:	
• Percentage (Silver, 1995)	1.3%
• Quantity	99

Note: This estimate will vary directly with the actual number of genes that are segregating in the founding pair.

Thus, if the parental strains differ in only three unlinked loci, there is a $.125^3 = .002$ likelihood (a one in 500 chance) that complete homozygosity will be accomplished in one generation. Obviously, with increasing levels of residual heterozygosity, this likelihood diminishes. The dashed line in Figure 3.2 illustrates the likelihood that original residual heterozygosity is eliminated by a given generation when the original residual heterozygosity is more typical of common crosses (more than 500 loci) and when linkage is considered.

The rate of genetic drift in mice.

The calculated rate of genetic drift depends on the estimate of the spontaneous mutation rate. In mice, this rate is difficult to measure accurately because of the sample size needed for a reliable estimate. Drake *et al.* (1998) calculated a mutation rate of about 1.1×10^{-5} per zygote using data from control groups (1,485,036 progeny) in the extensive radiation studies performed at Oak Ridge, Harwell, and Neuherberg. In these studies, female mice that were homozygous-recessive for 7 marker traits were mated with males that were homozygous for all the corresponding wild-type alleles. Spontaneous mutations in the male's germ cells that inactivated one of these wild-type alleles would produce offspring that expressed the recessive trait, revealing the mutation.

Assuming 25,500 genes in the mouse genome, these results indicate that the rate of spontaneous mutation is 1 per 3.57 zygotes. Thus, new germline mutations will arise at the rate of 1 every 1.8 offspring. With strict sister–brother mating, and assuming no selective pressure, one quarter of these spontaneous mutations will eventually be fixed to homozygosity in the strain; the other three quarters will become extinct. The result is that, on the average, *one mutation that is severe enough to alter the function of the gene product will become fixed in an inbred strain about every 7 generations due to genetic drift*. This is an underestimate of the actual rate because mutations with inconspicuous effects were not included in the estimate of the mutation rate.

Using a very different strategy, based on comparison of mouse and rat sequence data at putatively neutral sites (4-fold degenerate sites in exons and unconstrained sites in introns), and an estimate of 80,000,000 generations separating the 2 species, Chamaray and Hurst (2004) estimate that $2–3 \times 10^{-9}$ silent mutations occur per silent site (i.e., base pair), per generation. Assuming the same rate for coding sequence, and that 2% of the 2.7×10^9 base pairs in the mouse genome are part of coding sequences, we estimate a rate of genetic drift affecting coding sequence of about one new mutation every 6–9 generations, an estimate that is remarkably similar to the one based on Drake *et al*. (1998).

At The Jackson Laboratory, we address the issue of residual heterozygosity by maintaining every inbred strain through a single lineage of sister–brother matings (our pedigreed foundation stocks). Mice that are distributed (our "production" mice) are from populations that were expanded from this foundation stock. Thus, inbreeding of the parental line continues in an unbroken lineage of sibling matings. As a result, many of our inbred strains are well past generation F60, with virtually no residual heterozygosity present.

3.B.1.d.2. Genetic drift

Random spontaneous mutations occur constantly. The change in the genome of a breeding population due to the accumulation of unselected random mutations over generations is called genetic drift. Each new mutation is propagated in the breeding population until it becomes either extinct or fixed in the genome. Thus, even fully inbred strains in which the original residual heterozygosity has been eliminated will carry a background "load" of constantly changing segregating loci, and their fixed genomes will slowly change over time. The steady-state level of segregating loci due to genetic drift in a closed breeding population is determined by the background mutation rate (see sidebar at left), the number of breeders contributing to each generation, and the breeding scheme. If a breeding stock is maintained through random matings, the number of different segregating loci in an inbred strain will be relatively high and fixation or extinction of new mutations will be relatively slow. For inbred strains that are maintained through a single line of sibling matings, a new mutation either goes extinct or becomes fixed rapidly. In each inbred strain, a new mutation will become fixed about once every six to nine generations (sidebar).

At The Jackson Laboratory, we minimize the number of breeders contributing to each generation by maintaining the pedigreed foundation stock through a single lineage of strict sister–brother mating. This practice minimizes the steady-state level of heterozygosity and maximizes the rate of extinction and fixation of new mutations. Thus, even though we cannot prevent genetic drift, our "production" mice are as genetically homogeneous as possible. An additional strategy we use to minimize genetic drift for our most popular strains of JAX® Mice is to freeze, at a given generation, embryos and gametes, which are used to re-establish the strain every five generations. Typically, the quantity of frozen stock is sufficient for at least 10 years, reducing the rate of genetic drift more than 25 fold. (For details, see 8.B.2.b, "Our Genetic Stability Program.")

3.B.1.d.3. Genetic contamination

Probably the greatest source of heterozygosity in inbred strains is due to genetic contamination caused primarily by human error (for example, by mistakenly mating the wrong mice together). The Jackson Laboratory employs a rigorous Genetic Quality Control Program that minimizes the chances a contamination event will occur, and, should one occur, to minimize the chances it will be propagated through the inbred strain. (See Chapter 8, "Genetic Quality Control: Preventing Genetic Contamination and Minimizing Genetic Drift.")

3.B.1.d.4. Copy number variation (CNV)

Copy number variations (CNVs) are defined as genomic alterations involving duplication or deletion of more than 1 kb of DNA (Freeman *et al*., 2006). CNVs can result in gene copy number differences between individuals (i.e., zero, one, three, or four copies of a gene, rather than two). CNVs have recently been implicated in a diverse group of human diseases, including nervous system disorders (Lee and Lupski, 2006), mental retardation (Sharp *et al*., 2006), and cancer (La Starza *et al*., 2007). Although some CNVs involve sizable fragments of a chromosome and include many genes, CNVs are difficult to detect without specific tests. As a result, their ubiquity in mammalian genomes was not fully realized until recently. In fact, CNVs may arise quite frequently. For example, evaluation of CNV differences among monozygotic twins (Bruder *et al*., 2008) demonstrates that, within every generation, a new CNV may appear in 5–10% of the individuals.

Graubert *et al*. (2007) evaluated CNVs across mouse strains; they identified an average of 22 CNVs per strain, with an average size of 271.5 kb. Recent data, however, which are based on the higher resolution platforms available for human studies, suggest that the average CNV size may be much smaller, and that many CNVs would have been undetected in the general screening studies performed to date in mice. Full characterization of the extent of inter-strain CNVs will require additional work with emerging technologies.

What can researchers do to minimize the impact of genetic drift and copy number variation (CNV) on interpretation of results?

One way to help clarify the effect of genetic drift or copy number variation (CNV) on interpretation of published data is for researchers to cite the facility that maintained the breeding colony of the mice used in the study and to include the date when the mice or the breeding stock were obtained. If future studies identify mutations, including CNVs, in the stock that affect the phenotype of interest, and if the approximate date the mutation arose can be identified, the original results can be interpreted correctly by future investigators in this new context.

Recently, Watkins-Chow and Pavin (2008) investigated copy number variation *within* the C57BL/6J strain (000664). They identified two CNVs, both on Chr 19, that were segregating within the strain. They investigated one of the CNVs in more detail. Thirteen percent of the mice were homozygous for this CNV; 64% were heterozygous and 23% were negative. This ratio is in concordance with the Hardy-Weinburg equilibrium, which demonstrates that the CNV is freely segregating within the strain. This identification of two CNVs in the small portion of the screened C57BL/6J genome demonstrates that individual mice of highly inbred strains are *not* isogenic and that numerous CNVs may be segregating within even carefully maintained inbred strains. Collectively, CNVs such as the two reported could account for some of the phenotypic variability observed within inbred strains and previously attributed to environmental, epigenetic, or stochastic differences.

Presumably, CNVs occur in all inbred strains. Although possible effects of CNVs on results of experiments cannot be eliminated, they can be minimized. The same breeding and genetic stability programs at The Jackson Laboratory that minimize segregating heterozygosity due to genetic drift in inbred strains also minimize the number of segregating CNVs.

At present, CNVs must be treated experimentally as a source of random variation. As information about CNVs accumulates for inbred mice, however, their stringently controlled genetic backgrounds will make it possible to evaluate the often subtle influence of CNVs on phenotypic expression. In the future, the similarity of CNVs between inbred strains of mice and humans can be exploited to model the role of CNVs on phenotypic variation in humans.

3.B.1.e. Nomenclature

Inbred strains are designated using uppercase alphanumeric characters. Nomenclature for a substrain reflects the inbred strain as well as information about how the substrain has diverged, for example, the investigator or institution that developed the substrain and the institution that currently maintains it. Table 3.4 provides several examples of inbred strain and substrain names and their interpretation.

Table 3.4. Examples of nomenclature for inbred strains and substrains.

Name	Definition	Convention
C57BL DBA SJL NZW	Inbred strains C57BL (line C, female 57, BLack); DBA (Dilute Brown non-Agouti); SJL (Swiss, Jim Lambert); NZW (New Zealand, White)	Uppercase alphanumeric characters, starting with alphabetic character, which may represent information about parental strains, such as location of origin, strain originator, phenotype.
129 201	Inbred strains 129 and 201.	Nomenclature exception: Numbers starting the inbred strain name, acceptable due to long-established use before current nomenclature rules.
DBA/1LacJ (001140)	Substrain of DBA/1J that was transferred to The Laboratory Animal Center at Carshalton, UK (Lac); now maintained at The Jackson Laboratory (J).	Inbred strain name, forward slash (/), and alphanumeric information representing provenance. (Laboratory and investigator codes are assigned by the Institute for Laboratory Animal Research [ILAR], U.S. National Academy of Sciences, Washington, D.C. The codes are available on their website (http://dels.nas.edu/ilar_n/ilarhome/search_lc.php).
CBA/CaGnLeJ (001143)	Substrain of CBA inbred strain, originated by T. C. Carter (Ca), transferred to E. L. and M. C. Green (Gn), and then transferred to P. W. Lane (Le); now maintained at The Jackson Laboratory (J).	
C57BL/6NCrl	Substrain of C57BL/6J that was sent to the National Institutes of Health (N); now maintained at Charles River Laboratories (Crl).	

Often, abbreviations for inbred strain and substrain names are used in manuscripts and when designating F1 hybrids. Table 3.5 lists the most common of these abbreviations.

Table 3.5. Abbreviations of inbred mouse strain and substrain names used in hybrid names.

Abbreviations and strains	
129P	129P substrains
129P1	129P1/ReJ (001137)
129P2	129P2/OlaHsd
129P3	129P3/J (000690)
129S	129S substrains
129S1	129S1/Sv-*Oca2*$^{+}$*Tyr*$^{+}$*Kit*$^{Sl\text{-}J}$/J (000090)
	129S1/SvImJ (002448)
129S2	129S2/SvPas
129S4	129S4/SvJae
129S5	129S5/SvEvBrd
129S6	129S6/SvEvTac
129S7	129S7/SvEvBrd-*Hprt*$^{b\text{-}m2}$
129S8	129S8/SvEv-*Gpi1*^{c} *Hprt*$^{b\text{-}m2}$/J (002027)

Abbreviations and strains	
129X1	129X1/SvJ (000691)
A	A strains
AHe	A/HeJ (000645)
AK	AKR strains
B	C57BL
B6	C57BL/6 strains
B6Ei	C57BL/6JEi (000924)
B10	C57BL/10 strains
BR	C57BR/cdJ (000667)
C	BALB/c strains
C3	C3H strains
C3Fe	C3HeB/FeJ (000658)
C3Sn	C3H/HeSnJ (000661)
CB	CBA strains
CBACa	CBA/CaGnLeJ (001143)

Abbreviations and strains	
CBy	BALB/cByJ (001026)
D1	DBA/1 strains
D2	DBA/2 strains
HR	HRS/J (000673)
L	C57L/J (000668)
NZB	NZB strains
NZW	NZW strains
R3	RIIIS/J (000683)
SJ or J	SJL/J (000686)
SM	SM/J (000687)
SW	SWR strains
W	NZW strains

Numbers in parentheses are JAX® Mice stock numbers.

3.B.1.f. Research examples

Variations in the effects of drugs among different inbred mouse strains: a contribution to drug evaluation and the identification of genes that predict differences in drug efficacy.

Crowley *et al.* (2005) compared responses to the selective serotonin reuptake inhibitor citalopram, an antidepressant, among eight inbred mouse strains using a tail suspension test. DBA/2J (000671), BALB/cJ (000651), and BTBR strains from The Jackson Laboratory were the most responsive, and C57BL/6J (000664) and A/J (000646) were the least responsive. The strain distribution of the antidepressant-like activity did not correlate with the strain distribution of effects on other behaviors, indicating that the patterns of sensitivity to citalopram are "behaviorally specific and unlikely to result from pharmacokinetic variables." This strain survey identified the more appropriate strains for screening antidepressants and identified parental strains that can be used to map quantitative trait loci (QTL) that regulate serotonin reuptake inhibitors. In a follow-up study, Crowley *et al*. (2006) performed a mapping cross between a citolopram-sensitive strain (BALB/cJ) and a citolopram-insensitive strain (A/J). Three QTLs were identified (one each on Chr 7, Chr 12, and Chr 19). Three candidate genes at the Chr 19 QTL were sequenced. The researchers found two polymorphisms in the vesicular monoamine transporter-2 gene, suggesting the possibility that this gene may regulate response differences to anti-depressant serotonin reuptake inhibitors. Such QTL studies in mice identify possible genes for human pharmacogenetic studies of therapeutic responses to anti-depressant drugs. Identification of such genetic variants in humans patients would allow for more individualized, rationally-designed, and ultimately, more successful drug treatments.

Interaction of dietary fat, body composition and bone mineral density characterized using strain panels.

Strain comparisons provide an opportunity to identify possible genetic influences on relationships among phenotypes and relationships between treatment and outcome variables. Evaluations of such relationships in most genetically heterogeneous populations are complicated because genetic and random sources of variance are confounded for each genotype. In contrast, with inbred strains, random variation can be estimated directly, and its effect minimized, because multiple copies of the same genotype can be sampled. Although studies using less than six inbred strains generally are not sufficiently powerful for hypothesis testing, results from an increasing number of large strain surveys are being made available to the public. For example, the Mouse Phenome Database (MPD; www.jax.org/phenome) now provides data from hundreds of strain comparison studies of a wide range of phenotypes.

Data from two of these studies, involving a total of 51 inbred strains, were used by Li *et al.* (2008) to examine genetic effects on the relationships of dietary fat, body composition, and bone density (BMD) in mice. Using structural equation modeling, the researchers demonstrated that greater body fat is associated with lower BMD under conditions of a high fat diet, but not a low fat diet. One lifestyle implication suggested by this model is that, to maintain or increase BMD, lowering body fat, through exercise for example, may be more important for people on a standard Western diet than for people on a very low fat diet.

3.B.2. Wild-derived inbred strains

Because the majority of standard inbred strains were developed from domesticated mice originally obtained from mouse fanciers, genetic diversity was limited. To introduce allelic diversity, some researchers have imported wild mice directly into the laboratory.

3.B.2.a. Definition, characteristics, and value

Wild-derived inbred strains originate from mice that are

- trapped in the wild,
- imported into the laboratory without domestication, and then
- inbred by intercross sibling mating for at least 20 generations.

Most of the wild-derived strains used for research come from the subspecies *Mus musculus domesticus*, *M. m. musculus*, *M. m. castaneus*, and *M. m. molossinus*, whereas standard laboratory strains are primarily mixes of *M. m. domesticus* and *M. m. musculus*. (For detail on the origins of laboratory mice, refer to 2.A, "Origins and basic genetic characteristics of the laboratory mouse.") Also see Silver (1995), Morse (2007), and Tucker (2007).

Other *Mus* species, such as *M. caroli*, are available as inbred strains; however, crosses of these mice with the *M. musculus* species result in postfertilization defects. These defects limit the usefulness of non-*M. musculus* species because they cannot be used with the powerful genetic tools, such as those used for mapping, that require outcrossing. Examples of outcrossing difficulties include the following: *M. m. cervicolor* x *M. m. musculus* embryos generated from artificial insemination do not undergo the first cleavage event. *M. dunni* x *M. m. musculus* hybrids fail to thrive at the blastocyst stage. Postnatal viability of *M. caroli* x *M. m. musculus* pups is severely compromised. Crosses of *M. spretus* with *M. m. domesticus* produce viable offspring; hybrid females are fertile, but hybrid males are sterile. (Silver, 1995).

Individuals of a given wild-derived inbred strain are genetically virtually identical, as in any inbred strain. However, because their progenitors were so different genetically from the progenitors of the classical inbred strains, the wild-derived inbred strains provide considerable genetic diversity. It is this genetic diversity—compared to traditional inbred strains—that makes them such valuable tools for evolution and systematics research. Also, progeny from crosses between wild-derived and common inbred strains are especially useful for genetic mapping. In addition, several wild-derived inbred strains naturally carry fused chromosomes, called Robertsonian chromosomes, which are used for mapping, genetic structure-function studies, and to provide genetic markers. (See 3.E, "Mice with chromosomal aberrations," later in this chapter.)

3.B.2.b. Considerations

Considerations for the use of wild-derived inbred strains include the following:

- Little normative data exist for wild-derived inbred strains.
- As with any inbred strain, any experimental result from a study of a single wild-derived strain cannot be applied to "all mice" or even to "all wild mice."
- Wild-derived inbred mice are especially sensitive to variations in their environment caused by noise, vibration, and replacement of regular caretakers. This may contribute to some of the variation in breeding performances of wild-derived strains compared to standard inbred strains.
- One consideration relates specifically to handling: Because the mice retain many of their "wild" alleles, they are very quick and take advantage of any opportunity to escape, which makes them a challenge to care for.

For additional information on husbandry considerations for wild-derived inbred mice, see 9.C.3, "Caring for wild-derived inbred mice."

3.B.2.c. Maintenance breeding strategies

Often, the progenitors of wild-derived inbred strains are either one pair or a trio. Once a wild-derived stock is fully inbred, it is maintained and monitored as any other inbred strain.

3.B.2.d. Nomenclature

Nomenclature for wild-derived mice follows conventions similar to those of inbred strains; however, a wild-derived inbred strain name always designates the investigator who trapped or introduced the wild mice into the laboratory and then inbred them. Even so, unambiguous identification of a strain as wild-derived can be made only by reviewing the strain details. Table 3.6 provides examples of wild-derived strain names and their interpretation.

Table 3.6. Examples of nomenclature for wild-derived inbred strains.

Name	Definition	Convention
CAST/EiJ (000928)	Inbred substrain of wild *M. m. castaneus*, developed by E. M. Eicher (Ei); now maintained at The Jackson Laboratory (J).	Uppercase alphanumeric characters, starting with alphabetic character, which represent information about parental strains; forward slash (/); and ILAR investigator and laboratory code(s) (http://dels.nas.edu/ilar_n/ilarhome/search_lc.php).
MOLD/RkJ (000734)	Inbred substrain of wild *M. m. molossinus* from Japan, developed by T. H. Roderick (Rk); now maintained at The Jackson Laboratory (J).	As above.

3.B.2.e. Research examples

Identification of loci that regulate lifespan.

Klebanov *et al*. (2001a) mapped genetic determinants of maximum lifespan, a life history trait, using two 4-way cross populations, each of which included one wild-derived inbred strain—either CAST/EiJ (000928) or MOLD/RkJ (000734). These studies identified two significant loci, *Leg1* and *Leg2*, each of which accounted for at least 10% of the variance in maximum life span. At each of these loci, the allele from only a single strain—the wild-derived strain within the cross—accounted for the association of lifespan with the genotype at that locus. In both cases, the allele from the wild-derived strain was associated with longer lifespans. No allele from the six standard inbred strains in the study, at any locus evaluated, contributed a comparable effect on lifespan. (Flurkey *et al*. [2007b] speculated that the inadvertent selection for fecundity and other characteristics during domestication of the progenitors of standard laboratory strains may have minimized genetic variation for life history traits such as lifespan.) Unfortunately, the two wild-derived strains themselves are not particularly long-lived, presumably because other genes limit their lifespan. *Leg1* and *Leg2* congenics, using genetic backgrounds from long-lived strains, are currently being evaluated for lifespan.

Discovery of alleles in wild-derived mice for extended female reproductive lifespan.

Flurkey *et al*. (2007a), studying female reproductive aging, found that the very long reproductive lifespan of mice imported from a South Pacific island (an outbred stock, Pohn, mice from Pohnpei) diminished significantly within eight generations in the laboratory, even though breeders in the Pohn stock were not sibling-mated. However, outcrossing late generation Pohn mice to a standard laboratory strain (C57BL/6J [000664]) produced PohnB6F1 hybrid mice with a longer female reproductive lifespan than previously reported for any *M. musculus* F1 hybrid, including other hybrids of C57BL/6J. By using this "F1" strategy, the researchers unmasked alleles that delay female reproductive senescence.

3.B.3. F1 and F2 hybrids

A major advantage of inbred strains is the genetic homogeneity of its members. But what if a researcher wants to study the interaction of *different* alleles of the same gene? Or work with an animal that is more robust and that expresses hybrid vigor? Outcrossing two inbred strains to create hybrids of the first and second filial generations (F1 and F2) produces mice that combine the genetic consistency of inbred strains with controlled genetic diversity and hybrid vigor.

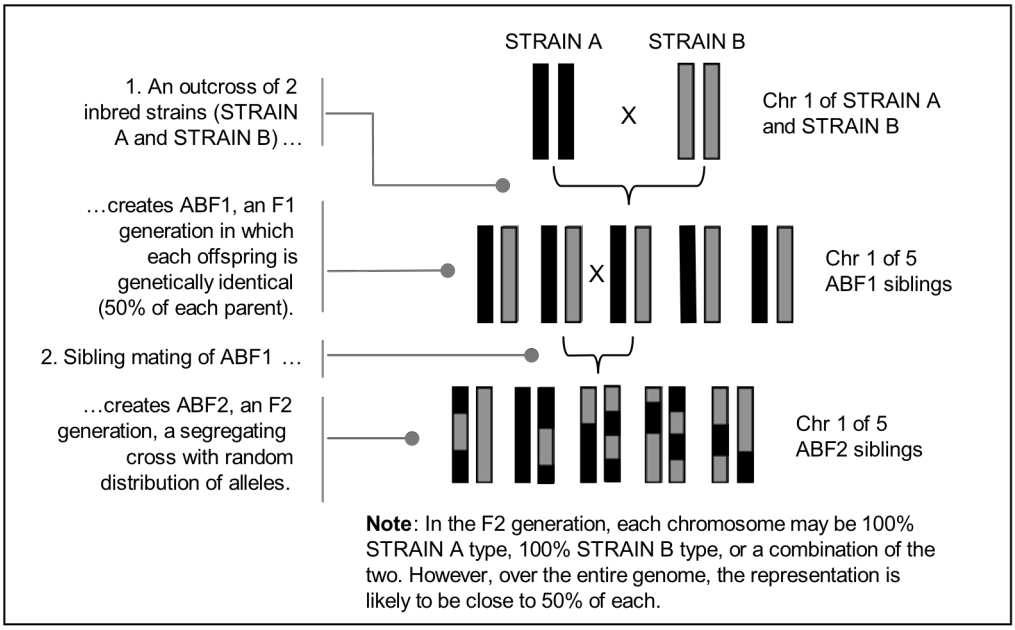
Evaluation of F1 and F2 hybrids is a critical first step in determining the genetic characteristics of any phenotype, such as its mode of inheritance. (Even Mendel used this strategy with his peas.) Although both F1 and F2 hybrids involve controlled genetic diversity, the different types of diversity provide different types of information.

3.B.3.a. Definition, characteristics and value

3.B.3.a.1. F1 Hybrids

An F1 hybrid is the result of the first outcrossed generation of two inbred strains. F1 hybrids from the same two parental strains are genetically identical to each other, with the exception of the sex chromosomes (Figure 3.3). Reciprocal F1s, which are created by reversing the strains of the dams and sires, differ phenotypically and genetically from each other. Differences include Chromosome (Chr) Y, the male's Chr X, mitochondria, maternal and paternal imprinting, and other maternal influences (including litter size, composition of milk, and maternal behavior).

Figure 3.3. Genetic structure of F1 and F2 hybrids, using Chr 1 as an example.



F1 hybrids are particularly useful for the following:

- To study heterosis, the expression of a phenotype that does not appear in either parent, but that results from the interaction of two different alleles of a single gene. The most common example of heterosis is hybrid vigor, which results from two processes:
  - Physiological "buffering," the concept that an F1 hybrid presents a broader array of responses to various stresses because each of the two alleles for a given gene may be optimal for different environmental challenges.
  - Prevention of phenotypic expression of deleterious recessive alleles that are randomly fixed in the parental inbred strains.

  Thus, compared to their inbred parents, F1 hybrids generally live longer, breed better, and are more disease resistant.

- To determine the mode of inheritance—whether a phenotype is characterized by dominance, additivity, or heterosis.
- To create or enhance expression of polygenic diseases (such as lupus or diabetes), by combining dominant or additive genetic determinants at different loci. This process is an example of negative epistasis.
- To study phenomena that affect the health of the mice, such as deleterious mutations or radiation. F1 hybrids usually are more robust than either parental strain.
- To minimize "noise" in bioassays, such as those involving nutrients, drugs, pathogens, or hormones. The physiological buffering capacity of F1 hybrids minimizes the interactions with non-relevant sources of variation that could interfere with the readout of the assay.
- To maintain the health of mixed background mutant mouse stocks when the mutation severely impairs health or fertility. This strategy is most often used with transgenic strains. The stock may be either continuously or periodically refreshed through outcrosses of the mutant carrier with an F1 hybrid. Within these crosses, females are often the F1 hybrid because they tend to be good, productive mothers.

3.B.3.a.2. F2 Hybrids

An F2 hybrid is the first intercrossed generation of F1 hybrid siblings. Each F2 hybrid is genetically unique, containing, on the average, a 50:50 mix of the genotype of each parental strain, but in a random configuration (Figure 3.3). As a consequence,

- genetic variance among individuals is produced, increasing the variance for a phenotype in the population, and
- recessive traits are expressed, permitting the study of recessive alleles on a mixed genetic background.

F2 hybrids are particularly useful for the following:

- To determine whether a phenotype is regulated by one or multiple genes and to characterize its genetic complexity.
- To map a trait.
- As approximate controls for many targeted mutant stocks that are maintained on a mixed genetic background (for example, outbred stocks with a mixed C57BL6 and 129 background, commonly used for genetically engineered mice).

3.B.3.b. Considerations

Considerations for the use of F1 and F2 hybrids include the following:

- F1 hybrids are often used in tissue transplantation studies because they provide a common environment for solid organs transplanted from either parental strain. However, transplanting bone marrow, which contains hematopoietic stem cells (HSCs), is not straightforward due to hybrid resistance. F1 hybrids may or may not accept HSCs from either parent, or they may initially accept the HSCs but slowly reject the tissue. This rejection occurs, not because of classic acquired immunity, but because of innate immune system differences determined by natural killer cells (Kumar *et al.*, 1997). Thus, when using F1s as hosts in bone marrow transplantation studies, it is imperative to conduct preliminary tests to determine the extent of hybrid resistance.
- Normative data for F1 hybrids are much less extensive than for popular inbred strains.
- A single F1 genotype represents a narrow range of the *M. musculus* genome in only one configuration. As with any model based on a single genotype—such as an inbred strain—experimental results from studies using a single F1 hybrid genotype should not be considered typical of laboratory mice in general.
- In F2 hybrids, heterozygosity is reduced by 50% compared to parental F1 hybrids. This reduces hybrid vigor.
- F2 hybrids and other segregating populations are often used for mapping studies, requiring storage of tissue (at -80 C) for DNA analysis. It is advisable to keep the samples for possible future analysis as new information (e.g., about mutations) or new technologies emerge.

3.B.3.c. Maintenance breeding strategies

Unlike inbred strains, F1 and F2 hybrids are not maintained as "strains." Rather, they are constantly recreated—F1 hybrids from their inbred parents, and F2 hybrids from their F1 parents and their inbred grandparents. Thus, a steady supply of F1 or F2 hybrids requires maintenance of breeding colonies of the progenitor inbred strains.

Note: To meet the needs of researchers who use F1 and F2 hybrids and cannot or choose not to maintain parental stocks, some suppliers of mice provide a selection of F1 and F2 hybrids for distribution. For information on hybrid mice available from The Jackson Laboratory, call Customer Service at 1-800-422-6423 (North America) or 1-207-288-5845 (International).

3.B.3.d. Nomenclature

An F1 hybrid name is a combination of abbreviations of the parental strain names with an "F1" designator; an F2 hybrid name is the same as the F1 but with an "F2" designator. For all hybrids, the abbreviation of the female strain is listed first. Because the abbreviations typically do not designate substrains, when authors first mention an F1 or F2 in publications, they must provide the full strain or substrain designation of both parental strains. Thereafter, use of an abbreviation is acceptable. For a list of approved inbred strain and substrain abbreviations, refer to Table 3.5, "Abbreviations of inbred mouse strain and substrain names used in hybrid names," presented previously in this chapter. Table 3.7 provides several examples of F1 and F2 hybrid names and their interpretation.

Table 3.7. Examples of nomenclature for F1 and F2 hybrids.

Name	Definition	Convention
B6D2F1/J (100006)	F1 hybrid of C57BL/6J (B6) female and DBA/2J (D2) male; generated at The Jackson Laboratory (J).	Inbred parental strain or substrain abbreviations (female strain listed first), "F1," forward slash (/), and ILAR investigator and laboratory codes (http://dels.nas.edu/ilar_n/ilarhome/search_lc.php).
CByD2F1/J (100015)	F1 hybrid of BALB/cByJ (CBy) female and DBA/2J (D2) male; generated at The Jackson Laboratory (J).	As above.
B6129SF1/J (101043)	F1 hybrid of C57BL/6J (B6) female and 129S1/SvImJ (129S) male; generated at The Jackson Laboratory (J).	As above.
B6129SF2/J (101045)	F2 hybrid produced by breeding 2 B6129SF1 hybrids; generated at The Jackson Laboratory (J).	F1 hybrid name, with "F2" replacing "F1."

3.B.3.e. Research examples

Allelic diversity of F1 and F2 hybrids clarifies role of inheritance in cancer.
Within just a few years of the demonstration that Mendel's laws were applicable to the inheritance of at least some phenotypes in mammals, Little and Tyzzer (1916) used F1 and F2 hybrids of a stock of Japanese waltzing mice and stocks of "common" mice to investigate the mode of inheritance of susceptibility to a transplantable tumor. All the Japanese waltzing mice were susceptible, but none of the common mice were. All F1 offspring of crosses with the Japanese waltzing mice were susceptible to the tumor, demonstrating that the susceptibility phenotype was dominant. However, only 1.6% of the mice in the F2 generation were susceptible. Little and Tyzzer illustrated how this non-Mendelian pattern of inheritance for tumor-susceptibility—revealed in the F2 generation—could result from influences on the phenotype by multiple genes, each of which is inherited in a Mendelian fashion. This study was the first to analyze phenotypic segregation in F2 hybrids to study the genetics of disease in mammals. It established a standard strategy for genetic analysis that is routinely used even today.

A single cross (F1 and F2 hybrids) illustrates polygenic inheritance and maternal effects.
Reifsnyder *et al*. (2000) demonstrated that the risk of type 2 diabetes was much greater in F1 hybrids of NZO/HlLtJ (002105) and NON/ShiLtJ (002423) mice than in purebred NZO/HlLtJ mice. (NON/ShiLtJ mice do not develop diabetes although they express impaired glucose tolerance.) This indicated that dominant or additive factors contributed by both parental strains combined to enhance the expression of diabetes in the F1 mice. The distribution pattern of diabetes frequency in the F2 generation confirmed that the induction of diabetes was polygenic. An influence of the direction of the cross on diabetes outcome was identified in the F1: when the dam was from the NZO/HlLtJ strain, the incidence of diabetes in the pups was greater than when she was from the NON/ShiLtJ strain. Cross fostering studies showed that pups of either cross gained weight more rapidly when the foster mother was NZO/HlLtJ rather than NON/ShiLtJ. The more rapid weight gain was associated with a higher risk for development of diabetes in F1 offspring from either cross. This maternal effect provides a model for the role of juvenile obesity in the enhancement of diabetes risk. This model was discovered because of the careful design of the breeding strategy.

3.B.4. Multi-strain crosses

Although F2 hybrids are typically used to study complex traits, their allelic diversity derives from only two genotypes. To provide genetic diversity that more closely models a natural population, researchers use multi-strain crosses—structured crosses involving more than two parental strains. Multi-strain crosses are especially useful to comprehensively study the genetics of complex traits such as behaviors and body composition.

3.B.4.a. Definition, characteristics, and value

Multi-strain crosses are structured, intercrossed generations of different F1 hybrids created in various combinations from three or more inbred strains. An example of a multi-strain cross is a 4-way cross, in which two different F1 hybrids are outcrossed.

Exactly what are commercially available outbred stocks? How do they differ from the multi-strain crosses described here?

Commercially available, appropriately outcrossed stocks, commonly called outbred stocks, are maintained using breeding strategies that maximize diversity. However, unlike multi-strain crosses derived from inbred strains, mice of an outbred stock have a varying and uncontrolled genetic relationship to each other. The main advantage of outbred mice is that they are less expensive than strains that are genetically well defined.

Although outbred stocks are sometimes used as heterogeneous models, F1, F2, and defined multi-strain crosses provide greater reliability as well as opportunities to study the genetics of any phenotype of interest. In addition, multi-strain crosses actually provide a greater genetic diversity compared to outbred stocks that are not routinely refreshed.

Properly outcrossing a stock requires very careful breeding of a large number of mice in each generation. Without the addition of new genetic diversity, as outcrossing proceeds, the stock becomes progressively more inbred. In fact, the majority of established outbred stocks may be considered incompletely inbred stocks.

The first step in creating a multi-strain cross is to choose the parental strains. Criteria are based on the nature of the study. For example, to make a population of mice for a selection study, investigators would choose strains with a wide range of values for the target phenotype. To create a population that reflects a broad variety of genetic influences, they would choose strains that are as unrelated to each other as possible. The Mouse Phenome Database (MPD) provides a comprehensive collection of phenotypic data for mouse strain comparisons. For an overview of the MPD, see 6.C.2, "Other databases." Access the MPD at www.jax.org/phenome.

Multi-strain crosses are created by

- outcrossing the inbred parental strains (in various combinations) to create an F1 generation, and
- selectively intercrossing different F1s to create the first segregating generation (which we call the S0 generation).

Sometimes, investigators are interested in generations of multi-strain crosses subsequent to the S0 generation. For example, with a 4-way cross, the S1 generation is the first generation in which a segregating locus can be homozygous. To retain as much diversity as possible in the S1 and subsequent generations, the strategy would be to pair mice for breeding from the most distantly related families.

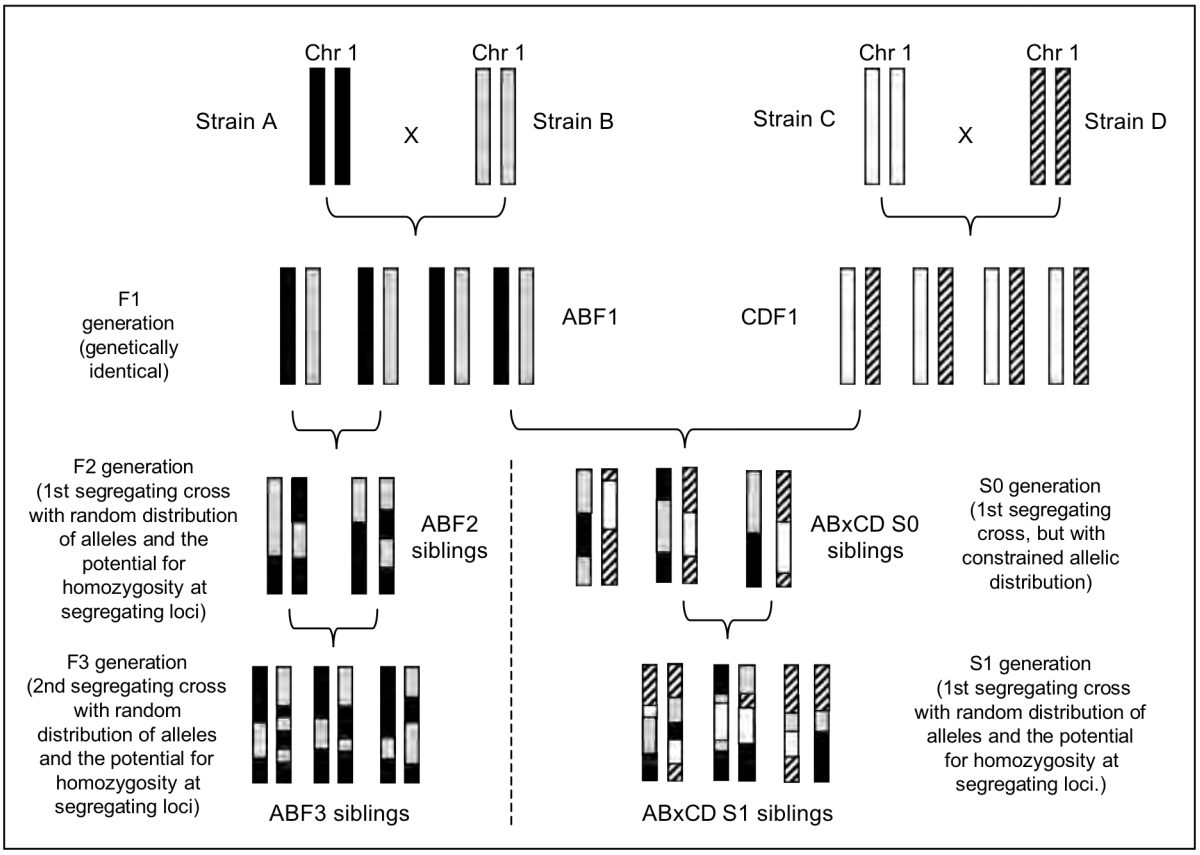
The strength of a multi-strain cross depends on the careful choice of the parental inbred strains that make up the F1 generation. By choosing strains that are distantly related, the multi-strain cross population produces a wide range of genotypes—in a virtually infinite assortment—that represents the species far more comprehensively than other inbred or bilineal models.

Multi-strain crosses offer additional advantages:

- They provide a population that avoids biases from idiosyncrasies of single genotypes.
- The population can be reproduced from the parental strains at any time.
- Their extensive heterozygosity promotes hybrid vigor (similar to F1 and F2 hybrids).

Figure 3.4 illustrates the differences in genetic diversity between a bilineal and a 4-way cross.

Figure 3.4. Differences in genetic diversity between crosses of two vs. four inbred strains.



3.B.4.b. Considerations

Considerations for the use of multi-strain crosses include the following:

- Because of the increased genetic variability, a larger sample size may be necessary to maintain a given statistical power (Festing, 1999).
- The use of multi-strain crosses generally precludes studies that require complete histocompatibility.
- Little background information is available for most multi-strain crosses.
- Multi-way crosses are often used for mapping studies, requiring storage of tissue (at -80 C) for DNA analysis. It is advisable to keep the samples for possible future analysis as new information (e.g., about mutations) or new technologies emerge.

3.B.4.c. Maintenance breeding strategies

As with F1 and F2 hybrids, S0 and S1 generations must be continually created from progenitors. However, multi-strain crosses are often maintained as stocks, especially when the population was selected for a specific trait.

3.B.4.d. Nomenclature

No specific nomenclature rules exist for segregating crosses from three or more inbred progenitors. Thus, it is the responsibility of the researcher to clearly explain the crosses in materials and methods and designate an appropriate abbreviation for use in the remainder of the publication. Figure 3.4 provides an example of such nomenclature, which was defined in Klebanov *et al*. (2001b).

3.B.4.e. Research examples

Identification of genetic correlations between diverse phenotypes.

To resolve questions concerning the feasibility of artificial selection for increased lifespan in mice and whether female reproductive longevity might provide an effective selection criterion, Klebanov *et al*. (2001b) used the S0 and S1 generations of three 4-way crosses. The investigators determined the heritability of lifespan and calculated genetic correlations between maternal reproductive lifespan and total lifespan of parents and offspring. They found that, "in sufficiently diverse mouse populations, selection for increased longevity should be possible and that the direct selection for parental lifespan will be a more efficient strategy than selection for female reproductive lifespan."

An intervention study using a highly, genetically diverse—yet reproducible—population that models the diversity of the human genome.

The Jackson Laboratory is currently participating in a multi-site program designed to assess the effectiveness of treatments that may increase lifespan in mice. The National Institute on Aging Interventions Testing Program (ITP) was established to evaluate agents that are proposed to increase lifespan and delay the appearance of age-related disease in genetically heterogeneous mice. Mice from the S0 generation of a 4-way cross (CByB6F1/J [100009] females x C3D2F1/J [100004] males) are being used to test the treatments because these mice exhibit a broad range of age-related pathologies that model a human population more accurately than inbred mice do. Mice in the first cohort studied (Strong *et al*., 2008) were treated with one of four agents. Two are anti-inflammatory agents: aspirin and nitroflurbiprofen. Two are anti-oxidents: 4-OH-alpha-phenyl-N-tert butyl nitrone (4-OH-PBN) and nordihydroguaretic acid (NDGA). NDGA and aspirin increased median lifespan of male mice significantly (12% and 8%, respectively), but none of the treatments increased maximum lifespan. Nitroflurbiprofen and 4-OH-PBN had no effect on lifespan. None of the treatments affected either mean or maximum lifespan among female mice. Measures of blood levels of NDGA or aspirin and its salicylic acid metabolite suggest that the observed lack of effects of NDGA or aspirin on lifespan in females could be related to sex differences in drug disposition or metabolism. Although the effect of NDGA and aspirin are small, further studies using a range of doses will determine if greater effects are possible. If one, or even multiple, inbred strains or F1s had been used, results would have a more limited relevance because of potential interactions of the treatment with the relatively narrow range of age-related pathologies that characterize any homogenic population.

3.C. Strains with single-locus mutations (spontaneous, induced, and genetically engineered mutant strains; congenic and conplastic strains)

Researchers first studied genetic variants and observable, single-locus mutations that arose spontaneously in non-inbred stocks. These phenotypic deviants provided a way for researchers to study the function of the altered gene, especially its relationship to disease phenotypes. The development of inbred strains made it even easier for researchers and technicians to identify and study phenotypic deviants.

This observational approach is useful for spontaneous mutations that affect visible phenotypes, such as coat color, size, morphology, and behavior. However, spontaneous mutations are rare. Furthermore, those with effects that are not obviously visible can be discovered only by screening; and, the cost–benefit ratio of screening for spontaneous mutations is very high. Thus, to increase the rate of mutations so that screening can be efficient and cost-effective, researchers now often induce random mutations using various techniques such as ionizing radiation (e.g., X-rays) or chemical mutagenesis.

The development of technology to sequence and manipulate DNA directly, combined with the availability of the complete sequence of the mouse genome, has provided researchers with the tools to insert, delete, or alter virtually any desired gene in the genome. Consequently, researchers now often study gene function using "reverse genetics," i.e., altering known genes to produce precise single-locus mutants, and then studying the phenotypic consequences.

For purposes of discussion in this handbook, we group strains with single-locus mutations as follows:

- Mice with spontaneous and induced mutations and mice that are genetically engineered (knockout, knockin, transgenic). In this category, the mutations are carried on the genetic backgrounds on which they arose or were engineered.
- Congenic and conplastic mice. In this category, the mutations or variant alleles are transferred through directed breeding to a new genetic background.

What is the relationship between single-locus mutations and modifier genes?

The effect of a mutation is usually attributed entirely to the locus where the mutation occurred, suggesting that the phenotype is under the regulation of that single locus. Often, however, when the mutation is transferred to a different genetic background (as when creating a congenic strain), a variant of the phenotype appears—due to the presence of genes that interact with the mutation to alter the phenotype on the new background. These genes are usually called "modifier" genes.

Of course, the same genes exist in the original strain as well, but with different alleles. In the original strain, however, the mutant phenotype is attributed entirely to the mutation. It is only when the mutation is transferred to a new background, and when a different phenotype appears, that we notice the interaction of the mutation with the modifier genes. For the sake of illustration, assume that a mutation was discovered in strain A. When the mutation was transferred to strain B, a different phenotype appeared and modifier genes were identified. If, however, the mutation had arisen on strain B, the modified mutant phenotype would have been attributed exclusively to the mutation and *not* to interaction with modifier genes. If this mutation was then *transferred* to strain A, the existence of modifier genes would have been recognized.

Indeed, researchers should keep in mind that the consequence of any mutation on any genetic background is almost always the product of both a direct effect of the mutation and interactions of the mutation with specific genes at other loci. This is true even on the genetic background on which the mutation arose or was originally created.

3.C.1. Spontaneous, induced, and genetically engineered mutant strains

3.C.1.a. Definitions, characteristics, and value

3.C.1.a.1. Spontaneous and induced random mutations

The random nature of spontaneous and induced mutations has a very distinct implication: Researchers can make discoveries about the genetic regulation of a phenotype without the bias of a hypothesis. This permits novel discoveries of unanticipated genetic relationships.

Creating new mouse strains based on spontaneous mutations: The Phenotypic Deviant Search program and the Mouse Mutant Resource at The Jackson Laboratory

Animal care technicians at The Jackson Laboratory are trained to identify unexpected, deviant phenotypes, which could indicate the occurrence of a spontaneous mutation. Technicians set aside these "deviant" mice and their littermates, and researchers meet weekly to view the selected mice. Phenotypes of interest are tested for heritability by individual researchers or by the Mouse Mutant Resource group, which also registers mutations from investigators outside of The Jackson Laboratory. Following further characterization, the mice are offered to the global research community. To date, more than 500 strains of JAX® Mice have been developed from mice identified via the deviant search.

We are very serious about recognizing the contribution our caretakers make to our deviant search program. Animal care technicians who discover mutations that result in publications receive formal acknowledgment in the manuscripts.

For more information about the Mouse Mutant Resource, visit http://mousemutant.jax.org/index or email mmr@jax.org.

Spontaneous mutations

Spontaneous mutations that cause observable phenotypes generally are discovered by conscientious animal care technicians or researchers who are very familiar with the phenotypes of specific strains and who observe something unique in a specific mouse. If a phenotype is of interest, the mouse and its relatives are bred to determine whether the phenotype is heritable.

Induced random mutations

Induced mutations in mice are typically produced by such treatments as chemicals or ionizing radiation. For chemically-induced mutations, male mice are injected with a chemical that is known to cause point mutations (N-ethyl-N-nitrosourea [ENU], for example). For radiation-induced deletions, males are irradiated. Following treatment, the males are bred with non-treated females, and their offspring are screened for phenotypes of interest. Because treatment produces numerous mutations in each mouse, a desired phenotype often is produced by complex genetics. These phenotypes are generally "lost" as the line is propagated and the critical constellation of mutations is broken up. As a result, if a line still expresses a deviant phenotype after about five generations, this usually indicates that this deviant phenotype is driven by a single locus mutation.

3.C.1.a.2. Genetically engineered mutations

Genetic engineering technology allows researchers to create mice with specific mutations for designated genes. In this handbook, we organize genetically engineered mice into two categories:

- Targeted mutant mice produced using homologous recombination, in which a targeted endogenous gene is altered. This classification includes knockout (KO) mice, in which a gene is made non-functional, and knockin mice, in which the function of a gene is altered.
- Transgenic mice, in which a functional exogenous gene—possibly from another species—is inserted randomly into the genome of a mouse.

Targeted mutant mice by homologous recombination

Homologous recombination involves genetic modifications—either "knocking out" an exon or "knocking in" a novel DNA sequence or gene—to the genome of embryonic stem (ES) cells. Successfully modified ES cells are identified through a selection process and injected into host embryos. Embryos are then transferred into pseudopregnant host females. When the ES cells contribute to the cellular makeup of the developing fetus, the result is a chimera. Chimeras comprise both ES-derived cells and cells from the original embryo. These chimeras are usually bred with the host strain to test for germline transmission of the targeted mutation. When a chimera produces progeny with the mutation, it is called "going germline." Germline chimeras produce heterozygotes for the targeted mutation. Thus, these heterozygotes must be intercrossed to produce a homozygous "knockout" or "knockin" mouse.

Transgenic mice

To create transgenic mice, multiple copies of a genetically-engineered transgene are injected into a fertilized egg. The transgene usually includes the structural components of the gene and a promoter region that specifies when and where that gene is expressed. For example, a promoter could specify that a gene would be expressed only in fat cells, and only when the animal was under stress. Often, an intron is included in the engineered transgene, which helps stabilize the gene once it is inserted. Typically, a transgene will insert as multiple tandem copies. Two or more different transgenes also can be co-injected simultaneously. Co-injected transgenes typically insert in the same genome location.

A few words about 129 strains, ES cells, and nomenclature…

A large number of mutant mice produced through homologous recombination involve embryonic stem (ES) cells from 129 inbred strains. When using a 129 substrain, it is critical that the genotype match that of the 129 ES cells and the DNA library used for the construct. Failure to do so results in mixed 129 genotypes. For a list of the 129 strains from which the principal ES cells were derived, see Appendix B, "129 Strains—Nomenclature and Related ES Cell Lines."

Because of the complex history of the 129 strains, nomenclature was problematic, which hampered replication of studies (Simpson *et al*., 1997; Threadgill *et al*., 1997). In 1999, Festing *et al*. developed new nomenclature to distinguish the 129 parental lines and related 129 strains. The International Committee on Standardized Genetic Nomenclature for Mice approved the new nomenclature. For an overview of the new rules, refer to Appendix B, "129 Strains—Nomenclature and Related ES Cell Lines." For full nomenclature details, visit www.informatics.jax.org/mgihome/nomen/strain_129.shtml.

Examples of genetically engineered mutant mice

- T cell receptor (TCR) transgenic mice are used to study interactions of the T lymphocyte with specific antigens (von Boehmer *et al*., 1988). Most T lymphocytes in the TCR transgenic mice target a single antigen that is specified by the researcher when the transgenic mouse is created. This enables researchers to study the interaction of a specific antigen with T lymphocytes because it greatly amplifies a cellular-level phenomenon impossible to observe by other means. However, the repertoire of the T cell system is artificially—and extremely—restricted in these mice. Most of their T cells react with the same antigen, and no other antigens. Therefore, these mice are very immunocompromised.
- Tissue-specific knockouts can be achieved with a Cre-lox strategy, involving both homologous recombination and transgenic technologies. A structural component of the gene to be knocked out is surrounded by loxP sites using homologous recombination. The loxP sites are targets of a Cre recombinase, which cuts the DNA strand at that site. The Cre recombinase can excise, invert, or translocate the "floxed" fragment (the fragment flanked by the lox sites), depending on the orientation of the loxP sites. The gene for the recombinase is physically linked to a tissue-specific promoter, and a mouse is made transgenic for this "construct." A Cre-lox mouse is produced by crossing mice that have a tissue-specific Cre recombinase with mice that have a target gene flanked by loxP sites (a "floxed" locus). It is important to note that, although all cells of the mouse carry the engineered gene, expression of the recombinase is limited only to the tissue designated by the promoter. As an example, to restrict expression of a transgene to the brain and other neuronal tissue, a promoter for a neuron-specific protein, nestin, can be used. Figure 3.5 provides a schematic overview of Cre-lox technology.

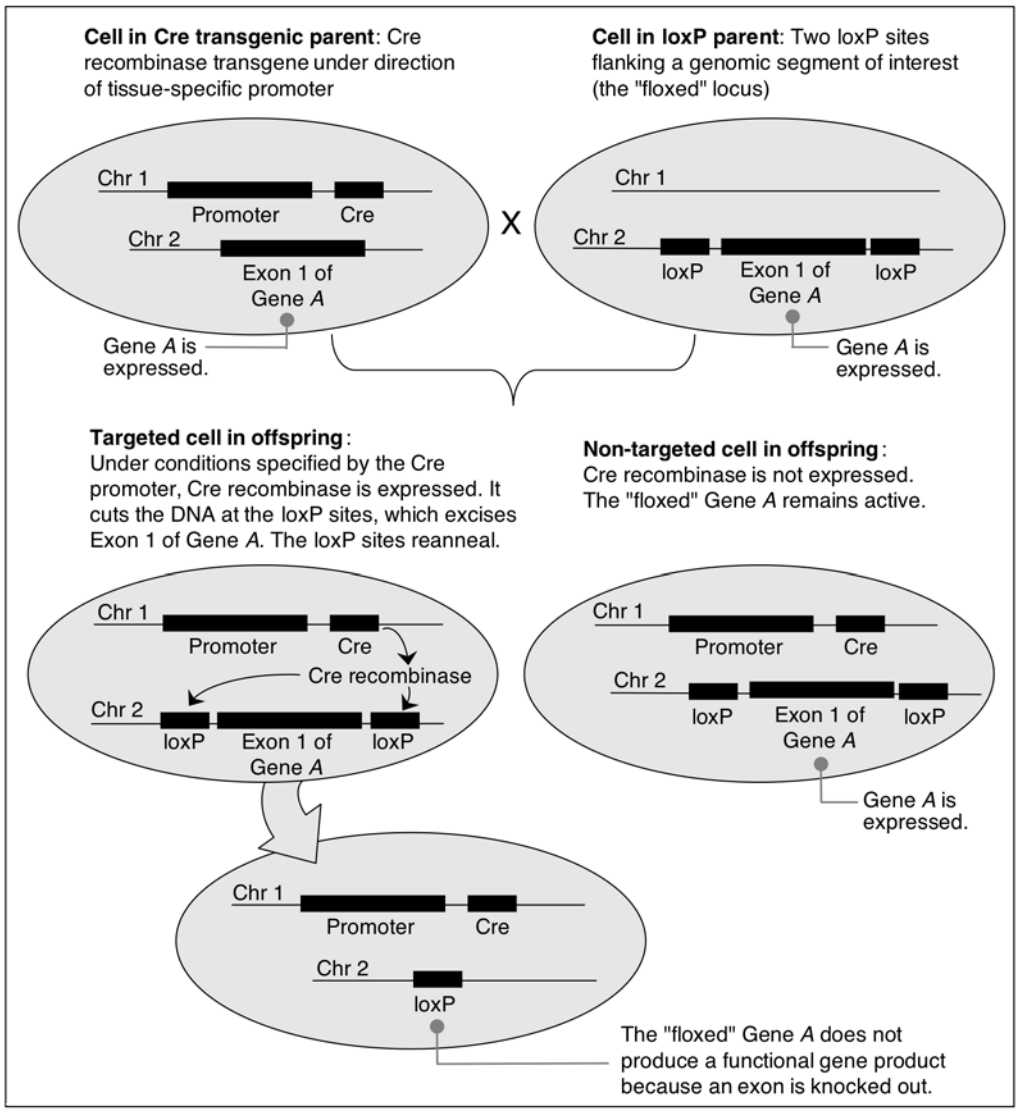
Creating humanized mice: compounding mutations to enable human tissue transplants

Despite the similarities between mice and humans at the genetic, physiologic, and anatomical levels, some human diseases can be studied only in human tissue. (Viruses, for example, are typically species-specific.) Humanized mouse models meet the need for hosts of human tissue. Such models are severely immunocompromised, permitting the transplantation and survival of human tissue in mice so that pathogenesis and treatment of uniquely human diseases can be studied. Leonard Shultz has spent much of his research career at The Jackson Laboratory constructing a succession of immunodeficient mouse models, each of which was a better host for human tissue than the one before.

His most recent model, the NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ strain of JAX® Mice (005557), is arguably the most versatile immunodeficient mouse model available. This strain and other similar humanized mouse models are now being used for studies of human hematopoiesis, innate and adaptive immunity, infectious diseases, cancer biology, regenerative medicine, and autoimmunity, including type 1 diabetes (Shultz *et al*., 2007; Pearson *et al*., 2008).

Such humanized mice illustrate the value of a broad understanding of the various types of mutant mice. The NOD mouse has inherent immunodeficiences in its innate immune system, representing strain variation. The *scid* mutation, which severely impairs adaptive immunity, was a spontaneous mutation. The *Il2rg* knockout was specifically engineered to produce immunodeficiency. It is because of the combination of these complementary deficiencies that such unique mouse models could be created.

Figure 3.5. Schematic of Cre-lox technology illustrating an excision of a target site.



- The FLP-FRT system is similar to the Cre-lox System. It involves the use of flippase (FLP) recombinase, derived from the yeast *Saccharomyces cerevisiae* (Sadowski, 1995). FLP recognizes a pair of FLP recombinase target (FRT) sequences that flank a genomic region of interest.
- A more recently developed (and simpler) way of suppressing expression of an endogenous gene is by constructing an RNA-mediated interference (RNAi) transgenic. When a transgene produces a strand of RNA that is complementary to an endogenous mRNA, the complementary strand hybridizes with the endogenous RNA; the double stranded RNA is degraded by endogenous mechanisms, leading to post-transcriptional suppression of gene expression. RNAi transgenic technology permits tissue- or temporal-directed suppression of gene expression, without the requirement to outcross to another strain that carries a targeted mutation, as with Cre-lox and FLP-FRT systems (Dykxhoorn *et al.,* 2003). RNAi technology holds great promise for mammalian application (Behlke, 2006), but it currently has some drawbacks. The most serious is that, often, insufficient RNAi is produced to completely suppress the target gene.

3.C.1.b. Considerations

Considerations for the use of spontaneous, induced, and genetically engineered mutant strains include the following:

- A mutant phenotype of the strain may necessitate special husbandry.
- For strains based on random mutations, initially the gene is unidentified, and it will remain so until it is positionally cloned or tested by complementation.
- When inducing random mutations, the initial mutational load can impair breeding.
- For transgenic mice
  - Multiple copies of the transgene frequently insert, sometimes at multiple sites. Because the site of insertion is random, endogenous genes might be knocked out, which could create a cryptic KO mouse. This happens in approximately 10–15% of transgenics.
  - The expression of the transgene may be lost through generations of breeding, typically as a result of DNA methylation or copy number loss. The best strategy to minimize this loss is to maintain the line in forced hemizygosity.
  - Over-expression of a transgene, including marker transgenes, may have unintended consequences, including cell lethality (Leiter *et al.*, 2007).
  - A successfully inserted transgene may not be expressed. To increase the probability of expression, it is helpful to include an intron in the transgene.
- For mice used in a bigenic system:
- It may be necessary to maintain parental strains. For example, with Cre-lox models, often both the Cre and loxP parental strains must be maintained.
- Genotyping sometimes is complicated by a need to differentiate homozygotes from hemizygotes (e.g., typically floxed genes must be homozygous for the system to work).
- For Cre-lox Systems, both the Cre and the loxP lines must be genotyped; however, even the presence of both Cre and loxP sites is no guarantee that the Cre-lox System will work. Similarly, the fact that the Cre-lox System worked in one generation is no guarantee that it will work in subsequent generations.
- The promoter driving the Cre may give only mosaic cell expression in a tissue.

3.C.1.c. Breeding mutant mice and selecting controls

When developing and expanding a line of mutant mice, several issues must be clarified:

- The heritability and mode of inheritance of the phenotype.
- The genetic background of the new mutant strain.
- The breeding scheme used to expand and maintain the mutant strain and perhaps create controls.

Also, as with any breeding of inbred strains, preventive measures must be taken to guard against genetic contamination and minimize genetic drift.

3.C.1.c.1. Heritability and mode of inheritance

Regardless of the origin of a random mutation, the first step in development of a mutant mouse line is to determine heritability of the phenotype, whether it is monogenic, and whether it can be carried homozygously. Heritability is "proof" that a phenotype is genetically determined.

Analysis of heritability also includes determining the mode of inheritance—dominant, recessive, or additive—and the degree of the penetrance. (For details about heritability, refer to 2.B.1, "Terminology.")

Don't some inbred strains carry mutations? Why aren't the mutations part of the name?

Probably all inbred strains carry fixed mutations. The mutations might have been in the progenitors, or they might have arisen during inbreeding or strain maintenance. For example, in the C3H family, most strains carry the retinal degeneration 1 mutation (*Pde6b*^{rd1}), which causes blindness; a few carry the toll like receptor 4 mutation (*Tlr4*$^{Lps\text{-}d}$), which causes immunodeficiency.

Neither mutation is specified in C3H strain names, such as C3H/HeJ (000659), which carries both mutations, or C3HeB/FeJ (000658), which does not carry *Tlr4*$^{Lps\text{-}d}$. (This is yet another reason why it is so important to learn the genetic details about the mice you are studying. For JAX® Mice, such details are provided on the strain datasheets, available at www.jax.org/jaxmice/query.)

Historically, when a mutation arises spontaneously in the parental lineage and becomes homozygous, it becomes part of the genotype of the parental lineage, and the name of the strain is not changed. However, when a new strain is specifically created to carry a mutation, even if the strain is coisogenic to the parental strain, the mutation *is* designated in the new strain name.

3.C.1.c.2. Strain background considerations

Once investigators have characterized the heritability of a mutation, they must consider the genetic background they will use to carry the mutation. If the mutation arose or was created on a well-characterized strain, and if the strain characteristics do not interfere with the study of the mutation, it can be maintained on the original strain. If, however, the original background is mixed or not well characterized, or if the strain characteristics could interfere with the study of the mutation, it can be moved to another background, creating a congenic (see 3.C.2). Generally, C57BL/6 is the preferred background because it is a well-characterized, robust strain.

3.C.1.c.3. Breeding schemes and choices for controls.

When choosing a breeding scheme, an important consideration is whether the priority is to create as many mutant mice as quickly as possible or to expand the line more slowly but create littermate controls at the same time. Table 3.8 describes the breeding options, several of which can be used to produce control animals along with the mutant mice.

Note: Transgenic mice that are hemizygous are usually maintained by backcrossing to the parental strain.

Table 3.8. Expansion and maintenance breeding schemes for strains with single-locus mutations.

Conditions related to the mutation		Applicable breeding schemes	
Fertility	**Mode of inheritance**	**Description (genotypes)**	**Offspring**
Mutants of both genders are fertile	Dominant or semi-dominant	Heterozygous mutant x homozygous wild-type (*M*/+) x (+/+)	(*M*/+), (+/+)*
		Heterozygous mutant x heterozygous mutant (*M*/+) x (*M*/+)	(*M*/*M*), (*M*/+), (+/+)*
		Homozygous mutant x homozygous mutant (*M*/*M*) x (*M*/*M*)	(*M*/*M*)
	Recessive	Heterozygous carrier x heterozygous carrier (*m*/+) x (*m*/+)	(*m*/*m*), (*m*/+)*, (+/+)*
		Homozygous mutant x homozygous mutant (*m*/*m*) x (*m*/*m*)	(*m*/*m*)
Mutants of only one gender are fertile	Dominant or semi-dominant	Heterozygous mutant x homozygous wild-type (*M*/+) x (+/+)	(*M*/+), (+/+)*
		Homozygous mutant x homozygous wild-type (*M*/*M*) x (+/+)	(*M*/+)
	Recessive	Homozygous mutant x heterozygous carrier (*m*/*m*) x (*m*/+)	(*m*/*m*), (*m*/+)*
Mutants of neither gender are fertile†	Dominant or semi-dominant	Not applicable	
	Recessive	Heterozygous carrier x heterozygous carrier (*m*/+) x (*m*/+)	(*m*/*m*), (*m*/+)*, (+/+)*

M = dominant mutant allele; *m* = recessive mutant allele; + = wild-type allele.

* Can be used as littermate controls. See Table 3.9 for details.

† If fertile ovaries can be obtained from mutant females, ovarian transplants can be used to expand the mutant stock. This may be more efficient than intercross matings because fewer animals are needed. (For more detail on ovarian transplants, see 13.E.2, "Assisted reproductive techniques [ARTs].")

For breeding schemes that do not produce controls, other options exist. Table 3.9 highlights these options.

Table 3.9. Choices of controls for specific mutant genotypes.

Mutant genotypes	Suitable controls
(*M*/*M*)	• If a mutant's genetic background is an inbred strain, that inbred strain (coisogenic to the mutant strain) is a suitable control as long as the mutant strain has not become a substrain. • If the mutant's genetic background is mixed, F2 hybrids between the 2 parental strains are approximate controls. Although it is unlikely that an F2 hybrid population will have the same genetic mix as the mutant line, an F2 control is the best option if littermate controls cannot be used.
(*M*/+)	Same as for (*M*/*M*), plus… • Wild-type (+/+) littermates are ideal controls.
(*m*/*m*)	Same as for (*M*/*M*), plus… • For fully recessive mutations, heterozygote (*m*/+) siblings or wild-type (+/+) littermates are suitable controls. (Typically, these controls are designated (+/?) because they cannot be distinguished from each other without genotyping.)

M = dominant mutant allele; *m* = recessive mutant allele; + = wild-type allele.

A recessive mutation that is fatal or that causes a critical phenotype such as sterility also can be carried in a *balanced stock*, in which the mutation is linked to a marker gene (often one for coat color) so that mutant mice and carriers of the mutation can be identified by sight. Balanced stocks are infrequently used today because of the prevalence of genotyping; however, once a balanced stock is created, it is very convenient to use. Several strains of JAX® Mice are maintained as balanced stocks. For details on breeding a balanced stock, refer to Appendix I, "Using a Balanced Stock to Carry a Recessive Mutation That Is Sterile or Lethal, Including Embryonic Lethal."

3.C.1.c.4. Breeding considerations to minimize genetic drift

When maintaining a mutant strain, breeding strategies must minimize the creation of genetic differences between the mutant strain and the background strain on which is resides. Use these guidelines:

- *If the mutation is on a stable inbred strain*, backcross to the parental strain about every 10 generations. This will prevent creation of a substrain.
- *If the mutation must be kept on a mixed background*, choose breeders to minimize inbreeding at each generation, and backcross offspring to the F1 hybrids between the two parental strains about every 10 generations. This will prevent creation of recombinant inbred lines.

To learn when a specific strain of JAX® Mice was most recently refreshed, contact Technical Support at 1-800-422-6423 (North America) or 1-207-288-5845 (International).

3.C.1.d. Nomenclature

Nomenclature for strains with single locus mutations includes information about the background strain or substrain followed by information about the gene and mutant allele. Note that the designation for the mutation is the same whether it is carried homozygously or heterozygously. For full strain details, check with the supplier of the strain. For JAX® Mice, refer to the strain datasheet (www.jax.org/jaxmice/query). Table 3.10 provides several examples of nomenclature for single locus mutations.

Table 3.10. Examples of nomenclature for strains that carry single locus mutations.

Name	Definition	Convention
C57BL/6J-$A^{w\text{-}J}$/J (000051)	Substrain of C57BL/6J (000664), in which the agouti gene (*A*) is homozygous for the mutated $A^{w\text{-}J}$ allele; maintained at The Jackson Laboratory (J).	Background strain, hyphen (-), symbol for the mutated allele (italicized), forward slash (/), and ILAR code(s) (http://dels.nas.edu/ilar_n/ilarhome/search_lc.php).
C3H/HeJ-*Mgrn1*^{md}/J (000223)	Segregating inbred strain, in which the *Mgrn1* gene is heterozygous for the *Mgrn1*^{md} allele; maintained at The Jackson Laboratory (J).	As above.
B6129PF1/J-$A^{w\text{-}J}/A^{w}$ (100409)	F1 hybrid, created from a C57BL/6J-$A^{w\text{-}J}$/J (000051) female and a 129P3/J (000690) male (which is homozygous for the A^{w} allele); the *A* gene in the F1 is heterozygous for the $A^{w\text{-}J}$ allele and the A^{w} allele; generated at The Jackson Laboratory (J).	As above, with optional allelic information following ILAR code(s).
B6;129S4-*Nos1*^{tm1Plh}/J (002633)	Targeted mutation one of the *Nos1* gene (*Nos1*^{tm1Plh}), on a mixed C57BL6/J and 129S4/SvJae background (B6;129S4); developed by P. L. Huang (Plh); maintained at The Jackson Laboratory (J).	Background strain (strain abbreviations separated by a semi-colon [;] indicate less than 5 or unknown number of filial generations); hyphen (-); gene (italicized), number of targeted mutation (*tm#*) and allele (italicized superscript); forward slash (/), and ILAR code(s) (http://dels.nas.edu/ilar_n/ilarhome/search_lc.php)
C57BL/6-Tg (CAG-EGFP)1Osb/J (003291)	Transgenic mouse (Tg) on a C57BL/6 background, with insert of gene enhanced green fluorescent protein under the direction of promoter for beta actin, (CAG-EGFP); 1st line created at the Research Institute for Microbial Diseases, Osaka University (Osb); maintained at The Jackson Laboratory (J).	Background strain; transgene symbol "Tg"; insert designation, line number and developer; forward slash (/); and ILAR code(s) (http://dels.nas.edu/ilar_n/ilarhome/search_lc.php).
STOCK-Tg (B19-RNAi:Il3)241Ckn/J (002182)	Transgenic (Tg) mouse on a background derived from 3 or more founder strains (STOCK), in which an *Il3* antisense RNA transgene is expressed under control of the B19 parvovirus promoter; 241st line created by D. A. Cockayne (Ckn); maintained at The Jackson Laboratory (J).	As above, with "STOCK" designation to represent mixed background of 3 or more founder strains.

3.C.1.e. Research examples

A spontaneous mutation used to study the role of leptin in autoimmunity.
A spontaneous mutation in the leptin receptor (*Lepr*$^{db\text{-}5J}$) of NOD/ShiLtJ (001976) mice, which are prone to type 1 diabetes, resulted in a new strain: NOD/ShiLtJ-*Lepr*$^{db\text{-}5J}$/LtJ (004939). Research with this strain led to the demonstration that altered leptin signaling suppressed the progression of type 1 diabetes in NOD mice (Lee *et al.,* 2005). Had these investigators not been so observant of the new phenotype, they would have missed the opportunity to discover the surprising role of a fat tissue hormone (leptin) in the modification of an autoimmune disease.

ENU technology used to create a model for hyperlipidemia.
To discover new mouse models of cardiovascular disease, Svenson *et al*. (2008) used ENU mutagenesis followed by high-throughput phenotyping. The researchers identified a new missense mutation in the LDL receptor (Ldlr), which they named Wicked High Cholesterol (WHC). When they compared mice bearing the WHC mutation to the widely used *Ldlr* knockout (KO) mice, they observed notable phenotypic effects of the mutation, such as accelerated atherosclerotic lesion formation and reduced hepatosteatosis in the WHC mutant after a short exposure to an atherogenic diet. The WHC mutant carries, on a C57BL/6J (000664) genetic background, a single-base mutation in the *Ldlr* gene that substitutes a G with an A in exon 14 at nucleotide 2096. As a result, cysteine V at amino acid residue 699 is substituted by a tyrosine (C699Y) in the extracellular EGF-precursor homology domain of the LDL receptor. Although the EGF precursor homology domain does not participate in lipid binding, the physiological effects of this mutation demonstrate that this domain influences the function of the receptor, presumably through influences on tertiary structure. The WHC mutant provides a useful new tool for understanding the pathophysiology of atherosclerosis and for evaluating additional genetic modifiers regulating hyperlipidemia and atherogenesis. The creation and discovery of WHC provides a good example of how ENU mutagenesis technology—generating single nucleotide mutants that do not appear naturally—helps researchers understand structure function relationships as well as overall function of the genes that are mutated.

Cre-lox technology used to demonstrate the relationship of fat to insulin metabolism.
Blüher *et al*. (2003) used a Cre-lox System to study the influence, on energy metabolism, of insulin signaling in fat tissue. They created mice in which the insulin receptor was "floxed," i.e., in which an exon of the insulin receptor was surrounded by lox sites. By crossing these mice with mice bearing a Cre-recombinase transgene under the regulation of the AP2 promoter, which limits expression of the recombinase to white fat, the researchers produced mice in which white fat had no insulin receptors. As a result, the amount of fat tissue was greatly diminished. Because these Cre-lox mice were more insulin-sensitive than normal, a role for white fat in the development of insulin resistance, which promotes type 2 diabetes, was demonstrated. Interestingly, these Cre-lox mice also lived about 15% longer than the various controls (mice with only the Cre-recombinase transgene, mice with only the floxed insulin receptor, and mice of the same mixed genetic background with neither mutation), demonstrating a role for even normal levels of white fat in limiting lifespan.

3.C.2. Congenic and conplastic strains

A congenic strain is one in which a short chromosomal segment—containing a gene with an allele of interest—is transferred to a different genetic background through selective breeding using either phenotypic or genotypic screening. It is important to note that this chromosomal segment contains other linked genes as well.

Historically, congenic strains were generated to help understand the effects of genetic variants on transplantation biology. Most strains were major histocompatibility complex (*H2*), minor histocompatibility complex (*H*), non-histocompatibility alloantigen, and cellular marker congenics. Today, uses include the study of mutations on a standardized genetic background.

Why are there so many B6.129 congenic strains?

A large number of congenic mice are produced using knockout and knockin mutants that were created from 129 ES cells. Chimeras often are made by combining genetically engineered 129 ES cells with embryos from a strain that can be distinguished from 129 by coat color. C57BL/6J (000664) is such a strain. When the chimeras are mated with C57BL/6J mice, the chimeras that have gone "germline" produce agouti offspring, which are B6129F1 hybrids. The chimeras that have not gone germline produce only black offspring, which are C57BL/6J inbred mice.

When the B6129F1s are mated together to propagate and expand the stock, a genetically mixed background is produced. To move the mutation to a standardized genetic background, often a congenic is made on the C57BL/6J background—resulting in a B6.129 congenic.

Congenic mice are particularly useful for the following purposes:

- To "clean up" a genetic background. By putting the allele of interest on a standardized inbred strain, researchers can quantify the effects of the allele on a well-defined and reproducible background.
- To study an allelic series on the same genetic background. This allows researchers to directly compare the effects of the various alleles for a specific gene on one, well-defined background.
- To study the effect of a given allele on multiple, standardized backgrounds (i.e., C57BL/6J [000664], C3H/HeJ [000659], DBA/2J [000671]. This allows researchers to study the effects of modifying genes.
- To support gene mapping and positional cloning studies.

We include conplastic strains in this discussion about congenics because a conplastic strain is a mitochondrial congenic, a mouse that contains the nuclear genome of one strain and the mitochondrial genome of another.

3.C.2.a. Definitions, characteristics and value

3.C.2.a.1. Congenic strains

Congenic strains are produced by

- transferring a chromosomal segment (including the allele of interest) from a mouse of one strain or stock to an inbred strain through outcrossing (generation N1, which is the same as an F1 generation), and then by
- backcrossing mice with the allele or phenotype of interest for nine more generations (N2–N10), generally reversing the gender of the inbred strain and the carrier breeders at least once to assure that both Chr Y and the mitochondrial chromosome are the recipient type.

A congenic strain has been backcrossed for at least 10 generations. Statistically, at this point, 99.8% of the allelic differences between the donor and recipient strains that are unlinked to the congenic locus are expected to be the recipient type in the congenic (Figure 3.6) (Silver, 1995). Note that the amount of donor genome linked to the selected gene or marker is reduced at a much slower rate, approximately equivalent to 200 cM/N, where N is the number of backcross generations for $N > 5$ (Silver, 1995).

It can take up to three years to create a fully congenic strain (N10). Therefore, researchers sometimes use *incipient* congenics (N5–N9) in their initial studies because at that point in the backcrossing process, at least 94% of the genetic background, on the average, is recipient type.

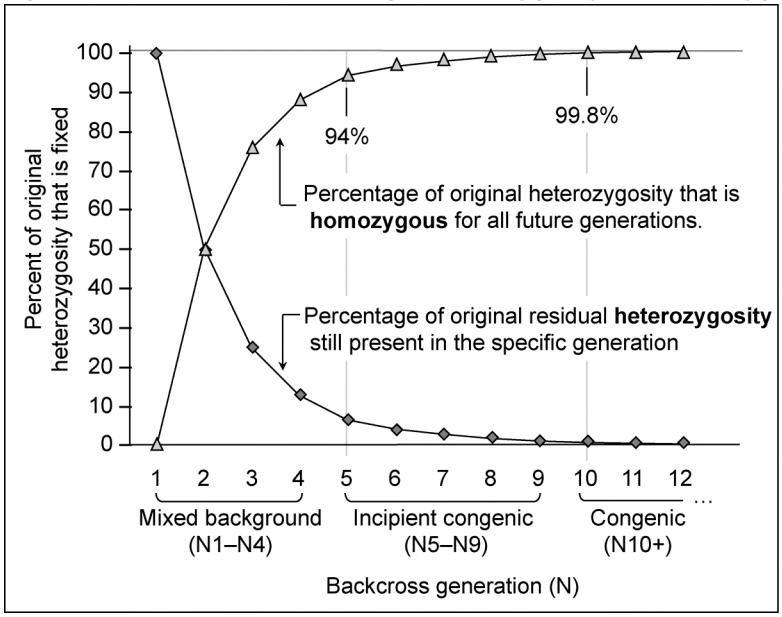
After N10, the breeding strategy depends on whether the congenic strain is to be kept as a fully inbred congenic strain, in which the donor allele is homozygous, or as a segregating inbred

congenic strain, in which the donor allele is kept heterozygous at the congenic locus. (A segregating inbred congenic strain is useful when the congenic allele is dominant or additive and a researcher wishes to produce carriers and non-carriers within the same cross.)

A fully inbred congenic strain requires a filial intercross (F1) after the N10 generation, followed by filial breeding (F2) of offspring that are homozygous for the donor allele. Note that although the genetic background for the inbred congenic strain is virtually completely recipient type, the congenic locus includes passenger genes that will be donor type.

A segregating inbred congenic strain is maintained after N10 by continued backcrossing to the parental strain. In the segregating congenic, the genes that are linked to the congenic locus, and that have allelic differences between the donor and recipient, will also be heterozygous.

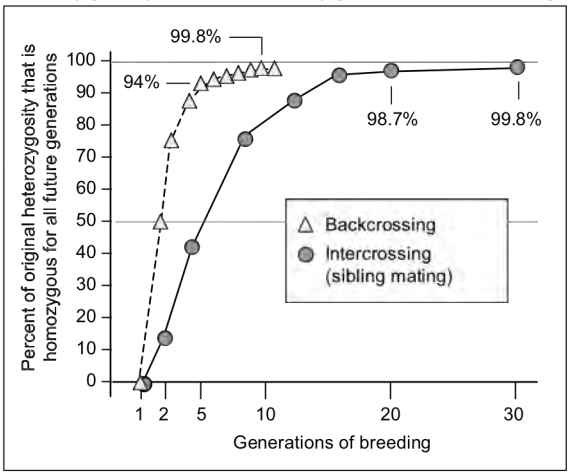
Figure 3.6. Effects of backcrossing on homozygosity and heterozygosity.



The effects of backcrossing on homozygosity and residual heterozygosity (unlinked to the congenic locus) throughout the process of creating congenic mice. The symbols represent residual heterozygosity at any given generation, expressed as the percent of the original heterozygosity in the N1 generation. The filled triangles and diamonds represent mean values. For any individual mouse, the value will probably differ from the mean.

It takes only 10 generations to create an inbred congenic, while it takes 20 generations to create a standard inbred strain. This is because in creating a congenic, one of the parents is always 100% homozygous for the recipient genotype. Figure 3.7 illustrates this comparison.

Figure 3.7. Effects of backcrossing vs. intercrossing (inbreeding) on percentage of original heterozygosity that is homozygous for all future generations.



By the 10th generation of backcrossing, the congenic strain is homozygous (recipient type) for 99.8% of the original genetic differences from the donor strain that are not linked to the congenic locus. For inbreeding by sibling mating, it takes 30 generations of inbreeding to reach that percentage of homozygosity.

Creating a congenic strain using phenotyping

Today, because genetic markers are available for most mutations, congenic mice are commonly created using selective breeding based on genotyping. Traditionally, however, congenics were created for a known monogenic trait by selecting for the desired phenotype at every generation. This approach is sometimes used today, especially when a genetic marker is unavailable. Selection based on phenotypes requires multiple strategies, based on the characteristics of the mutation. These strategies are described in Appendix H, "Transfer of a Mutant or Variant Allele to a New Genetic Background by Phenotypic Selection."

Creation of speed congenics using marker-assisted backcrossing

Speed congenics are congenic mice created using a breeding strategy that reduces the number of backcross generations necessary to fix (to the recipient type) the required 99.8% of the genetic differences between the donor and recipient strains. This strategy involves genotyping using single nucleotide polymorphism (SNP) markers and/or microsatellite (SSLP) markers to select the most suitable mice, i.e., those with the greatest amount of the recipient genotype, for backcrossing. At The Jackson Laboratory, we recommend placing markers about every 10 cM. Speed congenic technology can reduce the number of required backcrosses from 10 to 5 and save 1-1/2 years. Speed congenics provide another benefit: any residual heterozygosity is well-defined.

Are speed congenics worth the expense?

A drawback with creating congenics using traditional backcrossing is that the process takes about 2.5–3 years. This can be a major impediment to progress of a research program. In contrast, creating a speed congenic takes just about 1.5 years. Although the process to create a speed congenic is more expensive, the time savings—and the fact that the strain is so well-defined—might make a speed congenic the preferred option.

For information on the speed congenic service at The Jackson Laboratory, visit www.jax.org/jaxservices/speedcongenic. Or call us at 1-800-422-6423 (North America) or 1-207-288-6294 (International). We will analyze your needs and help you determine whether a speed congenic is the right choice for your research program.

3.C.2.a.2. Conplastic strains

A conplastic strain has the nuclear genome of one strain and the mitochondrial genome of another. Conplastic strains are used mostly to study the role of mitochondrial genes in energy balance and disease risk.

A conplastic strain is developed by

- initial outcrossing of a female mitochondrial donor with a male recipient (generation N1) followed by
- continued backcrossing, mating one female of each generation (the mitochondrial donor) to a male of the recipient strain for at least 9 additional generations (N2–N10).

The effects of backcrossing on homozygosity and residual heterozygosity of conplastic strains is the same as for congenics (Figure 3.7), except that for conplastic strains, few, if any, passenger genes are involved because only the small number of genes encoded by the mitochondrial DNA are transferred.

3.C.2.b. Considerations

In a congenic strain, a short chromosomal segment—containing multiple genes rather than a single gene—is transferred to an inbred strain by successive backcrossing. Along with the allele of interest, this segment includes donor-type alleles that are commonly referred to as "passenger genes" or "linked genes." The transfer of these donor-type alleles has important implications for research:

- Although the congenic strain is very closely related to the recipient strain, the strains will differ at locations in addition to the gene of interest. Therefore, the strains are not coisogenic.
- Variants of phenotypic expression in the congenic strain might result from an interaction of the recipient background with the allele of interest or from an interaction of the recipient background with passenger genes from the donor. Thus, a variant phenotype observed in a new congenic strain cannot be attributed exclusively to an interaction of the target gene with the recipient genotype.

3.C.2.c. Maintenance breeding strategies

Following the creation of an inbred congenic strain or segregating inbred congenic strain, maintenance of congenic strains is the same as for single locus mutations (see 3.C.1.c.3, Table 3.8, "Expansion and maintenance breeding schemes for strains with single-locus mutations." Conplastic strains are maintained by backcrossing females of the conplastic strain to males of the recipient strain.

For detailed information on breeding strategies for specific congenic or conplastic strains of JAX® Mice, refer to the strain datasheet, available at www.jax.org/jaxmice/query.

3.C.2.d. Controls

For congenic mice, the selection of controls is based on the nature of the mutation. Considerations are the same as those specified for random mutations (see 3.C.1.c.3, Table 3.9, "Choices of controls for specific mutant genotypes"). For conplastic mice, controls are the recipient inbred strain.

3.C.2.e. Nomenclature

Nomenclature for congenic strains includes information about recipient and donor strains as well as the locus of interest. Nomenclature for conplastic strains includes names of the mitochondrial donor strain and the recipient strain. Numbers of generations of backcrossing and inbreeding (designated by N for backcross generation and F for filial generation) generally are not reflected in the name, but are important pieces of information that should be provided by the supplier. For example, N6F20 designates that a strain has been backcrossed 6 times followed by 20 generations of sister–brother mating. A question mark indicates that data are not available. For example, N(?)F25 means that the number of backcross generations is unknown, but the mice have been inbred for 25 generations. Table 3.11 provides several examples of congenic and conplastic strain names.

Table 3.11. Examples of nomenclature for congenic and conplastic strains.

Name	Definition	Convention
B6.129P1-*Lama2*^{dy}/J (000631)	Congenic strain, where locus of interest (*Lama2*^{dy}) of the 129P1 donor strain has been backcrossed to recipient strain C57BL/6J (B6) at least 5 times (.) at The Jackson Laboratory (J).	Abbreviation of recipient strain, period (.), abbreviation of donor strain, hyphen (-), locus of interest (in italics), forward slash (/), and ILAR code(s) (http://dels.nas.edu/ilar_n/ilarhome/search_lc.php). ***Note***: Congenic strain names, which include a period (.), are assigned at backcross generation 5 (N5). Congenics from N5 to N9 are called incipient congenics. A full congenic requires 10 backcross generations. For details about the number of backcross generations for a particular strain, check with the supplier. For JAX® Mice, refer to the strain datasheet at www.jax.org/jaxmice/query.
B6.Cg-*A*^{y}/J (000021)	Congenic strain, where locus of interest (*A*^{y}) from a mixed donor stock (Cg) was backcrossed to recipient strain C57BL/6J (B6) at least 5 times (.) at The Jackson Laboratory (J).	As above, with "Cg" representing a mixed donor stock.
C57BL/6J-mt$^{PWD/Ph}$/ForeJ (0005761)	Conplastic strain, in which the mitochondrial genome (-mt) of PWD/Ph was transferred to C57BL/6J by J. Forejt (Fore); maintained at The Jackson Laboratory (J).	Strain of nuclear genome, mitochondrial symbol (-mt), strain of mitochondrial genome (superscripted), ILAR code(s) (http://dels.nas.edu/ilar_n/ilarhome/search_lc.php).

3.C.2.f. Research examples

Use of congenic strains in discovery of function of *Lith1* and *Lith2* genes.

Congenic strains were instrumental in the discovery of *Lith1* and *Lith2* genes, both of which determine cholesterol gallstone formation (Paigen *et al.,* 2000). Researchers started with two inbred strains, C57L/J (000668) and AKR/J (000648) that differed with respect to gallstone formation (C57L/J forms gallstones readily when fed a lithogenic diet; AKR/J does not). They created an F1 hybrid from the two strains and backcrossed it to the resistant strain, AKR/J. They fed these mice a lithogenic diet and mapped the phenotype "gallstone formation." Two loci were identified: *Lith1*, which mapped to Chr 2; and *Lith2*, which mapped to Chr19. Confirmation that these genetic regions actually contained a gene or genes that regulates gallstone formation required further work. By moving the chromosomal regions containing the gallstone formation genes from the C57L/J strain to the AKR/J strain, i.e., by constructing the congenic strains AK.L-$Lith1^{C57L/J}$ and AK.L-$Lith2^{C57L/J}$, the researchers conferred susceptibility to the previously resistant AKR/J strain. This directly demonstrated that the *Lith1* and *Lith2* loci contained genes that controlled gallstone formation. Once the genes underlying these QTLs are identified, the "roadmap" for discovery of orthologous human LITH genes will be available, and the putative roles in cholesterol gallstone formation can be tested in selected human populations.

Use of conplastic strains to modify free radical production.

Gusdon *et al*. (2007) used conplastic strains of mice to study the affect on mitochondrial function of a variant in a mitochondrially encoded gene that retards the development of type 1 diabetes. The researchers transferred the "*a*" variant of the NADH dehydrogenase subunit 2 gene (*mt-Nd2*^{a}), carried by mitochondria from ALR/LtJ (003070) mice, to the NOD/ShiLtJ (001976) genetic background by creating the NOD/ShiLtJ-mt$^{ALR/LtJ}$/Mx conplastic mouse. They also created the reciprocal ALR/LtJ-mt$^{NOD/ShiLtDvs}$/Mx conplastic, which places mitochondria with the common *mt-Nd2*^{c} variant on the ALR background. Gusdon *et al*. (2007) demonstrated that mitochondria carrying the *mt-Nd2*^{a} variant produced a lower level of free radicals *in vitro* than mitochondria with the *mt-Nd2*^{c} variant, whether the nuclear genome was ALR- or NOD-type. This work demonstrates that conplastic strains with ALR/LtJ mitochondria may be valuable as a means of determining if reduction of free radical generation can moderate other diseases such as atherosclerosis and hypertension, as well as type 1 diabetes.

3.D. Recombinant strain panels

3.D.1. Overview

Three of the categories of laboratory mice are associated with strain panels that are developed from outcrosses of inbred strains: recombinant inbred (RI); recombinant congenic (RC); and chromosome substitution (CS; also called consomic) strains, including genome tagged strains. These strain panels generally are based on the outcrossing of two founder strains to create an F1 generation, followed by intercrossing or backcrossing or a combination of both to create a set of new inbred strains. The mice of each inbred strain panel contain portions the genetic makeup of the founder strains recombined in multiple, unique—and very well-defined—configurations.

RI, RC and CS inbred strain panels extend the advantages of standard inbred strains:

- Because the *founders* are well-characterized inbred strains, investigators can take advantage of previous research and accumulated data to choose phenotypes that are differentially regulated in the founder strains, and, therefore, whose genetics can be studied using the panel.
- Because each of the multiple strains *within* a panel is inbred, investigators can take advantage of the powerful research capabilities afforded by inbred strains. In particular, researchers can draw on the accumulated information about phenotypes and genotypes for each of the strains in the panel.
  - Because extensive genetic data and maps exist for most recombinant strain sets, mapping a newly-characterized trait often requires no additional genotyping.
  - Because the genotype of a euthanized mouse is not lost, phenotypes that require terminal—but multiple—sampling can be studied. For example, longitudinal information on invasive measures can still be generated for a given genotype.
  - Because data acquired for a recombinant strain set are cumulative, researchers can compare their results for one phenotype with information for other phenotypes from the same set of strains to identify phenotypes that are genetically correlated—without mapping.
- For mapping studies, specific recombinant strains identified as critical for initial mapping can be crossed for fine mapping. This approach requires less phenotyping and genotyping than more traditional fine mapping strategies.
- Quantitative trait loci (QTLs) that are mapped using recombinant strain sets can be quickly verified and refined by generating sets of either intercross or backcross lines between the strains with recombinations in critical QTLs.
- Data obtained from several recombinant strain sets can be compared or combined, particularly if the sets have a founder strain in common or if they segregate for alleles of common origin in the genomic region(s) of interest.
- A recombinant panel can be used to map a quantitative trait that has a great deal of non-genetic variance. Because many "copies" of each genotype are available, a precise mean value of the phenotype for a given genotype can be determined by evaluating multiple individuals for each recombinant strain of the panel. The same principle applies for phenotypes with incomplete penetrance.

Table 3.12 provides an overview and comparison of RI, RC, and CS panels. Detail on each panel type follows the table.

Table 3.12. Comparison of recombinant inbred (RI), recombinant congenic (RC), and chromosome substitution (CS) panels.

Panel type	Development scheme	Specific uses and limitations
Recombinant inbred (RI): Set of inbred strains, in which about 50% of the genome of each RI strain is from each founder strain in unique combinations.	• Outcross 2 inbred strains to create F1s. • Intercross F1s to create F2s. • Intercross sibling pairs from F2 generation into multiple inbred lines. • Continue intercrossing to F20.	Uses: • Determining whether one or multiple genes regulate a phenotype. • Mapping for monogenic traits without genotyping. • Easy identification of genetic correlations by comparison of the strain distribution of a new phenotype with the published strain distributions of other phenotypes. • Mapping a phenotype with large non-genetic variation or incomplete penetrance. Limitations: • Less useful for complex traits because allele combinations that are needed for expression of the trait are broken up within each RI line.
Recombinant congenic (RC): Set of inbred strains in which a small amount—25% or less—of the genome of each RC strain is from a donor strain. For each RC strain, this amount is randomly drawn from the donor genome.	• Outcross 2 inbred strains to create F1s. • Backcross N2 and N3 generations. • Intercross N3 sibling pairs for 14 more generations (F1–F14) into multiple inbred lines.	Uses: • Mapping for monogenic and complex traits. • Constructing a single-locus congenic strain (from a single RC strain). Limitation: • Few RC strain panels exist.
Chromosome substitution (CS): Set of inbred strains in which individual chromosomes have been replaced by homologous chromosomes from the donor strain.	• Outcross 2 inbred strains to create F1s. • Starting with F1s, backcross progeny for 9 additional generations. With each backcross, use marker-assisted selection for a single, complete donor chromosome. • After N10, intercross until all markers for the target chromosome are donor-type.	Uses: • Rapid mapping of phenotype to a chromosome. • Analysis of complex traits (multiple QTLs can be identified). • Fine mapping of QTL using one CS strain as a parent and a founder strain as the other parent. • Identification of modifier genes. Limitations: • Initially, a phenotype is mapped to an entire chromosome. • Initially, cannot discriminate between single and multiple QTLs when they are on one chromosome. • Few CS strain panels exist.

3.D.1.a. Considerations

Considerations for the use of recombinant strain panels include the following:

- Phenotypic variance for a complex trait may emerge among strains of a recombinant panel, even when the founder strains do not express the variance, because unique combinations of alleles can produce epistatic effects within one or more of the recombinant strains. This epistatic variance then can be analyzed by performing crosses involving the individual affected strains and the founder strains.

  Coat color provides a very clear example of how this epistatic variance can affect phenotypic expression. When RI lines are constructed, coat color alleles that differ between the founder strains at multiple loci, and which may or may not be expressed, assort randomly. Thus, coat colors of mice from different strains within a panel may vary, and some may even differ from either founder strain.
- During propagation of recombinant lines, mutations may occur spontaneously and be bred by chance to homozygosity, as with any inbred strain. This possibility may confound genetic analyses because the mutation is not shared by other strains of the panel or by either of the founder strains. As a preventive measure, The Jackson Laboratory preserves many RI lines as cryopreserved embryos.
- Initial characterization of a phenotype across inbred strains of a panel involves the assessment of numerous strains, ideally at the same age and at the same time. It is usually impossible to use such a balanced design, however, because acquisition and breeding of the strains is difficult to completely synchronize. The best alternative is to include mice of a readily available control strain (usually a parental strain of the panel) every time any mice of the panel are tested. Thus, the degree of uncontrolled variation in the phenotype over time can be evaluated and statistically controlled. This strategy increases the number of control mice that must be evaluated, but minimizes misleading results.

3.D.1.b. Comparison to F2 hybrids

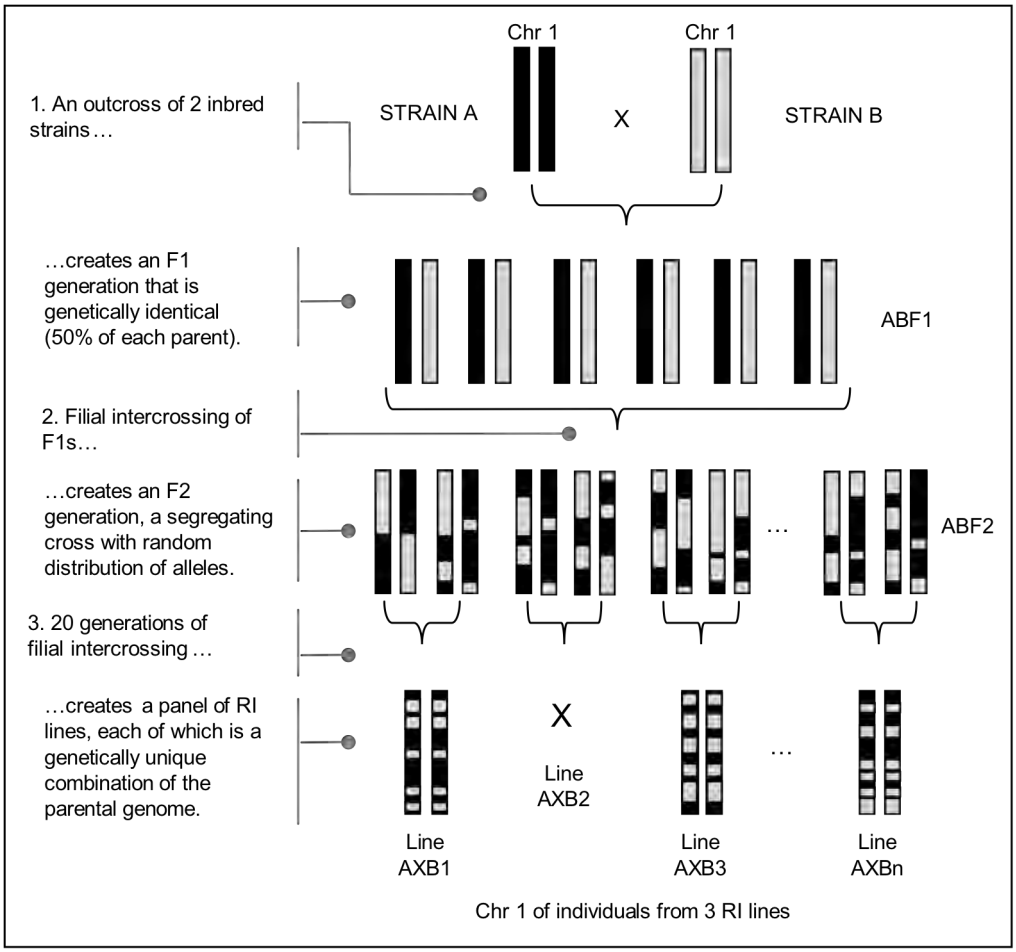
Mice from an F2 generation also provide genetic diversity and genome reorganization. Therefore, sometimes their use is compared with that of RI, RC or CS strain panels. But the two categories have very different characteristics: Mice of each F2 generation must be created from an F1 generation, which must be created from two inbred strains. The genome of each F2 hybrid is unique and is not reproducible; it must be evaluated for each F2 mouse. In contrast, the genomes of individual mice within any inbred strain of a panel are identical by definition and perpetually renewable. The genome of each strain need be determined only once.

3.D.2. Recombinant inbred (RI) strain panels

3.D.2.a. Definition, characteristics and value

A recombinant inbred (RI) strain panel comprises a set of inbred strains—RI lines—each of which contains genetic contributions from two different inbred founder strains in a unique, random distribution throughout the genome. The genome of each line comprises 50% of the genome of each founder strain, but in a unique combination (Bailey, 1971; Taylor, 1978). Figure 3.8 provides an overview of creation of an RI strain panel and an example of the genetic variance that can occur on Chr 1.

Figure 3.8. Creation of recombinant inbred (RI) lines; example of effect on Chr 1.



Due to inbreeding depression, not all strains survive. Thus, some line numbers are missing in the final panel. For example, in the illustration, line AXB2 did not survive.

The original purpose of the RI strain panel was as a mapping tool to enable rapid mapping of a phenotype at a time when this process was very difficult. Researchers created RI panels from founder strains with phenotypic differences that were already mapped (C57BL/6J [000664] and DBA/2J [000671], for example), which provided a scaffold of genetic markers with known map locations and associated strain distribution patterns. They could then evaluate any new phenotype in the RI lines and compare its strain distribution to those of known markers. Strain distributions that correlated between a new and a known marker would designate the map location of a gene that regulated the phenotype. Thus, if a trait differs between two inbred strains for which an RI panel exists, it may be possible to map a gene regulating that trait without additional genotyping—simply by phenotyping the lines in the panel. Furthermore, if enough strains are available, an RI panel can have about four times the resolution of an F2 cross (because of recombinations that occurred when the RI line was created).

Today, researchers also use RI lines to test hypotheses of causality between phenotypes. If two phenotypes (for example, obesity and mammary tumor incidence) appear in Strain A, but not in Strain B, an investigator might propose that the two phenotypes are causally related—that obesity promotes mammary tumor formation. They could test this hypothesis by evaluating an RI panel for the two phenotypes. If the strain distributions of the two phenotypes differ, they would conclude that the phenotypes are not directly related and that their association in the founder strains may be coincidental. If the strain distributions coincide in a sufficient number of strains to provide statistical significance, researchers would conclude that the phenotypes are genetically linked. Although this does not *prove* that the phenotypes are regulated by the same gene, it does provide a basis for *proposing* that they are. Three possibilities exist: one phenotype may be directly caused by the other; both phenotypes may be regulated by the same gene; or the phenotypes may be regulated by closely linked genes. Additional studies, such as fine mapping, targeted mutagenesis, or phenotypic modification, are necessary to distinguish among these possibilities.

As with any genetic mapping resource, the greater the number of different samples (in this case, the number of RI strains in a set) that are used to analyze a trait, the more precisely the trait can be mapped. Thus, large sets of RI strains offer obvious advantages over small ones. It is generally observed that, when mapping a single locus trait, at least 13 RI strains within a panel must be evaluated to obtain statistical significance.

Variations:

Sometimes, RI panels are created from crosses other than F1 hybrids of two inbred parental strains. Examples:

- Advanced intercross lines, generated from multiple generations of intercrosses before inbreeding, which breaks the genome into smaller segments than in a traditional RI strain and which allows for more precise mapping.
- Multi-strain crosses (for example, an 8-way cross), which provide much greater genetic diversity but require more lines in the mapping panel.

3.D.2.b. Maintenance breeding strategies

RI lines are maintained by sibling incrossing, as other inbred strains are. Due to genetic variation and inbreeding depression, it is not unusual for reproductive performance to vary from line to line within an RI panel. Some lines may reproduce very poorly.

3.D.2.c. Nomenclature

Nomenclature for RI strains includes abbreviations for the two founder strains, separated by an X, the number of the RI line, and other information. The abbreviation of the female strain used in the initial cross is listed first. All members of an RI set are serially numbered, regardless of how many laboratories produced them. Many abbreviations used for RI strains differ from those used for hybrid strains. For clarification, refer to strain information from the supplier. Table 3.13 provides several examples of RI strain names and their interpretation.

Table 3.13. Examples of nomenclature for recombinant inbred (RI) strains.

Name	Definition	Convention
BXD1/TyJ (000036) BXD2/TyJ (000075) BXD5/TyJ (000037)	The first (1), second (2) and fifth (5) lines in an RI panel derived from a cross between a C57BL/6J (B) female and a DBA/2J (D) male; developed by B. A. Taylor (Ty); maintained at The Jackson Laboratory (J).	Abbreviation for strain of female founder, "X," abbreviation for strain of male founder, line number in the panel, forward slash (/), and ILAR code(s) (http://dels.nas.edu/ilar_n/ilarhome/search_lc.php) ***Note***: Not all lines survive inbreeding; thus, gaps in the sequence of line numbers sometimes appear.
CX8B/EiJ (001568) CX8D/EiJ (001569)	The (B) and (D) lines in an RI panel created by crossing a Balb/cWt (C) female with a C58/J (8) male; developed by E. Eicher (Ei); maintained at The Jackson Laboratory (J).	As above with grandfathered exception: line designated by alphabetic letter.

3.D.2.d. Research examples

An RI panel used to study the genetics of angiogenesis.

Rogers *et al*. (2004) used composite and multiple interval mapping in a BXD RI panel (based on a C57BL/6J [000664] and DBA/2J [000671] cross) to identify novel QTLs on Chrs 4, 13, 5, and 18 that regulate the induction of angiogenesis by the vascular endothelial growth factor (VEGF). Polymorphisms in the associated genes may influence individual susceptibility to angiogenesis-related diseases such as cancer, macular degeneration, atherosclerosis, and arthritis.

An RI panel used to discover loci that control transcript expression of acute phase proteins.

Vazquez-Chona *et al*. (2005) used mice from two panels of RI strains based on C57BL/6J and DBA/2J inbred strains. One RI panel was developed at The Jackson Laboratory, the other by Peirce *et al*. (2004). Vazquez-Chona and colleagues found three loci that control transcript expression of acute phase proteins in the brains of these mice. The genotype at one locus, on Chr 12, was highly correlated with the expression of classic acute phase genes. Within this locus they identified the inhibitor of DNA binding 1 (*Id2*) as a candidate upstream regulator.

The Collaborative Cross—an RI panel used to map complex traits across inbred lines.

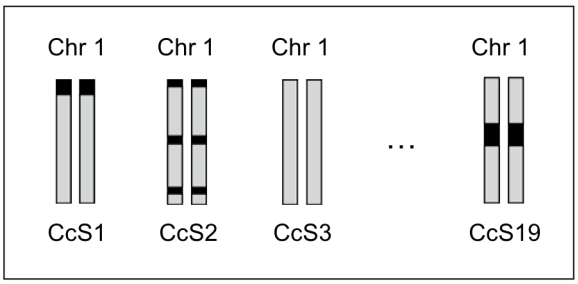
The Collaborative Cross, designed specifically for complex trait analysis, is an RI panel developed by crossing eight, genetically diverse, inbred strains of JAX® Mice (Churchill *et al*., 2004). About 1,000 strains will be produced to complete the project; 650 lines are presently in production (Chesler *et al*., 2008). The project is under the supervision of The Complex Trait Consortium, an international group of scientists that includes several from The Jackson Laboratory. With a defined, stable, and reproducible population of about 135,000 recombinations, mapping resolution will be high enough to dissect virtually any complex trait and characterize its epistatic and gene-environment interactions. By providing a large, common set of genetically defined mice, the Collaborative Cross will become a focal point for cumulative and integrated data collection on diverse phenotypes, facilitating a systems approach to mammalian genetic analysis. The availability of mice from a fully genotyped panel is expected to greatly reduce the barrier to entry for new studies, particularly for nongeneticists. As another component of the project, F1 (RIX) progeny, as a group, will be a source of virtually unlimited, yet reproducible, combinatorial diversity that will represent the genetic structure of human populations far better than any other conventional mouse crosses.

3.D.3. Recombinant congenic (RC) strain panels

3.D.3.a. Definitions, characteristics and value

Although RI panels are very useful for studying monogenic traits, the most common human diseases are polygenic—requiring a specific allelic configuration of multiple genes. To apply the advantages of inbred strains to the study of polygenic traits, researchers developed recombinant congenic (RC) strain panels (Demant and Hart, 1986). An RC strain panel comprises a set of inbred strains, each of which contains small segments of an inbred donor genome transferred to an inbred recipient genome. The creation of an RC strain panel is very similar to that of an RI panel, with one major difference: following the initial outcrossing of the donor and recipient strains, lines of the RC strain panel are backcrossed, typically for two generations**,** before intercrossing (Demant and Hart, 1986). In an RC panel of 18 strains created using two backcross generations, about 90% of the donor genome is represented, but in each individual strain, only about 12.5% of the donor genome, on the average, is represented. As a result, an allele that participates in determining a complex trait can be separated from the other alleles influencing that trait, and its effect can be studied in "isolation." In principle, the RC panel transforms a multigenic trait into a series of single gene traits. Figure 3.9 provides an example of the genetic variation among RC strains.

Figure 3.9. Chr 1 in four lines of the CcS RC strain panel.



The CcS RC panel was constructed from the BALB/cHeA (recipient) and STS/A (donor) inbred strains. Adapted from Groot *et al.* (1992).

An important consideration in using RC strains is that, in comparison to RI strains, less of the donor genome is represented in each RC strain. Therefore, to obtain the same degree of coverage of the donor genome, a greater number of RC strains is necessary.

Variations:

RC panels also can be developed using alternative strategies:

- The number of backcrosses can vary. Increasing the number of backcrosses decreases the proportion of the donor genotype in each strain. Note: Because backcrossing increases homozygosity twice as fast as does sibling intercrossing, an RC strain is considered inbred when the sum of the number of intercross (F) generations plus twice the number of backcross (N) generations (including the initial cross) is at least 20.
- During development of an RC strain panel for a particular complex trait, selection strategies are used to identify breeders. As with congenic strain development, selection can be based either on phenotype or genotype. But unlike congenic strain development, for which selection is made on only the *donor* genotype or phenotype, selection for RC strains can involve phenotypes or genotypes of the recipient as well as the donor. When selecting on phenotype, because the trait is complex, multiple loci will be involved in producing the trait. These loci will be isolated (along with some irrelevant loci) in the RC strains. Further mapping studies and crosses of specific RC strains can then be used to identify the genetic interactions that regulate the phenotype. When selecting on genotype, loci regulating the trait are identified in a preliminary cross. These loci are selected in various combinations during backcrossing to produce the RC strain panel. Phenotypic variation among the individual RC strains can then be related to a particular combination of loci within that strain.

Standard inbred strains can be considered a large RC panel.

Recently, some in the scientific community have started to think of the classical inbred strains as a set of recombinant congenics that "developed" with a majority of genetic material (~85–95%) from *M. domesticus* and a small amount (~5–15%) from *M. musculus*. This concept permits the potential to apply recombinant congenic analysis for mapping to any phenotype that has been characterized for a large number of inbred strains (Yang *et al.*, 2007).

3.D.3.b. Maintenance breeding strategies

RC strains are maintained by sibling incrossing, as other inbred strains are. Reproductive performance may vary among strains within a set.

3.D.3.c. Nomenclature

Nomenclature for RC strains includes the two founder strains and the line number. The strain of the female is listed first. Table 3.14 provides several examples of RC strain names and their interpretation.

Table 3.14. Examples of nomenclature for recombinant congenic strains.

Name	Definition	Convention
NONcNZO5/LtJ (004455) NONcNZO10/LtJ (004456)	The fifth (5) and tenth (10) lines of an RC panel derived from a cross between a NON/ShiLtJ (NON) female and a NZO/HiLtJ (NZO) male; developed by E. Leiter (Lt); maintained at The Jackson Laboratory (J).	Abbreviation for strain of female founder, "c," abbreviation for strain of male founder, line number in the panel, forward slash (/), and additional information, including ILAR code(s) (http://dels.nas.edu/ilar_n/ilarhome/search_lc.php). ***Note***: Not all lines survive inbreeding; thus, gaps in the sequence of line numbers may appear.
CBcNO7A/LtJ (003052)	The (A) subline of the seventh (7) line of an RC panel derived from a cross between a CBA/JLsLt (CB) female and a NOD/Shi (NO) male; developed by E. Leiter (Lt); maintained at The Jackson Laboratory (J).	As above, with uppercase alphabetic subline indicator (A).

3.D.3.d. Research examples

Use of an RC panel to characterize the polygenic regulation of induced-colon cancer.

The genetic regulation of 1,2-dimethylhydrazine (DMH)-induced colon tumors was analyzed using an RC panel between BALB/cHeA (resistant) and STS/A (susceptible). An RC panel was used because the trait is polygenic. The CcS/Dem RC strains differed widely in the development of DMH-induced colon tumors, indicating that a limited number of genes with a major effect are responsible for the high susceptibility of the STS/A strain. The data for the number of tumors and the size of the tumors had different strain distribution patterns, indicating that these sub-phenotypes were regulated by different sets of genes (Moen *et al.,* 1991). Further studies of the susceptible strains identified four loci (susceptibility to colon cancer loci: *Scc-1*, *-2*, *-3*, *-4*) that mapped to four different chromosomes (Groot *et al.*, 1992).

Use of an RC panel to characterize control of a trait by genes with low penetrance.

The genetic regulation of N-ethyl-N-nitrosourea-induced lung cancer was studied in an RC panel derived from B10.O20 (resistant) and O20 (susceptible) inbred strains. From F2 crosses of five OcB strains with O20 mice, 730 F2 hybrids were typed and scored for tumor number and size. Thirty lung cancer susceptibility (*Sluc*) loci (14 of which were previously reported) and 25 two-way interactions between loci were identified. Thus, advanced crosses of specific RC lines with one of the parental strains elucidated the genetic complexity of lung cancer and identified many new loci and interactions among loci (Tripodis *et al.,* 2001).

Use of an RC panel to study the interaction of genetic elements that regulate a complex trait.
To study genetic interactions affecting type 2 diabetes (T2D), a series of 10 RC strains was constructed between NZO/HlLt mice, which develop a polygenic T2D, and NON/Lt mice, which express genetically determined risk factors for T2D, but do not develop frank diabetes. The strains were constructed on the NON/Lt background by selecting for combinations of NON and NZO loci that were known QTLs for obesity and diabetes. All ten strains gained significantly more weight than the NON/Lt founder strain, but none were as obese as the NZO/HlLt founder strain. Strain-specific T2D incidence ranged from 0–100% and was influenced by the number of specific diabetogenic QTL. Some obese strains did not develop T2D, demonstrating that obesity alone does not cause T2D; other conditions are necessary. In contrast, the NONcNZO10/LtJ (004456) strain manifested a 100% incidence of T2D without the extreme obesity of the NZO founder strain. Thus, this RC panel captures alleles that interact with obesity to either enhance—or diminish—risk for T2D, and models the polygenic nature of T2D in humans (Reifsnyder and Leiter, 2002).

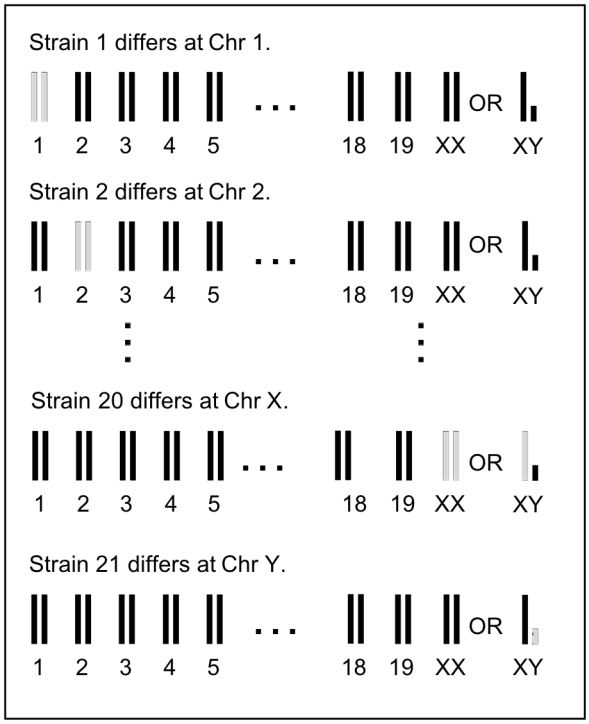
3.D.4. Chromosome substitution (CS) strain panels and genome tagged mice

3.D.4.a. Definitions, characteristics and value

3.D.4.a.1. Chromosome substitution (CS) strains

A CS strain (sometimes called a consomic strain) is an inbred strain in which one of its chromosomes is replaced by the homologous chromosome of another inbred strain via a series of marker-assisted backcrosses (Nadeau *et al.,* 2000; Table 3.12). In principle, a complete CS panel consists of 22 strains, each with the same background genotype, but each having one of 22 replacement chromosomes (Chr 1–19, Chr X, Chr Y, or the mitochondrial chromosome) from a single donor genotype (Figure 3.10). Occasionally, replacement of a specific chromosome results in infertility; therefore, multiple strains, each with a portion of the donor chromosome, are produced. Sometimes investigators develop a single CS strain to study one donor chromosome on a recipient background.

Figure 3.10. Chromosomal differences among strains of a CS strain panel.



Each strain in the CS strain panel differs from the recipient strain by one chromosome only. The 22-strain panel includes replacements of all 22 chromosomes, one per strain. In the figure, black represents the recipient; gray represents the replacement from the donor. Not represented in the figure is the conplastic CS strain, which differs only at the mitochondrial chromosome.

When constructing a CS strain, progeny must be genotyped at every backcross generation to ensure that recombination has not occurred between the donor chromosome and its recipient counterpart. Traditionally, Chr Y has been transferred onto a new strain without genotyping because recombination of Chr Y is rare. After 10 backcross generations, a CS line will be homozygous and recipient-type for 99.8% of the differences between the founders—excluding the substituted chromosome.

Analysis of a CS strain panel allows researchers to rapidly associate regulation of a phenotype with a particular chromosome or chromosomes. The term used to identify the mapped trait is quantitative trait chromosome (QTC). A QTC may carry more than one locus that affects a trait.

Other considerations when using a CS strain panel include the following:

- CS strain panels are useful for analysis of Mendelian (monogenic) traits as well as complex (polygenic) traits—multiple QTCs can be identified in a single screen. In contrast, RI panels can be used for *direct* mapping of monogenic traits only (Nadeau *et al.,* 2000).
- CS panels facilitate fine mapping. Once a QTC is identified, mice from the appropriate CS strain can be backcrossed to the host strain, and progeny with recombinations in the donor chromosome can be identified. Using this method, the location of a QTL can be resolved (fine-mapped) relatively easily compared to mapping with F2 hybrids.
- CS strain panels can be used to identify modifier genes that affect the phenotypic expression of a mutation by crossing mutant mice to mice of different strains of the CS panel. Differences in phenotypic expression among the different F1 hybrid progeny would identify the location of a modifying locus.
- Because genetic background noise inherent to genotyping crosses is minimized in CS strain panels, and because non-genetic variance can be minimized by phenotyping multiple mice from each strain, efficient confirmation of weak QTCs is possible, and tests for dominance or additive modes of inheritance are efficient.
- CS strain panels are particularly sensitive to errors that are introduced by unbalanced designs, i.e., by an uneven distribution of mice from each strain among different sampling times. For example, if a majority of the control mice (mice of the parental strain) were evaluated for the phenotype in the first month of the study, and a majority of mice from some of the CS strains evaluated in the fourth month, effects of evaluation-time differences will be confounded with the genetic differences between the control and CS strains. Sampling-time variance can result from seasonal effects, unrecognized variations in the phenotyping tools or procedure, and random differences in environmental variables. Both false positive and false negative results will occur. Studies using a CS panel are especially prone to this problem because of the difficulty in coordinating breeding among the 20–25 strains of the panel. The best solution is to include a sufficient number of control mice at each evaluation time to provide an appropriately-sized group for statistical comparison. If this strategy is prohibitive, the distribution of control mice among sampling times should be as balanced as possible. If these conditions cannot be met, results of a CS panel survey in which different mice were evaluated at different times must be considered preliminary until they can be confirmed by direct comparison of the control group with the affected CS strain(s).
- Mice of a CS strain that exhibit the trait can be used to produce, within a few generations, a series of congenic strains that subdivide the chromosome into segments and thus refine the position of the causative locus. This is achieved by backcrossing to mice of the recipient strain, identifying recombinant progeny in an N2 generation, and determining which regions of the chromosome are associated with the variant phenotype.
- The CS strain panel (C57BL/6J Chr #$^{PWD/Ph}$/ForeJ), developed from a wild-derived founder (PWD/Ph) and a standard laboratory strain (C57BL/6J [000664]) provides greater genotypic variance—and potentially greater phenotypic variance—than other recombinant panels.

3.D.4.a.2. Genome tagged mice

Genome tagged mice are similar to CS strains, except that a portion of a chromosome—rather than an entire chromosome—from a donor strain is maintained on a standard recipient background. Genome tagged mice are created to provide greater precision with regard to the locus of interest. However, a greater number of strains are required for a complete panel. A set of genome-tagged mice for a given chromosome can easily be produced from a single CS strain.

3.D.4.b. Maintenance breeding strategies

CS strains and genome-tagged mice are maintained with sibling intercrossing, as with other inbred strains.

3.D.4.c. Nomenclature

Nomenclature for CS strains includes the name of the recipient strain and the number and donor of the transferred chromosome. (The superscripted donor name is distinguishable from an allele symbol because it is capitalized and non-italic.) Table 3.15 provides several examples of consomic strain names and their interpretation.

Table 3.15. Examples of nomenclature for consomic strains.

Name	Definition	Convention
C57BL/6J-Chr $1^{A/J}$/NaJ (004379) C57BL/6J-Chr $X^{A/J}$/NaJ (004398)	Two strains of a CS strain panel, in which Chr 1 and Chr X from strain A/J (Chr $1^{A/J}$, Chr $X^{A/J}$) were transferred to C57BL/6J by J. Nadau (Na); maintained at The Jackson Laboratory (J).	Recipient strain, hyphen (-), chromosome number, donor strain (superscripted), forward slash (/), ILAR code(s) (http://dels.nas.edu/ilar_n/ilarhome/search_lc.php).
BALB/cByJ-Chr $Y^{C57BL/6By}$/J (001452)	CS strain, where Chr Y from C57BL/6By was transferred to BALB/cByJ; maintained at The Jackson Laboratory (J).	As above.

3.D.4.d. Research examples

A CS panel used for the discovery of a QTL for pubertal timing.

The genetic regulation of pubertal timing in mice is poorly understood, at least partly because relatively large non-genetic effects amplify variance. Krewson *et al*. (2004) used a C57BL/6J-Chr $\#^{A/J}$/NaJ CS strain (CSS) panel to identify QTCs for the more rapid pubertal development in A/J (A; 000646) compared to C57BL/6J (B6; 000664) mice. In an initial survey, a total of 4–20 females per CS strain, from at least two litters per strain, were evaluated for the timing of vaginal opening (VO). CSSs for Chr 6 and Chr 13 each displayed an earlier time of VO than B6 mice. From these initial studies, appropriate sample sizes for accurate estimates of the timing of VO were determined. Additional studies then confirmed these two differences. F1 mice (B6 X CSS) for Chr 6 and Chr 13 displayed phenotypes that were intermediate between the CSS and B6 strains, indicating that the trait was inherited in a codominant manner. Nathan *et al*. (2006) studied the C57BL/6J-Chr $6^{A/J}$/NaJ (B6-6^{A}; 004384) strain in greater detail. Linkage analysis of N2 mice from a B6 X B6-6^{A} cross identified a QTL on the distal end of Chr 6 that regulates VO in mice. The QTL was confirmed and its location refined by generating and phenotyping a panel of 12 congenic strains from a B6 X B6-6^{A} cross. Additional analysis of the QTL demonstrated that the effects of the responsible gene(s) are gender specific and without parent-of-origin effects. These results demonstrate that the genetic regulation of a phenotype with large non-genetic variance can be studied reliably using mice of a CSS panel.

Genome tagged mice used to explore the genetics of behavioral variation.

Gale *et al*. (2008) studied a panel of genome-tagged mice that consisted of more than 60 congenic strains, each carrying a different, small (20–30 cM) segment of the DBA/2J (000671) genotype on the C57BL/6J (000664) background. The panel was designed to cover almost the entire DBA/2J genotype. A total of 97 loci were mapped for a variety of complex behavioral traits including hyperactivity, anxiety, avoidance, and conditional fear. This study mapped a much larger number of loci, many to a greater precision, than generally is possible with standard mapping crosses. In addition, more than half the strains with significant QTLs exhibited phenotypes that differed from either of the parental strains. These results indicate that, in traditional mapping crosses such as an F2 cross, epistasis can interfere with detection of loci for complex traits because its effects differ for each individual mouse, increasing the variance and decreasing the sensitivity for detection of loci that influence complex traits. In contrast, as with any inbred strain, epistatic effects are constant within a strain of genome tagged mice.

3.E. Mice with chromosomal aberrations

Chromosomal aberrations are rearrangements of the normal chromosomal structure. They occur spontaneously or can be induced, and can generally be observed cytologically. Most chromosomal aberrations carried on laboratory mice were discovered in wild mice.

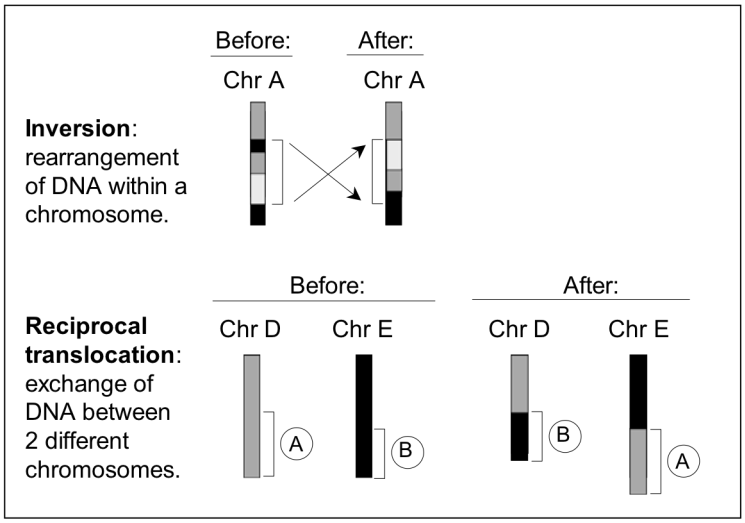
3.E.1. Definitions, characteristics and value

Six types of chromosomal aberrations are preserved in inbred strains:

- Inversions: rearrangements of DNA segments within chromosomes, where the segment is reversed end to end.
- Insertions: the insertion of a fragment from one chromosome into another.
- Robertsonians: two *different* chromosomes joined at their centromeres to produce a bi-armed chromosome.
- Reciprocal translocations: exchanges of DNA segments between two *different* chromosomes. Generally reciprocal translocations produce either semi-sterility or complete male sterility.
- Trisomies: deviations from the normal diploid number of chromosomes in which triplicate copies of one chromosome or a portion of that chromosome exists in the cell.
- Chr Y aberrations.

Figure 3.11 provides simple examples of two types of chromosomal aberrations.

Figure 3.11. Schematic: inversion and reciprocal translocation.



Chromosomal rearrangements have been used as dominant markers for linkage studies and for marking tissues in experiments involving chimeras or transplantations. Today, many strains with chromosomal aberrations are used in fluorescence *in situ* hybridization (FISH) gene mapping studies and in meiotic nondisjunction studies. Chromosomal aberrations are also used to study the relationship of chromosomal structure or gene location to gene function. And, some chromosomal aberrations in the mouse are useful for modeling the effect of chromosomal aberrations on fertility.

Mice with chromosomal aberrations are also used to create other research models. Robertsonian chromosomes in combination can be used to produce whole chromosome trisomies for specific mouse chromosomes. An example is the mouse used to study Down syndrome, B6EiC3Sn *a/A*-Ts(17^{16})65Dn (001924).

Often, chromosomal aberrations are transferred to a standard inbred background for study just as other mutations are. Sometimes the aberrations are carried and studied on an undefined stock. To produce mice trisomic for a specific chromosome, a combination of different strains is required.

3.E.2. Maintenance breeding strategies

Some strains that carry a chromosomal aberration can be maintained either as homozygotes or heterozygotes that segregate for the aberrant chromosome. Several have fertility and sterility problems; these strains require special breeding schemes and handling. For details on specific strains of JAX® Mice with chromosomal aberrations, refer to the strain datasheet (www.jax.org/jaxmice/query).

3.E.3. Controls

Appropriate controls for segregating aberrations are wild-type littermates; controls for homozygous aberrations maintained on standard inbred backgrounds are mice of the specific inbred strain background. Because of their origin from wild mice, Robertsonian chromosomes maintained homozygously, and not on a standard inbred background, do not have a genetically similar control.

3.E.4. Nomenclature

Nomenclature for mice with chromosomal aberrations includes the type of aberration and chromosome(s) involved, an aberration series number, and the researcher or laboratory that discovered or produced the aberration. Table 3.16 provides several examples of nomenclature of mice with chromosomal aberrations. Table 3.17 provides the aberration codes used in nomenclature for strains of JAX® Mice. For information on JAX® Mice with chromosomal aberrations, visit www.jax.org/jaxmice/type/chromosomal_abberati. For a complete list of aberration codes, refer to the full nomenclature rules at www.informatics.jax.org/mgihome/nomen/anomalies.shtml.

Table 3.16. Examples of nomenclature for strains with chromosomal aberrations.

Name	Definition	Convention
STOCK In(5)30Rk/J (000852)	An undefined stock (STOCK) carrying an inversion (In) in Chr 5 (5); the 30th inversion found by T. Roderick (Rk); maintained at The Jackson Laboratory (J).	Background strain or stock, abbreviation of aberration (see Table 3.17 for code translation), affected chromosome(s), series number of the aberration, ILAR code(s)* of discoverer or developer, forward slash (/), and ILAR code(s). (http://dels.nas.edu/ilar_n/ilarhome/search_lc.php.)
STOCK Rb(6.16)24Lub (000885)	An undefined stock (STOCK) carrying the Robertsonian translocation (Rb), where Chr 6 and Chr 16 are joined (6.16); the 24th (24) translocation found at Medizinische Hochschule Lubeck (Lub).	As above.
B6EiC3Sn *a/A*-Ts(17^{16})65Dn (001924)	Mutant strain B6EiC3Sn, segregating at the agouti locus (*a/A*), carrying a trisomy (Ts) for the centromeric end of Chr 17 and the distal telomeric end of Chr 16 (17^{16}); the 65th (65) trisomy found by M. T. Davisson (Dn).	As above.
CBA/CaH-T(14;15)6Ca/J (000655)	Inbred strain CBA/CaH carrying a reciprocal translocation (T) for Chr 14 and Chr 15 (14;15); the 6th (6) translocation found by T. C. Carter (Ca); maintained at The Jackson Laboratory (J).	As above.

Table 3.17. Abbreviations of chromosomal aberrations used in strain names of JAX® Mice.

Abbreviation	and definition	Abbreviation	and definition
In	Inversion	T	Reciprocal translocation
Is	Insertion	Ts	Trisomy
Rb	Robertsonian chromosomes (translocations)	Y	Chr Y abnormality

3.E.5. Research examples

A trisomy model used to test Down syndrome drugs.
Researchers at a University of Colorado at Denver and Health Sciences Center (UCDHSC; Costa *et al.,* 2007) have successfully treated a mouse model of Down syndrome with an FDA-approved drug used to improve memory retention of patients with Alzheimer's disease. The treatment has the potential for treatment of children and adults with Down syndrome. The strain, B6EiC3Sn *a/A*-Ts(17^{16})65Dn (001924) was developed by Dr. Muriel Davisson at The Jackson Laboratory (Davisson *et al.,* 1993). This strain has a mixed trisomy for part of Chr 17 and part of Chr 16, which results in symptoms of Down syndrome.

An undetected inversion that interfered with mapping.
During construction of a strain congenic for a segment of Chr 6 (B6.C3-6T), Rosen *et al*. (2004) noted that recombination was suppressed in the central region of Chr 6, as suggested by lack of recombination in more than 600 N10F2 mice. This absence of recombination frustrated attempts to fine map a quantitative trait locus (QTL) for bone density on Chr 6. Akeson *et al*. (2006) subsequently demonstrated the existence of an inversion, In(6)1J, in C3H/HeJ (000659) mice that encompasses about 20% of Chr 6 from ~73 Mb to ~116 Mb. As a result, linkage crosses using C3H/HeJ mice will show no recombination in this region of Chr 6. Because this inversion will hold this segment of Chr 6 intact, it could be useful for mutagenesis or breeding studies (Akeson *et al*. 2006).

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Chapter 4: Characteristics of Popular Strains of JAX® Mice, Including Reproductive Performance

Currently, The Jackson Laboratory strain database contains complete phenotypic and genotypic information for more than 4,000 strains of JAX® Mice—what it is about each strain that makes it unique and valuable to researchers for specific uses. Our objective for this chapter is to provide a summary of information for the most commonly used strains. This information includes the official and common strain names, the JAX® Mice stock number, relevant genes and alleles, and the most common characteristics and uses.

Information for each strain also includes "technician notes" whenever available. Technician notes, which are most often anecdotal, are contributed by animal caretakers and technicians at The Jackson Laboratory. Technician notes are important for two reasons: First, they often provide helpful hints for handling the mice. Second, they represent visual observations of specific phenotypes that may be unusual—but perfectly normal—for a specific strain. This knowledge can often prevent misidentification of normal mice as abnormal or ill.

The chapter is organized as follows:

For additional details on strains of JAX® Mice, see the following:

- www.jax.org/jaxmice/query
  Full details on all strains of JAX® Mice, as well as links to other strain-related information.
- www.jax.org/phenome
  Experimental data on many commonly-used strains of JAX® Mice.
- www.informatics.jax.org/external/festing/search_form.cgi
  Festing's characteristics of inbred mice and rats.

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4.A. Strain characteristics

129P1/ReJ	**Stock No. 001137**
Common name:	n/a **Generation:** F155 (18-OCT-06)
Former name:	129/ReJ;
Strain type:	Inbred strain
Appearance:	Pink-eyed, light bellied chinchilla; related genotype: *A^{w}/A^{w} Oca2^{p} Tyr$^{c\text{-}ch}$/Oca2^{p} Tyr$^{c\text{-}ch}$*
H2 haplotype:	*b*
Genes/alleles:	*Cdh23^{ahl}*: cadherin 23 (otocadherin); age related hearing loss 1 common names: Cdh23^{753A}; mdfw; *Disc1^{del}*; disrupted in schizophrenia 1; deletion common name: Disc1^{129S6}; *Poli^{d}*; polymerase (DNA directed), iota; deficient common name: Poli^{-}
Strain origin:	Developed by Dunn (1928) from crosses of coat color stocks from English fanciers and a chinchilla stock from Castle. Common origin with strain 101. Dunn to WL and ES Russell (1945), to Hunt, Wynder, and Runner at JAX (1947). After the 1947 Bar Harbor fire, Hunt returned a chinchilla pink-eyed (*Oca2^{p} Tyr$^{c\text{-}ch}$/Oca2^{p} Tyr$^{c\text{-}ch}$*) substrain to JAX (1948) and Wynder returned an albino pink-eyed (*Oca2^{p} Tyr^{c}/ Oca2^{p} Tyr^{c}*) substrain (1948). The original coat color genotype was reconstituted by crossing females of the former and males from the latter. Offspring of this cross were used to establish the R1 (WL Russell), Re (ES Russell), and Rr (MN Runner) substrains.
Source:	This non-dystrophic substrain descended from the 129/Re-*Lama2^{dy}*/+ subline and was established from tested offspring of full-sib *Lama2^{dy}*/+ x *Lama2^{dy}*/+ mating. Russell to JAX (1969), to barrier facility (1978) by fostering on C57BL/6J at F71. For a complete history, see Simpson *et al*., (1997).
Characteristics and uses:	• Homozygous for *Cdh23^{ahl}*, age-related hearing loss 1 mutation; onset between 3–5 months of age. • Co-isogenic with 129P1/ReJ-*Lama2^{dy}*/J (000641). • 5% spontaneous testicular teratoma. • High frequency of venous congestion in adrenals and uteri in old females. • High frequency of urinary calculi (Russell, 1966). • Highly susceptible to Sendai virus (Parker *et al*., 1978). • Widely used in production of targeted mutations due to availability of embryonic stem (ES) cell lines. 129 substrain must be matched to the ES cell line. • Research applications: cancer, neurobiology, reproductive biology, sensorineural.
Technician notes:	• Very good parents; don't need anything special to breed well. • Large, docile mice.

129P3/J — Stock No. 000690

Common name: n/a **Generation:** F178 (03-JAN-08)

Former name: 129/J; changed 15-DEC-04

Strain type: Inbred strain, segregating inbred strain

Appearance: Pink-eyed, light-bellied, light chinchilla; related genotype, *A^{w}/A^{w} Oca2^{p} Tyr$^{c\text{-}ch}$/Oca2^{p} Tyr^{c}* Albino; related genotype: *A^{w}/A^{w} Oca2^{p} Tyr^{c}/ Oca2^{p} Tyr^{c}*

H2 haplotype: *bc* (see Fischer Lindahl K, 1997)

Genes/alleles: *Cdh23^{ahl}*: cadherin 23 (otocadherin); age related hearing loss 1
common names: Cdh23^{753A}, mdfw

Disc1^{del}: disrupted in schizophrenia 1; deletion
common name: Disc1^{129S6}

Poli^{d}: polymerase (DNA directed), iota; deficient
common name: Poli^{-}

Strain origin: LC Dunn, Columbia University, NY in 1928 from crosses of coat color stocks from English fanciers and a chinchilla (*Tyr$^{c\text{-}ch}$*) stock from WE Castle. This strain has a common origin with strain 101.

Source: To WL Russell at JAX, to Runner at JAX. Russell sent stock to Hunt (1947), and Runner sent stock to Wynder (1947). Hunt to JAX (1948), an *Oca2^{p} Tyr$^{c\text{-}ch}$/Oca2^{p} Tyr$^{c\text{-}ch}$* substrain; and Wynder to JAX (1948), an *Oca2^{p} Tyr^{c}/ Oca2^{p} Tyr^{c}* substrain. The original coat color genotypes were reconstituted by crossing these two substrains. Offspring of the cross were used (1948) to establish the Rr, Re, and RI substrains. The JAX substrain was separated from the Rr substrain (1951) at F5 after the cross. For a complete history, see Simpson *et al.,* (1997).

Characteristics and uses:

- Homozygous for *Cdh23^{ahl}*, age-related hearing loss 1 mutation; onset prior to 3 months of age.
- Low tumor frequency; not susceptible to MMTV.
- 5% frequency of spontaneous testicular teratomas.
- High frequency of venous congestion of adrenals and uterus.
- Highly sensitive to estrogen.
- Highly resistant to radiation.
- Useful for ovary transplant and ova transfer studies.
- Carries a Type 1A Chr Y of Asian *M.m. musculus* origin (Tucker *et al*., 1992).
- Suppurative conjunctivitis and ulcerative blepharitis is common; corynebacterium have been found on conjunctival examination. This rare condition has been found only in 129P3/J and BALB/c substrains (Sundberg *et al*., 1991).
- Maintained by matings of *Oca2^{p} Tyr$^{c\text{-}ch}$/ Oca2^{p} Tyr^{c}* x *Oca2^{p} Tyr^{c}/ Oca2^{p} Tyr^{c}* , which produce chinchilla and albino progeny.
- Widely used in production of targeted mutations due to availability of embryonic stem (ES) cell lines. 129 substrain must be matched to the ES cell line.
- Research applications: cancer, cardiovascular, neurobiology, reproductive biology, sensorineural.

Technician notes:

- Very nervous and jumpy; gentle mice.
- Poor breeders. Around ½ of first litter born dead or die shortly after. Many missing litters. But good sized litters.
- Loud noises cause them to chew or eat litters; often find babies with missing limbs.
- Reduce amount of shavings to avoid mice pushing them in a pile under the water bottle, possibly drowning themselves.
- Change cages weekly; this seems to avoid diarrhea that occurs when cages changed less often.

129S1/SvImJ — Stock No. 002448

Common name: n/a **Generation:** F67 (03-JAN-08)

Former name: 129/SvImJ
129S1/Sv-p^+ Tyr^+ $Kitl^+$
129S3/SvIm
129S3/SvImJ

Strain type: Inbred strain

Appearance: White-bellied agouti; related genotype: A^w/A^w

H2 haplotype: *b*

Genes/alleles: *Disc1*^{del}; disrupted in schizophrenia 1; deletion
common name: Disc1^{129S6}

Gnat2^{cpfl3}: guanine nucleotide binding protein, alpha transducing 2; cone photoreceptor function loss 3
common name: Gnat2

Development: 129S1/SvImJ was developed to serve as a control inbred strain for many of the steel-derived ES cell lines (e.g. W9.5 and CJ7). SSLP marker analysis indicates that 129S1/SvImJ is identical to 129S1/Sv $+^p$ $+^{Tyr\text{-}c}$ $Kitl^{Sl\text{-}J}$/+ except for the region surrounding the *Kitl* gene on Chr 10. 129S1/SvImJ was derived from 129S1/Sv-$+^p$ $+^{Tyr\text{-}c}$ $Kitl^{Sl\text{-}J}$/+ (000090). The steel-Jackson mutation ($Kitl^{Sl\text{-}J}$, formerly $Mgf^{Sl\text{-}J}$) is segregating in 129S1/Sv-$+^p$ $+^{Tyr\text{-}c}$ $Kitl^{Sl\text{-}J}$/+. $Kitl^{Sl\text{-}J}$ was removed in 1995, at F26, by selective breeding to produce 129/Sv-$+^p$ $+^{Tyr\text{-}c}$ $+^{Kitl\text{-}Sl\text{-}J}$ (002448; see Simpson *et al*., 1997). This name was later shortened to 129/SvImJ. Subsequently designated 129S3/SvImJ (Festing *et al*., 1999), this strain was renamed 129S1/SvImJ in February 2001 to emphasize its relationship to Stock No. 000090.

Characteristics and uses:
- Homozygous for *Gnat2*^{cpfl3}, cone photoreceptor function loss 3, which affects bright light (photopic) vision.
- In response to challenge, develop immune-mediated nephritis characterized by proteinuria, glomerulonephritis, and tubulointerstitial disease (Xie *et al*., 2004).
- Widely used in production of targeted mutations due to availability of embryonic stem (ES) cell lines. 129 substrain must be matched to the ES cell line.
- Research areas: cancer, neurobiology, reproductive biology.

Technician notes:
- Chew a lot of grain and leave it on the bottom of the cage; may have to add grain to the wean cages more often than normal.
- May build up shavings under water bottle, especially when building nests, resulting in wet/drowned mice. It may be necessary to remove some shavings.

129X1/SvJ — Stock No. 000691

Common name: n/a **Generation:** F122 (03-JAN-08)

Former name: 129X1 (changed: 06-JUN-07)
129/SvJ (changed: 19-APR-07)

Strain type: Inbred strain

Appearance: Pink-eyed, light-bellied, light chinchilla; related genotypes: *A^{w}/A^{w}*, *Oca2^{p} Tyr$^{c\text{-}ch}$/Oca2^{p} Tyr^{c}*
Albino; related genotypes: *A^{w}/A^{w}*, *Oca2^{p} Tyr^{c}/ Oca2^{p} Tyr^{c}*

H2 haplotype: *bc*

Genes/alleles: *Cdh23^{ahl}*: cadherin 23 (otocadherin); age related hearing loss 1
common names: Cdh23^{753A}, mdfw
Disc1^{del}; disrupted in schizophrenia 1; deletion
common name: Disc1^{129S6}
Poli^{d}; polymerase (DNA directed), iota; deficient
common name: Poli^{-}

Strain origin: As 129/ReJ. 129/J, 129/ReJ, and 129/SvJ have a common origin.

Source: Runner to JAX (1947) and subsequently distributed to other members of the JAX staff, including ES Russell, Murray, Hummel, and Dagg. Hummel to Stevens in approximately 1953.

Characteristics and uses:

- Homozygous for *Cdh23^{ahl}*, age-related hearing loss 1 mutation; onset prior to 3 months of age.
- Testicular teratomas >1% (Stevens, 1973).
- Non susceptible to MMTV.
- Highly sensitive to estrogen at all ages.
- Maintained by mating mice of different colors, *Oca2^{p} Tyr$^{c\text{-}ch}$/ Oca2^{p} Tyr^{c}* X *Oca2^{p} Tyr^{c}/ Oca2^{p} Tyr^{c}*, which produces both chinchilla and albino offspring.
- Widely used in production of targeted mutations due to availability of embryonic stem (ES) cell lines. 129 substrain must be matched to the ES cell line.
- Useful for ovary transplant and ova transfer studies.
- Research applications: cancer, neurobiology, reproductive biology, sensorineural.

Technician notes:

- Gentle mice.
- Very "crawly"; sometimes hang off cage cover.
- Sometimes chew through filter hoods.
- Poor breeders. First 2–3 litters may be born dead or eaten; then they start breeding.
- Use Nestlets® with all new litters.
- Mice take shavings and push under water bottle, causing wet boxes; decrease shavings to avoid.

A/HeJ — Stock No. 000645

Common name: AHe; A Heston — **Generation:** F274 (03-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Albino; related genotype: *a/a* *Tyrp1^{b}/Tyrp1^{b}* *Tyr^{c}/Tyr^{c}*

H2 haplotype: *a*

Genes/alleles: *Hc^{0}*: hemolytic complement; deficient
common names: C5-, C5-d, C5-def, C5-deficient, hc^{o}

Strain origin: Developed by LC Strong in 1921 from a cross between a Cold Spring Harbor albino and a Bagg albino; thus, it is related to BALB/c (Strong, 1936). Majority of sublines trace to stock that Bittner obtained from Strong (1929). Some of this stock was sent to Heston (1940).

Source: Heston to JAX (1948) at F77.

Characteristics and uses:

- Low frequency of mammary and lung tumors in virgin females, but high percentage of mammary adenocarcinomas (large proportion acinar type) develop in multiparous females.
- High frequency of renal disease in older animals.
- Primary cleft palate sometimes found in newborns; easily induced with a variety of agents, including cortisone.
- Lung tissue very susceptible to induction of tumors by methylcholanthrene and urethane (Malkinson *et al.,* 1985).
- Hyperactive airway response to various chemicals (Ewart *et al.,* 1994; Levitt *et al.,* 1995; Takahashi *et al.,* 1995).
- Carries a Type 1A Asian *M.m. musculus* Chr Y (Tucker *et al.,* 1992).
- Small percent (4%) of nonproductive males are hermaphrodites; additional 17% of nonproductive males have abnormally small testes containing no sperm.
- In The Jackson Laboratory substrains, observation of rare spontaneous myoepitheliomas arising from myoepithelial cells of various exocrine glands.
- Low inter- and intra-strain aggression (Carlier *et al.,* 1991).
- Research applications: cancer, developmental biology, reproductive biology, immunology and inflammation.

Technician notes:

- Pretty, white coat.
- Very quiet, docile; a little curious, but in a shy way.
- Very easy mice to work with.
- Very non-productive, but if they have 1 litter, they seem to continue to breed.
- Often have eye problems. Sometimes, one or both eyes appear closed, but a week later they open.
- Sometimes born with their eyes open, which can result in damage.

A/J	**Stock No. 000646**
Common name:	A **Generation:** F278 (03-JAN-08)
Former name:	n/a
Strain type:	Inbred strain
Appearance:	Albino; related genotype: *a/a Tyrp1^{b}/Tyrp1^{b} Tyr^{c}/Tyr^{c}*
H2 haplotype:	*a*
Genes/alleles:	*Cdh23^{ahl}*: cadherin 23 (otocadherin); age related hearing loss 1 common names: Cdh23^{753A}; mdfw *Dysf^{prmd}*: dysferlin; progressive muscular dystrophy *Hc^{0}*: hemolytic complement; deficient common names: C5-, C5-d, C5-def, C5-deficient, hc^{o}
Strain origin:	Strong (1921) from a cross of an albino from Cold Spring Harbor stock and a Bagg albino; given to Cloudman (1928). A/HeJ and A/J have a common origin.
Source:	Cloudman to JAX (1947) at F73.
Characteristics and uses:	• Homozygous for *Cdh23^{ahl}*, age-related hearing loss 1 mutation; onset between 3–5 months of age. • Moderate mammary tumor frequency; high percentage of mammary adenocarcinomas (large proportion of acinar-type) in multiparous females. • Primary lung tumors in 50% of mice; lung tumors readily develop in response to carcinogens. • High frequency of renal disease in older animals. • Homozygous retrotransposon insertion in the dysferlin (*Dysf*) gene causes late onset (4–5 months) progressive muscular dystrophy (Ho M *et al.,* 2004). • Dysferlin mutation not present in RI lines with A/J mice (Ho M *et al.,* 2004). • Primary cleft palate sometimes found in newborn; easily induced with a variety of agents (Azziz and Ladda, 1990; Kalter, 1981). • Lower percentage of granulocytes than A/HeJ. • Low frequency of osteoporosis (Silberberg and Silberberg, 1962). • In The Jackson Laboratory substrains, observation of rare spontaneous myoepitheliomas arising from myoepithelial cells of various exocrine glands. • Research application: cancer, cardiovascular, developmental biology, internal/organ, neurobiology, sensorineural, immunology and inflammation. • Unusually long gestation length (unpublished communication, Corrow DJ)
Technician notes:	• Gentle mouse; easy to handle. • Very clean. • Small eyes, often with secretion that looks like they have a cold. • Males prone to rectal prolapses. • Large litters, good breeders. But breeders tend to bite pups. Breeders have some hair loss.

A/WySnJ — Stock No. 000647

Common name: A Woolley Snell **Generation:** F264 (05-DEC-07)

Former name: n/a

Strain type: Inbred strain

Appearance: Albino; related genotype: *a/a Tyrp1^{b}/Tyrp1^{b} Tyr^{c}/Tyr^{c}*

H2 haplotype: *a*

Genes/alleles:
Cdh23^{ahl}: cadherin 23 (otocadherin); age related hearing loss 1
common names: Cdh23^{753A}, mdfw
Hc^{0}: hemolytic complement; deficient
common names: C5-, C5-d, C5-def, C5-deficient, hc^{o}
Tnfrsf13c$^{Bcmd1\text{-}A/WySnJ}$: tumor necrosis factor receptor superfamily, member 13c; B-cell maturation defect 1, A/WySnJ
common names: Bcmd1$^{A/WySnJ}$

Strain origin: Developed by LC Strong (1921) from a cross between a Cold Spring Harbor albino and a Bagg albino. A/HeJ, A/J and A/WySnJ have a common origin. Strong to Bittner at JAX (1927).

Source: Bittner (1942) to Woolley at JAX (1942) to Snell at JAX (1951) at F84–86.

Characteristics and uses:
- Homozygous for *Cdh23^{ahl}*, age-related hearing loss 1 mutation; onset after 10 months of age.
- Frequency of facial clefting in A/WySnJ is 20–30%. Clefting reporting to be a 2 locus system: *clf1* and *clf2* (Juriloff and Mah, 1995). Highly susceptible to induction of congenital cleft palate by cortisone.
- In The Jackson Laboratory substrains, observation of rare spontaneous myoepitheliomas arising from myoepithelial cells of various exocrine glands.
- High incidence of spontaneous lung adenomas; lung tumors readily develop in response to carcinogens.
- High percentage of mammary adenocarcinomas (large proportion acinar-type) develop in multiparous females.
- Unlike A/J mice, A/WySnJ mice carry a spontaneous mutation in *Tnfrsf3c* and exhibit a significant loss of mature B cells (Miller and Hayes, 1991; Lentz *et al.,* 1996; Shulga-Morskaya *et al.* 2004)
- Research applications: cancer, developmental biology, immunology and inflammation, neurobiology, sensorineural.

Technician notes:
- Appear to be nice, fat, calm and clean-coated mice.

AKR/J — Stock No. 000648

Common name: AK **Generation:** F255 (03-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Albino; related genotype: *a/a Tyr^{c}/Tyr^{c} Soat1^{ald}/Soat1^{ald} hid/hid*

H2 haplotype: *k*

Genes/alleles:
Hc^{0}: hemolytic complement; deficient
common names: C5-, C5-d, C5-def, C5-deficient, hc^{o}
Obq3$^{AKR/J}$: obesity QTL 3; AKR/J
Obq4$^{AKR/J}$: obesity QTL 4; AKR/J
Rmcf^{s}: resistance to MCF virus; MCS sensitive
Soat1^{ald}: sterol O-acyltransferase 1; adrenocortical lipid depletion
common names: Acact-, ald
Thy1^{a}: thymus cell antigen1 theta: a variant
common names: Thy-1.1, Thy1.1, Thy1a
hid: hair interior defect

Strain origin: Detwiler to Furth (1928–36) as high leukemia strain. Random bred at Rockefeller Institute for several generations (Furth, 1946), followed by 9 generations of inbreeding by Mrs. Rhoades and an additional 12 by Lynch at the Rockefeller Institute (Lynch, 1954). Based on allele distribution, possibly shares a genetic origin with the RF strain (Atchley and Fitch, 1993).

Source: Lynch to JAX (1948) AT F22.

Characteristics and uses:
- High spontaneous frequency of lymphatic leukemia before 1 year; high viremic strain (DiFronzo and Holland, 1993).
- Viremic from birth, and express the ecotropic retrovirus AKV in all tissues.
- *hid* (hair interior defect) mutation causes alterations in hair development that is evident only microscopically.
- Mutation in *Soat1^{ald}* leads to truncated SOAT1 protein and adrenal cortical lipid depletion.
- Relatively resistant to aortic lesion formation on semi-synthetic high fat diet; hyporesponsive to diets containing high levels of fat and cholesterol.
- Low induction of colon carcinogenesis with 1,2dimethylhydrazine (DMH) (Rosenberg and Liu, 1995).
- Research applications: cancer, cardiovascular, developmental biology, diabetes and obesity, endocrine deficiency, internal/organ, metabolism, neurobiology, immunology and inflammation.

Technician notes:
- Heavy cage soiling.
- Gentle, easy to handle; but sometimes they will squeal when picked up.
- Occasionally non-productive, but good parents.

B6.129P2-*Apoe*^{tm1Unc}/J — Stock No. 002052

Common name: ApoE-KO; apoE-; apoE0; epsilon-; **Generation:** N12F17 (03-JAN-08)

Former name: n/a

Strain type: Congenic, mutant strain, targeted mutation

Appearance: Black; related genotype: *a/a*

H2 haplotype: *b*

Genes/alleles: *Apoe*^{tm1Unc}: apolipoprotein E; targeted mutation 1, University of North Carolina
Common names: AopE(-), APOE KO, apoE-, ApoE-KO, Apoe^{tm1Un}, apoE0, epsilon-

Development: The *Apoe*^{tm1Unc} mutant strain was developed in the laboratory of Dr. Nobuyo Maeda at The University of North Carolina at Chapel Hill. The 129-derived E14Tg2a ES cell line was used. The plasmid used is designated as pNMC109 and the founder line is T-89 in the primary reference. The C57BL/6J strain was produced by backcrossing the *Apoe*^{tm1Unc} mutation 10 times to C57BL/6J mice. Previously mice backcrossed 6 times (N6) to C57BL/6J mice were distributed solely. Mice from the N6 generation are homozygous for pink-eyed dilution Oca2^{p}, giving them pink eyes and a silver coat color. The E14Tg2a ES cell line carries this recessive mutation, which remained linked to the targeted *Apoe* gene on Chr 7 at this backcross generation. Mice from the N6 colony are no longer available for distribution. The pink-eyed dilution mutation was bred out of this strain by N12, the current backcross generation.

Characteristics and uses:

- Mice homozygous for *Apoe*^{tm1Unc} mutation show marked increase in total plasma cholesterol levels unaffected by age or sex.
- Fatty streaks in proximal aorta found at 3 months of age. Lesions increase with age and progress to lesions with less lipid but more elongated cells, typical of a more advanced stage of pre-atherosclerotic lesion.
- Moderately increased triglyceride levels reported in mice with this mutation on a mixed C57BL/6 x 129 genetic background.
- Aged APOE deficient mice (>17 months) develop xanthomatous lesions in the brain consisting mostly of crystalline cholesterol clefts, lipid globules, and foam cells. Smaller xanthomas seen in the choroid plexus and ventral fornix.
- Recent studies indicate that APOE deficient mice have altered responses to stress, impaired spatial learning and memory, altered long term potentiation, and synaptic damage.
- Research applications: diabetes and obesity, neurobiology (Alzheimer's), cardiovascular, atherosclerosis, lipid metabolism.

Technician notes:

- Good parents.
- Chewed ears may appear in breeders about 4 months of age.
- Malocclusion and hydrocephalus relatively common. Check for malocclusion in weanlings.

B6D2F1/J		**Stock No. 100006**
Common name:	B6D2F1	**Generation:** n/a
Former name:	n/a	
Strain type:	F1 hybrid	
Appearance:	Black; related genotype: *a/a Tyrp1^{b}/+ Myo5a^{d}/+*	
H2 haplotype:	*b/d*	
Genes/alleles:	See C57BL/6J (000664) and DBA/2J (000671)	
Development:	Hybrid strain created by crossing C57BL/6J (B6; 000664) female and DBA/2J (D2; 000671) male.	
Characteristics and uses:	• Mixed haplotype enables mice to accept tissue transplants from both parental strains. • Useful as a genetic background for transgenic/knockout creation as well as other deleterious mutations.	• Research applications: radiation, behavioral; drug safety and efficacy testing; bioassays of nutrients, drugs, pathogens, and hormones.
Technician notes:	• Easy to handle. • Parental breeding pairs are good producers. When having large litters, have increased appetite.	• May have small eyes, missing eye, cataracts, missing fur, occasional white belly spots.

BALB/cByJ — Stock No. 001026

Common name: CBy; BALB Bailey; BALB/c Bailey J; **Generation:** F243 (03-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Albino; related genotype: *A/A Tyrp1^{b}/Tyrp1^{b} Tyr^{c}/Tyr^{c}*

H2 haplotype: *d*

Genes/alleles:
Acads$^{del\text{-}J}$: acyl-Coenzyme A dehydrogenase, short chain; deletion, Jackson
common name: Bcd-1c

Cdh23^{ahl}: cadherin 23 (otocadherin); age related hearing loss 1
common names: Cdh23^{753A}; mdfw

Hld: hippocampal lamination defect

Mdmg1$^{BALB/cBy}$, mandibular morphogenesis 1

Strain origin: Halsey J Bagg albino to LC Strong in 1920 (Strong, 1978). The history of the BALB/c substrains has been well document (Potter, 1985), and the genetic difference between the BALB/c substrains characterized (Hilgers *et al*., 1985). Subline of BALB transferred from MacDowell, Cold Spring Harbor, to Muller, University of Texas, to Snell, University of Texas, (1932) to Scott (JAX), to Andervont (1935) to NIH (1951) at F72, to Bailey (1961) at F99.

Source: Bailey to JAX (1975) at F136 by hysterectomy derivation and fostering on C57BLKS/J.

Characteristics and uses:

- Low spontaneous mammary tumor frequency in females, but susceptible to MMTV.
- Higher reproduction rate than BALB/cJ.
- Carries an Asian *M.m. musculus*-origin Chr Y (Tucker *et al*., 1992).
- Suckling young susceptible to diarrhea in less than optimum environment.
- Males much less aggressive than BALB/cJ (Les, 1987).
- Spontaneous dystrophic cardiac calcinosis.
- High liver triglyceride levels.
- Increased wound healing time.
- Mid-range bone density levels.
- Moderate increase in food intake and weight on high-fat/high-calorie diet.
- Susceptible to toxoplasmosis.
- Differs from all other BALB/c sublines at the *Acads* (acyl-CoA dehydrogenease, short chain) locus by having a null allele.
- Useful for production of plasmacytoma, forming basis for development of ascites tumors and monoclonal antibodies.
- Research applications: cancer, immunology and inflammation, neurobiology, sensorineural.

Technician notes:

- Easy to handle.
- Occasionally jumpy due to weather.
- Eat a lot.
- Females may have ulcerated eyelids, rough appearing coats.
- Alopecia.
- Good sized litters; good parents.
- Higher reproductive rate than BALB/cJ. Pair or trio matings work well.
- Heavy cage soiling.

BALB/cJ	**Stock No. 000651**
Common name:	C; BALB **Generation:** F224 (03-JAN-08)
Former name:	n/a
Strain type:	Inbred strain
Appearance:	Albino; related genotype: *A/A Tyrp1^{b}/Tyrp1^{b} Tyr^{c}/Tyr^{c}*
H2 haplotype:	*d*
Genes/alleles:	*Hld*: hippocampal lamination defect
Strain origin:	BALB/cByJ and BALB/cJ have a common origin. Bagg acquired progenitors of this strain (then called BALB) in 1913 from an Ohio dealer. Bagg to Little and MacDowell, Cold Spring Harbor (1922), MacDowell to Muller at University of Texas at F12, Muller to Snell (who added the /c) (1932) at F25, Snell to Scott at JAX (1947).
Source:	Scott to JAX (1947) at F41.
Characteristics and uses:	• Low spontaneous mammary tumor frequency, but susceptible to MMTV. • Relatively resistant to the induction of colon carcinogenesis (Moen *et al.,* 1996). • Prone to other cancers later in life, including reticular neoplasms, primary lung tumors, and renal tumors. • Rare spontaneous myoepitheliomas arising from myoepithelial cells of various exocrine glands. • Very sensitive to radiation. • Susceptible to chronic pneumonia. • Plasmacytomas easily induced using pristane or silicone gels (Potter and MacCardle, 1964; Potter *et al.,* 1995). • Lower reproductive performance than other BALB substrains due to vaginal septa, which may cause dystocia. • Extremely high inter- and intra-strain aggression and lower reproductive performance than that found in BALB/cByJ mice (Les, 1987). • Research applications: cancer, cardiovascular, immunology and inflammation, neurobiology.
Technician notes:	• Highly aggressive; may kill cage mates. But easy for technicians to handle. • Coat may have an "oily," look, appearing dirty, yellowed, and unkempt. • Lumps, tumors. • May have temporary hair loss. Hair loss at weaning age resolves itself by 6 weeks of age. • With age, mice often develop a slight swelling around the edge of the eyelids. • Heavy cage soiling. • Urination often soaks fur around genital area, resulting in difficulty with sexing. • Moderate productivity rate. Good mothers. • Timed pregnancy and superovulation difficult with this strain. Many matings are not fertile although plugs are deposited. • If feed hopper is overfilled, mice will knock out large amounts of food.

BTBR T^+ *tf*/J		**Stock No. 002282**
Common name:	n/a	**Generation:** F?+34 (11-JAN-08)
Former name:	n/a	
Strain type:	Inbred strain, mutant strain	
Appearance:	Black and tan, tufted; related genotype: a^t/a^t T^+/T^+ *tf/tf*	
H2 haplotype:	*b*	
Genes/alleles:	T^+, brachyury; wild-type *tf*, tufted; tufted *Disc1*^{del}; disrupted in schizophrenia 1; deletion common name: Disc1^{129S6}	
Strain origin:	n/a	
Source:	n/a	
Characteristics and uses:	• Compared to other inbred strains, exhibit several symptoms of autism, including reduced social interactions, impaired play, low exploratory behavior, unusual vocalizations, and high anxiety. (McFarlane *et al*., 2008; Moy *et al*., 2007). • Exhibit 100% absence of the corpus callosum and a severely reduced hippocampal commissure. (Wahlsten *et al*., 2003). • Research applications: dermatology, developmental biology, neurobiology.	
Technician notes:	n/a	

BUB/BnJ	**Stock No. 000653**
Common name:	n/a **Generation:** F198 (11-DEC-07)
Former name:	n/a
Strain type:	Inbred strain
Appearance:	Albino; related genotype: *a/a Tyr^{c}/Tyr^{c}*
H2 haplotype:	*q2* (see Fischer Lindahl K, 1997; Shen *et al.*, 1982)
Genes/alleles:	*Cdh23^{ahl}*: cadherin 23 (otocadherin); age related hearing loss 1 common names: Cdh23^{753A}, mdfw *Gpr98^{frings}*: G protein-coupled receptor 98; frings common names: frings, Mass1^{frings} *Pde6b^{rd1}*: phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide; retinal degeneration 1 common names: rd, rd-1, rd1, rodless retina
Strain origin:	From albinos of unknown ancestry developed by JW Wilson at Brown University, with selection against circling which appeared at about F4 (Curtis, 1956).
Source:	Brown University to S Bernstein (Bn) at JAX at F46 and then to production facility in 1968.
Characteristics and uses:	• Carries the *Pdeb^{rd1}* retinal degeneration allele (Sidman and Green 1965). • 76% of males immunized at 6–8 weeks of age develop severe type II collagen induced arthritis with clinical and pathological features resembling rheumatoid arthritis in the human (Ortman *et al.,* 1994). • Relatively nicotine resistant (Pauly *et al.,* 1990; Marks *et al.,* 1991). • Homozygosity for *Gpr98^{frings}* results in susceptibility to audiogenic seizures prior to 25 days of age (Skradski *et al.,* 2001) and early onset hearing impairment by 3–4 weeks of age (Johnson *et al.,* 2005). • No detectable endogeneous ecotropic MuLV DNA sequences (Jenkins *et al.,* 1982). • High serum complement activity. • In response to challenge, BUB/BnJ mice develop immune-mediated nephritis characterized by proteinuria, glomerulonephritis, and tubulointerstitial disease (Xie *et al.,* 2004) • Carries a Western European Type 3 *M.m. domesticus* Chr Y (Tucker *et al.,* 1992). • Research applications: immunology and inflammation, neurobiology, sensorineural.
Technician notes:	• Docile, large mice. • Breeders occasionally chew ears.

C3H/HeJ — Stock No. 000659

Common name: C3; C3H Heston; **Generation:** F263 (03-JAN-08)

Former name: n/a

Strain type: Inbred strain, spontaneous mutation

Appearance: Agouti; related genotype: *A/A*

H2 haplotype: *k*

Genes/alleles:
Gria4^{spkw1}: glutamate receptor, ionotropic, AMPA4 (alpha 4); spike wave discharge 1
Pde6b^{rd1}: phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide; retinal degeneration 1
common names: rd, rd-1, rd1, rodless retina
Tlr4$^{Lps\text{-}d}$: toll-like receptor 4; defective lipopolysaccharide response
common names: lps^{d}, Tlr$^{Lps\text{-}d}$, TLR4-Mu, Tlr4^{-}, Tlr4^{d}
In(6)1J: inversion, Chr 6, Jackson 1

Strain origin: LC Strong originated the C3H strain in 1920 from a cross of a female Bagg albino x DBA male, with selection for high mammary tumor frequency (Strong, 1935). Significant differences among substrains. To Andervont, NCI (1930). A spontaneous mutation occurred in C3H/HeJ sometime between 1960 and 1968 at lipopolysaccharide response locus (mutation in toll-like receptor 4 gene, *Tlr4*$^{Lps\text{-}d}$) making C3H/HeJ mice endotoxin resistant while the other 3 C3H strains are endotoxin sensitive.

Source: Andervont to Heston, NCI (1941) at F35; Heston to JAX (1948) at F48.

Characteristics and uses:
- *Tlr4*$^{Lps\text{-}d}$ makes the strain susceptible to gram negative bacterial infections.
- Carries *Pde6b*^{rd1} retinal degeneration allele, which causes blindness by weaning age.
- Frequency of mammary tumors in both breeders and virgins lower than that of other C3H substrains (Outzen *et al*., 1985).
- Does not carry MMTV; but virgin and breeding females still may develop mammary tumors later in life.
- Hepatomas (72–91% in males at 14 months, 59% in virgin females, 30–38% in breeding females).
- High mortality in males exposed to turpentine or chloroform fumes.
- Carries Chr Y of *M.m. domesticus* origin (Tucker *et al*., 1992).
- When fed an atherogenic diet, fail to develop atherosclerotic aortic lesions.
- Low red and white cell counts.
- Lipopolysaccharide hyporesponsive because homozygous for *Lps*^{d} mutation, which occurred between 1960 and 1969 (Glode and Rosenstreich, 1976).
- Carries the In(6)1J inversion, encompassing about 20% of Chr 6 from ~73 Mb to ~116 Mb. Inversion has no apparent effect on phenotypes (Akeson *et al.,* 2006).
- Spontaneously develop alopecia areata (AA) at a reported incidence of approximately 0.25% by 18 months of age. AA can be surgically induced by grafting a small piece of skin from an older, donor animal with AA onto a younger, isogenic C3H/HeJ recipient.
- Research applications: cancer, cardiovascular, immunology and inflammation, neurobiology, sensorineural.

Technician notes:
- Active; can be jumpy; two-week-old mice very active. Aggressive; curious.
- Blind after 3 weeks of age.
- Fair amount of bent tails.
- Eat a lot; get fat as they age.
- Females sexually mature very early (25 days).
- Pair matings work best.
- Hair loss in older breeders.
- Cages wet from urine.

C3H/HeOuJ — Stock No. 000635

Common name: C3Ou; C3H Outzen **Generation:** F184 (03-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Agouti; related genotype: *A/A*

H2 haplotype: *k*

Genes/alleles: *Pde6b*^{rd1}: phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide; retinal degeneration 1
common names: rd, rd-1, rd1, rodless retina

Strain origin: C3H/HeJ, C3H/HeOuJ, and C3HeB/FeJ have a common origin. The C3H parent strain was developed by LC Strong in 1920 from a cross of a Bagg albino female with a DBA male followed by selection for high incidence of mammary tumors. Separated from C3H/HeJ in 1952 before occurrence of *Tlr4*$^{Lps\text{-}d}$ mutation.

Source: Normal littermates of C3H/HeJ-*Kit*$^{W\text{-}x}$ in JAX Foundation Stocks to Outzen at JAX (1981) at F93.

Characteristics and uses:

- Strain carries *Pde6b*^{rd1} retinal degeneration gene; mice will be blind by weaning age.
- Does not carry MMTV, but virgin and breeding females may still develop some mammary tumors later in life.
- Does not carry the *Tlr4*$^{Lps\text{-}d}$ mutation.
- High incidence of hepatomas in C3H mice (reportedly 72–91% in males at 14 months, 59% in virgin females, 30–38% in breeding females).
- Research applications: cancer, sensorineural.

Technician notes:

- Mice get fat as they age.
- White spots.
- Occasional hair loss in older breeders.
- Good breeders. Pair mating.

C3H/HeSnJ — Stock No. 000661

Common name: C3H Snell; C3Sn **Generation:** F198 (03-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Agouti; related genotype: *A/A*

H2 haplotype: *k*

Genes/alleles: *Pde6b*^{rd1}: phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide; retinal degeneration 1 common names: rd, rd-1, rd1, rodless retina

Strain origin: C3H/HeJ, C3H/HeOuJ, C3HeB/FeJ, and C3H/HeSnJ have a common origin. Strong (1920) to Andervont (1930) to Heston (1938) at F35.

Source: Heston to Dickie (JAX) (1947) at F48 to Snell in approximately 1964, to JAX.

Characteristics and uses:

- Parental substrain for Snell congenic strains.
- Does not carry MMTV.
- Does not carry the *Tlr4*$^{Lps\text{-}d}$ mutation.
- $CD8^+$ T-cell depletion.
- Does not carry the In(6)1J inversion found in C3H/HeJ (000659) (Akeson *et al.,* 2006).
- Research applications: neurobiology, sensorineural.

Technician notes:

- Jumpy, curious; get frightened if surroundings are noisy.
- Good breeders. Good parents.
- Large sized mice at weaning.

C3HeB/FeJ — Stock No. 000658

Common name: C3Fe; C3H Fekete; HeB **Generation:** F202 (03-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Agouti; related genotype: *A/A*

H2 haplotype: *k*

Genes/alleles: *Pde6b*^{rd1}: phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide; retinal degeneration 1; common names: rd, rd-1, rd1, rodless retina

Strain origin: C3H/HeJ, C3H/HeOuJ, and C3HeB/FeJ have a common origin. C3H/HeJ ova transferred to C57BL/6 by Fekete at JAX (1948). Original transplant animals to Hummel at JAX.

Source: Hummel to JAX (1950) at F3.

Characteristics and uses:

- Does not carry MMTV. Low frequency of mammary tumors.
- Calcareous heart deposits in almost all retired breeders.
- Does not carry the In(6)1J inversion found in C3H/HeJ (000659) (Akeson *et al.,* 2006).
- Does not carry the *Tlr4*$^{Lps\text{-}d}$ mutation.
- Ovarian tumor frequency after 19 months about 65% in female breeders, 22% in virgins.
- Research applications: sensorineural.

Technician notes:

- Jumpy.
- Good breeders. Good parents.
- Mature breeders tend to be chubby.

C57BL/6ByJ	Stock No. 001139
Common name:	C57 Bailey; B6By; Black 6 Bailey; B6 Bailey **Generation:** F200+F5 (28-DEC-07)
Former name:	n/a
Strain type:	Inbred strain
Appearance:	Black; related genotype: *a/a*
H2 haplotype:	*b*
Genes/alleles:	n/a
Strain origin:	C57BL/6J, C57BL/10J, and C57BL/6ByJ have a common origin. C57BL/6N was separated from C57BL/6J in 1951; C57BL/6ByJ was separated from C57BL/6N in 1961 (Bailey, 1978). C57BL/6ByJ carries a Type1A Asian *M.m. musculus* Chr Y (Tucker *et al.,* 1992). Histologically normal retinas found in this substrain (Sidman and Green 1965).
Source:	Bailey to JAX (1979) at F111.
Characteristics and uses:	• Carries the *Xmmv64*^{a} allele; C57BL/6J the *Xmmv64*^{o}. • Immunosuppression occurred in female C57BL/6J mice administered the k-selective agonist, U50,488H opioid, whereas C57BL/6ByJ were not suppressed (Eisenstein *et al.,* 1995). • C57BL/6J and C57BL/6ByJ have different isoforms of the *Std* (sulfotransferase, DHEA preferring) gene (Chapman, 1994). • Testis size in C57BL/6ByJ and C57BL/10J significantly smaller than in C57BL/6J substrain; based on 0.38% of body weight determined for 21 inbred strains (Chubb, 1992). • Low plasma cholesterol and triglyceride levels (Jiao *et al.,* 1990). • High level of plasma serotonin (1.7–2.2 mg/ml), more than twice the level found in BALB/cBy; they carry the plasma serotonin level *Spl*^{h} allele mapped to Chr 1 (Eleftheriou and Bailey, 1972). • Preference for ethanol, high preference for other sweet and sour compounds—sucrose, saccharin, acesulfame, dulcin, glycine, l-glutamine and d-phenylalanine—and low preference for salty compounds (Bachmanov *et al.,* 1996). • Intermediate tyrosine hydroxylase activity level compared to high activity in BALB/cJ and low activity in CXB-9/ByJ (Vadasz *et al.,* 1987). • All C57BL substrains are homozygous for *Bsp (*black spleen), thought to result in lipofuscinosis (melanosis, or blackening, of the anterior portion of the spleen), which is found in 4–57% of young C57BL mice (Chrichton and Shire, 1982). The pathological relevance is obscure (van der Heijden *et al.,* 1995. • 5 SNP differences distinguish C57BL6ByJ, C57BL/6J, and C57BL/6NJ (Petkov *et al*., 2004).
Technician notes:	• Calm mice, although 2- to 3-week-old mice can be jumpy. Jumpy if in noisy surroundings. • Occasionally poor breeders. Generally good parents. • Tend to get very large at end of breeding rotation. • Occasional barbering. • Prone to dermatitis.

C57BL/6J — Stock No. 000664

Common name: C57 Black; B6; B6J; Black 6 **Generation:** F226p (11-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Black; related genotype: *a/a*

H2 haplotype: *b*

Genes/alleles:
Cdh23^{ahl}: cadherin 23 (otocadherin); age related hearing loss 1
common names: Cdh23^{753A}; mdfw
Gluchos1$^{C57BL/6J}$: glucose homeostasis QTL 1; C57BL/6J
Gluchos2$^{C57BL/6J}$: glucose homeostasis QTL 2; C57BL/6J
Gluchos3$^{C57BL/6J}$: glucose homeostasis QTL 3; C57BL/6J
Nnt$^{C57BL/6J}$: nicotinamide nucleotide transhydrogenase; C57BL/6J

Strain origin: Little (1921) developed this strain from mating of littermates female 57 x male 52, from Miss Lathrop's stock. Sublines 6 and 10 separated prior to 1937 (Russell, 1978).

Source: Little to Fekete to Hall at F22, back to JAX (1948) at F24.

Characteristics and uses:

- Homozygous for *Cdh23*^{ahl}, age-related hearing loss 1 mutation; onset after 10 months of age.
- Mammary tumor frequency of 1% in breeders, none in virgins.
- Resistant to irradiation.
- Resistant to C57BL/10J transplantable tumors.
- High preference for alcohol, morphine, other opioids (Chadha *et al.,* 1991; Phillips *et al.,* 1994; Berrettini *et al.,* 1994; Belknap *et al.,* 1995; Grahame *et al.,* 1995; Watzl *et al.,* 1992).
- Highly susceptible to development of atherosclerotic lesions (Nishina *et al.,* 1993).
- Frequency of eye defects about 12% (Robinson *et al.*, 1993). Includes anophthalmia, microphthalmia, and cataracts.
- Frequency of hydrocephalus in weanlings is 1%, of malocclusion, 3%.
- 5 SNP differences distinguish C57BL6ByJ, C57BL/6J, and C57BL/6NJ (Petkov *et al*., 2004).
- Carries a Type 1A Asian *M.m. musculus* Chr Y (Tucker *et al.,* 1992).
- Presence of *Nnt* mutation, which diminishes initial insulin response to injected glucose (Toye *et al*., 2005), does not affect steady-state glucose clearance (Berglund *et al*., 2008) or impede development of diet-induced obesity with a high-fat diet (JAX® Services, 2008).
- Alopecia-areata like hair loss due to whisker chewing and barbering is common (Thornburg *et al.,* 1973; Militzer and Wecker, 1986), especially in older breeders and in crowded cages. Juvenile alopecia is common at about 4–5 weeks of age (first molt), but usually resolves itself by 6 weeks of age.
- Most widely used inbred strain. Commonly used for generation of congenics carrying both spontaneous and induced mutations. Other research applications: cardiovascular, developmental biology, diabetes and obesity, hematological, immunology and inflammation, neurobiology, sensorineural.

Technician notes:

- Occasionally hyperactive but easy to handle. Active barberers.
- Males may be aggressive in overcrowded cages or if cohorts are mixed after weaning.
- Occasional white spots in fur. Sometimes mice appear to have a dirty blue coat color.
- May have some yellow/tan hairs around the base of the tail, base of ears, and corners of eyes. This is considered normal.
- Good breeders. Good parents.
- Occasional rectal and uterine prolapse.
- Females may die bearing first litter or may cannibalize first litter. Not handling pups less than 7 days old may reduce this.
- Male spermatogenesis is slow; therefore, males do not breed well in polygamous matings. Pair or trio matings recommended.
- Sometimes build up shavings around water drip holes, resulting in leakage.
- Older males may gain weight and sometimes become non-productive.

C57BL/10J — Stock No. 000665

Common name: Black 10; Black 10 J
Generation: F239 (03-JAN-08)
Former name: n/a
Strain type: Inbred strain
Appearance: Black; related genotype: *a/a*
H2 haplotype: *b*
Genes/alleles: n/a

Strain origin: C57BL/6J and C57BL/10J have a common origin. Little (1921) developed this strain from mating of littermates male 57 x female 52 from Miss Lathrop's stock. Sublines 6 and 10 separated prior to 1937 (Russell, 1978). Little to Scott at JAX.

Source: Scott to JAX (1947) at F32.

Characteristics and uses:

- Low tumor frequency; resistant to some C57BL/6 tumors.
- Preference for alcohol, morphine and other opioids.
- Overall tumor incidence is 33% in males and 31% in females, most of which is due to lymphoma.
- Prone to dermatitis, a common problem in the C57BL strain.
- High lymphocyte phytohaemagglutinin response, good immune response to ovalbumin, poor response to DNP-keyhole limpet haemocyanin; resistant to induction of passive cutaneous anaphylaxis (IgG1- and IgE-mediated).
- Susceptible to immunosuppression of contact hypersensitivity by ultraviolet light; moderately susceptible to experimental allergic encephalomyelitis.
- Frequency of eye defects about the same as in C57BL/6J (Pierro and Spiggle, 1967; 1969).
- Frequency of both malocclusions and hydrocephalus is rarer than in C57BL/6J.
- Often used as a background strain for histocompatibility congenics.

Technician notes:

- Hair loss in weanlings very common.
- Hydrocephaly and white belly spots.
- Good mothers and fathers.
- Weanlings can be active, but breeders not active.

C57BL/10SnJ — Stock No. 000666

Common name: B10 Snell J; Black 10 Snell J
Generation: F62 (1995)
Former name: n/a
Strain type: Inbred strain
Appearance: Black; related genotype: *a/a*
H2 haplotype: *b*
Genes/alleles: n/a

Strain origin: C57BL/6J, C57BL/10J and C57BL/10SnJ have a common origin. Little (1921) strain from mating of littermates male 57 x female 52 from Miss Lathrop's stock. Sublines 6 and 10 separated prior to 1937.

Source: Little to WL Russell, to Scott at JAX at F26. Scott to Snell at F35–36 to JAX by hysterectomy derivation and fostering on C57BL/6J.

Characteristics and uses:

- Low tumor frequency.
- Resistant to some C57BL/6J tumors.
- Eye defects same as C57BL/6J.
- Frequency of both malocclusions and hydrocephalus is rarer than in C57BL/6J.
- Often used as a background strain for histocompatibility congenics.

Technician notes:

- Barbering in weanlings and breeders.
- Entire litters may be missing at weaning.
- Weanlings slightly to very jumpy.
- Eat a lot.
- Occasional hydrocephaly noticed at 4 weeks of age.
- Occasional rectal and vaginal prolapse.

C57BLKS/J		Stock No. 000662
Common name:	C57BL/Ks; C57Kaliss; Black Kaliss J	**Generation:** F147 (03-JAN-08)
Former name:	n/a	
Strain type:	Inbred strain	
Appearance:	Black; related genotype: *a/a*	
H2 haplotype:	*d*	
Genes/alleles:	*Cdh23*^{ahl}: cadherin 23 (otocadherin); age related hearing loss 1 common names: Cdh23^{753A}, mdfw	
Strain origin:	C57BL/6J sent to Biesele at Sloan-Kettering (1947). Pen bred 1 generation and returned to Kaliss at Sloan-Kettering. Taken to JAX (1948) and inbred.	
Source:	Kaliss to JAX (1965) by hysterectomy derivation and fostering on C3HeB/FeJ at F67. Genomic analysis of the C57BLKS inbred strain shows that 84% of the alleles in this strain are shared with C57BL/6 and 16% are shared with DBA/2J, indicating genetic contamination early in the strain's history (Naggert *et al.,* 1995).	
Characteristics and uses:	• Homozygous for *Cdh23*^{ahl}, age-related hearing loss 1 mutation; onset prior to 3 months of age. • High frequency of congenital malformation of toes. • High frequency of polycystic kidneys, microphthalmia (Dagg, 1963). • Diet-induced atherosclerotic lesions much more severe in C57BLKS/J than in C57BL/6J or many other inbred strains (Mu *et al.,* 1999).	• The mutations diabetes (*Lepr*^{db}) and obese (*Lep*^{ob}) each express a much more severe phenotype on the C57BLKS/J background than on the C57BL/6J background. • The *Cpe*^{fat} mutation causes severe obesity, hyperinsulinemia, and hyperglycemia on the C57BLKS/J background rather than the hyperinsulinemia, and mild obesity without hyperglycemia found on the HRS/J background (Collins *et al*., 2005). • Research applications: diabetes and obesity, neurobiology, sensorineural.
Technician notes:	• Quiet; easy to handle. • Barbering is common. • Malocclusion and hydrocephalus relatively common.	• Poor breeders. Trio mating works; good parents. • Small litter size.

C57BR/cdJ — Stock No. 000667

Common name: BR; Brown cd; C57 Brown; **Generation:** F253 (05-DEC-07)

Former name: n/a

Strain type: Inbred strain

Appearance: Brown; related genotype: *a/a Tyrp1^{b}/Tyrp1^{b}*

H2 haplotype: *k2* (Fischer Lindahl K, 1997; Shen *et al.*, 1982)

Genes/alleles: *Cdh23^{ahl}*: cadherin 23 (otocadherin); age related hearing loss 1
common names: Cdh23^{753A}, mdfw

Rmcf^{s}: resistance to MCF virus; MCF sensitive

Strain origin: Little (1921) developed this strain from a mating of littermates female 57 and male 52 that gave rise to C57BL and C57BR. Black and brown lines separated in the 1st generation. Substrain cd established in generation 13 from a cross of 2 brown branches, one of which had previously given rise to strain C57BR/a. Some given to Cloudman at JAX, to Heston at JAX (1938).

Source: Heston to JAX (1947) at F66.

Characteristics and uses:

- Homozygous for *Cdh23^{ahl}*, age-related hearing loss 1 mutation; results in early-onset hearing loss that is moderate at 7 weeks of age, severe by 20 weeks, and that progresses with increasing age (Henry, 1982; Zheng *et al.,* 1999).
- Low mammary tumor frequency; some hepatomas in females.
- Carries a Chr Y of Asian *M.m. musculus* origin (Tucker *et al.,* 1992).
- Highly atherogenic (Paigen *et al.,* 1990; Kuan *et al.,* 1992).
- Research applications: cardiovascular, neurobiology, sensorineural.

Technician notes:

- White belly spot.
- Poor breeders: small litters; missing litters.
- Need to be delayed mated; mating at wean age does not work very well.
- Occasionally, one pair will be very productive, with large litters.

C57L/J — Stock No. 000668

Common name: L; C57 Leaden; leaden **Generation:** F235 (03-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Leaden (grey); related genotype: *a/a* *Tyrp1^{b}/Tyrp1^{b}* *Mlph^{ln}/Mlph^{ln}*

H2 haplotype: *bc* (Shen *et al.*, 1982)

Genes/alleles:
Cdh23^{ahl}: cadherin 23 (otocadherin); age related hearing loss 1
common names: Cdh23^{753A}; mdfw
Mlph^{ln}: melanophilin; leaden
common names: leaden, ln
Obq3$^{C57L/J}$: obesity QTL 3; C57L/J
Obq4$^{C57L/J}$: obesity QTL 4; C57L/J
Rmcf^{s}: resistance to MCF virus; MCF sensitive

Strain origin: Murray at JAX (1933) mutation to leaden (*ln*) in F22 of a C57BR subline of which the nonleaden (brown) branch is now extinct. Stock was maintained by Cloudman at JAX.

Source: From Cloudman to Heston at JAX (1938), to JAX (1947) at F45.

Characteristics and uses:
- Homozygous for *Cdh23^{ahl}*, age-related hearing loss 1 mutation; onset prior to 3 months of age.
- High incidence of Hodgkin's-like reticulum cell neoplasm at 18 months of age; pituitary tumors in old multiparous females.
- Highly susceptible to experimental allergic encephalomyelitis (EAE).
- Frequent congenital cystic ovaries (Jagiello and Ducayen, 1973).
- Highly susceptible to diet-induced atherosclerosis and diet-induced cholelithiasis (gallstones) (Khanuja *et al.,* 1995).
- Carries a Chr Y of Asian *M.m. musculus* origin (Tucker *et al.,* 1992).
- Carries no detectable endogenous ecotropic MuLV DNA sequences.
- Low mammary tumor frequency.
- Extremely high hematocrit.
- Research applications: general purpose, cancer, cardiovascular, dermatology, immunology and inflammation, neurobiology, sensorineural.

Technician notes:
- Calm mice.
- May have white spot on belly.
- Like to have nesting material for litters.
- Poor–fair breeders. May become "non-productive" suddenly.

C58/J — Stock No. 000669

Common name: n/a **Generation:** F280 (27-NOV-06)

Former name: n/a

Strain type: Inbred strain

Appearance: Black; related genotype: *a/a*

H2 haplotype: *k2* (Fischer Lindahl K 1997; Shen *et al.*, 1982)

Genes/alleles: *Cdh23*^{ahl}: cadherin 23 (otocadherin); age related hearing loss 1
common names: Cdh23^{753A}; mdfw

Rmcf^{s}: resistance to MCF virus; MCF sensitive

Strain origin: MacDowell, Cold Spring Harbor (1921) from mating of littermates female 58 and male 52 of Miss Lathrop's stock.

Source: MacDowell to Brandenburg (University of California, Berkeley) at F75, Brandenburg to JAX (1948) at F77.

Characteristics and uses:

- Homozygous for *Cdh23*^{ahl}, age-related hearing loss 1 mutation; onset after 10 months of age.
- Aplasia of kidney in about 10% (Hummel *et al.*, 1966).
- Very high frequency of leukemia prior to one year of age (Mucenski *et al.,* 1988).
- Exhibit intermediate susceptibility to developing atherosclerotic aortic lesions on an atherogenic diet.
- Carries a Chr Y of Asian *M.M. musculus* origin (Tucker *et al.,* 1992).
- A high viremic strain (Jenkins *et al.,* 1982).
- Frequent polyovular follicles (Fekete, 1950).
- Research applications: general purpose, cancer, cardiovascular, neurobiology, sensorineural.

Technician notes:

- Small size at weaning.
- Occasional white spots on belly.
- Obesity may be associated with non-productivity.
- Poor breeders, but good parents. Go non-productive quite often. Must monitor birth dates to identify non-productives. Only on a 5-month breeding rotation.

CAST/EiJ — Stock No. 000928

Common name: n/a **Generation:** F92 (03-JAN-08)

Former name: Castaneus Eicher (Changed: 15-DEC-04)

Strain type: Wild-derived inbred strain (species: *M.m. castaneus*, Thailand)

Appearance: Agouti; related genotype: *A/A*

H2 haplotype: n/a

Genes/alleles: n/a

Development: Founders were trapped in a grain warehouse in Thonburi, Thailand, by Dr. Joseph T. Marshall. Mice were sent to Dr. Vernon Chapman and Dr. Frank Ruddle at Yale University, and from there, to Dr. Eva Eicher and Dr. Thomas Roderick at The Jackson Laboratory in 1971. Dr. Eicher's colony was maintained by inbreeding to generate CAST/Ei; Dr. Roderick's colony was maintained by inbreeding to generate CASA/Rk and CASB/Rk (Chapman and Ruddle, 1972).

Characteristics and uses:
- Research applications: genetics (evolution and systematics, gene mapping [numerous polymorphisms])

Technician notes:
- These mice are very difficult to handle.

CBA/CaH-T(14;15)6Ca/J — Stock No. 000655

Common name: n/a **Generation:** F164+3 (30-APR-08)

Former name: CBA/CaH-T(14;16)6Ca (Changed: 15-DEC-04)
CBA/CaH-T(14;16)6CaJ (Changed: 15-DEC-04)
CBA/CaH-T6/J (Changed: 15-DEC-04)

Strain type: Mutant strain, radiation induced mutation, chromosome aberration (translocation)

Appearance: Agouti; related genotype: *A/A*

H2 haplotype: *k*

Genes/alleles: T(14;15)6Ca: reciprocal translocation, Chr 14 and 15, Carter 6

Strain origin: A substrain of CBA/CaH. Lyon, Harwell, from crosses of translocation T(14;15)6Ca to CBA/Ca for 13 generations.

Source: Harwell to MC Green (1963), to JAX Foundation Stocks (1968).

Characteristics and uses:
- Histocompatible with CBA/CaJ.
- Does not have *Pdeb*^{rd1}.
- Homozygous for the marker translocation T(14;15)6Ca; results in nondisjunction at a rate of 4.4% in males, 22.2% in females.
- Used in tandem with the CBA/H strain in foreign body tumorigenesis studies in which the T6 chromosome was used as a marker to distinguish donor cells from host.
- Research applications: cancer, tissue/cell markers.

Technician notes:
- Somewhat jumpy.
- Clean mice.
- May have hair loss.

CBA/CaHN-*Btk*^{xid}/J — Stock No. 001011

Common name: n/a **Generation:** F88 (03-JAN-08)

Former name: CBA/NJ (Changed: 15-DEC-04)

Strain type: Inbred strain, mutant

Appearance: Agouti; related genotype: *A/A*

H2 haplotype: *k*

Genes/alleles: *Btk*^{xid}, Bruton agammaglobulinemia tyrosine kinase; X linked immune deficiency common name: xid

Strain origin: Strong (1920) from cross of Bagg albino female to DBA male. Sent from Little to Haldane and Grunebaerg (1932), to Carter (1947), to Harwell (1954). CBA/CaH from Harwell to NIH (1966). Derived from a subline of CBA/CaH bearing foam cell reticulosis (*fm*).

Source: NIH to CL Sidman at JAX (1983) at F51–3 to JAX (1983).

Characteristics and uses:

- Carries a mutation in the Bruton's tyrosine kinase gene (*Btk*); is a model of human X-linked immunodeficiency.
- B-lymphocyte-specific defect results in an inability to launch an antibody response to thymus-independent type II antigens, although they do produce normal amounts of antibody in response to some protein antigens.
- Low serum IgM and IgG3 and a reduced number of B-cells. Moreover, B-cells that are present have reduced surface IgM–IgD ratio, which suggests a disorder in B-cell maturation.
- The *Btk*^{xid} mutation, a recessive X-linked B-cell defect, which, in hemizygous males and homozygous females, prevents the mice from making antibody response to type III pneumococcal polysaccharide and other thymic-independent antigens, was discovered in the non-*fm* substrain at NIH.
- Research applications: immunology and inflammation.

Technician notes:

- Jumpy; startled by loud noise.
- Coat color may appear lighter agouti than normal.
- Poor breeders.
- Medium litter size.

CBA/CaJ	Stock No. 000654
Common name:	CBA Carter J; **Generation:** F199 (03-JAN-08)
Former name:	n/a
Strain type:	Inbred strain
Appearance:	Agouti; related genotype: *A/A*
H2 haplotype:	*k*
Genes/alleles:	*Rmcf*^{s}: resistance to MCF virus; MCF sensitive
Strain origin:	Strong (1920) from cross of Bagg albino female x DBA male. To Little at JAX.
Source:	From Little to Haldane and Gruneberg, University College, London (1932), to Royal Cancer Hospital (1933), to JG Carr and TC Carter, Institute of Animal Genetics, Edinburgh (1947), to MC Green (1950), to JAX Foundation Stocks by hysterectomy derivation and fostering on C57BL/6J (1966 and 1967).
Characteristics and uses:	• Histocompatible with CBA/H-T6J. • Low spontaneous incidence of leukemia but relatively high inducibility of myeloid leukemia in response to benzene and radiation exposure. • Carries viral proteins Mtv8, Mtv9, and Mtv14. • Males develop a mild adult onset diabetes-obesity syndrome characterized by hyperglycemia, hyperinsulinemia, and insulin resistance. Pancreatic beta cells do not degenerate, and circulating insulin levels remain high throughout life. • Carries a Chr Y of Type 2 Asian *M.m. musculus* origin (Tucker *et al.,* 1992). • Does not have retinal degeneration (*Pde6b*^{rd1}). • Not histocompatible with the CBA/J substrain, although *H2* locus is the same in both substrains (Green and Kaufer, 1965). • Research applications: general purpose, cancer, diabetes and obesity, reproductive biology.
Technician notes:	• Very jumpy, especially when disturbed. Hyperactive. • Occasional silvery coat colors in wean only. • Barbering common. • Clean mice. • Good parents; wean almost all pups born; very few missing.

CBA/J — Stock No 000656

Common name: CBA Jackson **Generation:** F274 (03-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Agouti; related genotype: *A/A*

H2 haplotype: *k*

Genes/alleles: *Pde6b*^{rd1}: phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide; retinal degeneration 1 common names: rd, rd-1, rd1, rodless retina

Strain origin: Strong (1920) from cross of a Bagg albino female x DBA male. To Andervont about 1940.

Source: Andervont to JAX (1948) at F67. Reintroduced to Foundation Stocks by hysterectomy derivation; fostered on DBA/2J (1975).

Characteristics and uses:

- Some hepatomas.
- Only CBA substrain that carries the *Pde6b*^{rd1} retinal degeneration allele (Sidman and Green 1965), resulting in blindness by weaning age.
- Fairly high mammary tumor frequency in breeding females at less than 1 year (Smith *et al.,* 1973).
- Develop a mild hearing loss late in life, with most of the hearing loss occurring in the higher frequencies (Sweet *et al.,* 1988).
- Susceptible to chronic whole body irradiation.
- Renal tubulointerstitial lesions at a high frequency (Rudofsky, 1978).
- Some CBA/J mice spontaneously develop exocrine pancreatic insufficiency syndrome (Eppig and Leiter, 1977; Leiter *et al.,* 1977).
- Relatively resistant to diet-induced atherosclerosis.
- Used to study granulomatous experimental autoimmune thyroiditis (G-EAT).
- Research applications: general purpose, cardiovascular, immunology and inflammation, internal/organ, metabolism, sensorineural.

Technician notes:

- Hyperactive mice. Jumpy, nervous.
- Ring tail.
- Missing fur.
- Weanlings may have silver gray coats.
- Entire litters may be missing at weaning.
- Good parents.
- Older breeders prone to seizures.
- Hair loss sometimes seen in breeders after 5–6 months of age.
- Occasional rectal prolapse.
- Cages may become quickly soiled due to urine.

CByJ.RBF-Rb(8.12)5Bnr/J — Stock No. 001802

Common name: n/a **Generation:** F65 (11-DEC-07)

Former name: CBy.RBF-Rb(8.12)5Bnr/J (Changed: 15-DEC-04)
Rb5Bnr (Changed: 15-DEC-04)

Strain type: Congenic, mutant strain, chromosome aberration (Robertsonian)

Appearance: Albino; related genotype: *Tyrp1*^{b}/*Tyrp1*^{b} *Tyr*^{c}/*Tyr*^{c}

H2 haplotype: n/a

Genes/alleles: Rb(8.12)5Bnr: Robertsonian translocation, Chr 8 and 11, Unversitat Bonn/Rhein 5

Development: Created by backcrossing the Robertsonian chromosome Rb(8.12)5Bnr on the BALB/cByJ genetic background; by Dr. Muriel Davisson at The Jackson Laboratory. Donor strain: RBF/DnJ.

Characteristics and uses:
- Strain created to facilitate selection of immunoglobulin-competent hybridomas (Taggert, 1983).
- Research applications: cancer.

Technician notes: n/a

CE/J — Stock No. 000657

Common name: Extreme Dilution **Generation:** F128 (21-NOV-06)

Former name: n/a

Strain type: Inbred strain, spontaneous mutation

Appearance: Greyish white; related genotype: A^{w}/A^{w} $Tyr^{c\text{-}e}/Tyr^{c\text{-}e}$

H2 haplotype: *k*

Genes/alleles:
Cdh23^{ahl}: cadherin 23 (otocadherin); age related hearing loss 1
common names: Cdh23^{753A}, mdfw
Hc^{0}: hemolytic complement; deficient
C5-, C5-d, C5-def, C5-deficient, hc^{o}
Tyr$^{c\text{-}e}$: tyrosinase; extreme dilution
common names: c^{d}

Strain origin: Knight (1920) from a mouse trapped in Illinois. Detlefsen studied genetics of the color type. Inbred by Eaton at least 15 generations; some sent to Woolley, Sloan-Kettering.

Source: Woolley to Speirs (1946); Speirs (2 gen. from Woolley) to Woolley and JAX (1948).

Characteristics and uses:
- Homozygous for *Cdh23*^{ahl}, age-related hearing loss 1 mutation; onset prior after 10 months of age.
- Resistant to amyloid induction and development of amyloidosis.
- Low mammary tumor frequency; 33% ovarian tumors in old age.
- Some sarcomas, wide range of tumor types; high adrenal cortical carcinoma frequency when gonadectomized at birth.
- Carries a Chr Y of Asian *M.m. musculus* origin (Tucker *et al.,* 1992).
- Research applications: general purpose, cancer, metabolism, neurobiology, sensorineural, immunology and inflammation.

Technician notes:
- Very, very jumpy mice. Hyperactive. Nervous, shy; easily frightened. Handle gently.
- Occasional kinky tails.
- Fair breeders.
- Usually chew ears of young mice.
- Get fat as they age.

CZECHII/EiJ — Stock No. 001144

Common name: n/a **Generation:** F16+62 (21-NOV-06)

Former name: CZECHII/Ei (changed: 15-DEC-04)

Strain type: Wild-derived inbred strain (species: *M.m. musculus*, Czechoslovakia)

Appearance: White-bellied agouti; related genotype: A^{w}/A^{w}

H2 haplotype: n/a

Genes/alleles: n/a

Strain origin: n/a

Source: n/a

Characteristics and uses:
- Research applications: genetics research (evolution and systematics, gene mapping (numerous polymorphisms)

Technician notes:
- These mice are very difficult to handle.

DBA/1J — Stock No. 000670

Common name: D1; **Generation:** F200 (03-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Dilute brown; related genotype: *a/a Tyrp1^{b}/Tyrp1^{b} Myo5a^{d}/Myo5a^{d}*

H2 haplotype: *q*

Genes/alleles: *Cdh23^{ahl}*: cadherin 23 (otocadherin); age related hearing loss 1
common names: Cdh23^{753A}, mdfw

Rmcf^{s}: resistance to MCF virus; MCF sensitive

Strain origin: The DBA strains are the oldest inbred strains. The name comes from the three coat color genes for which the DBA strains are fixed: dilute *Myo5a^{d}*, brown *Tyrp^{b}*, nonagouti *a*. Little began inbreeding in 1909 from a mouse colony segregating for coat color. During 1929 and 1930 crosses were made among substrains; several new substrains, including DBA/1 and DBA/2, were established. DBA/1 and DBA/2 differ at a large number of loci (including the MHC *H2* histocompatibility locus), which most likely results from residual heterozygosity in the strain when the substrains were separated.

Source: Fekete (1936) to JAX to Hummell (1947), Hummel to JAX (1948).

Characteristics and uses:

- Homozygous for *Cdh23^{ahl}*, age-related hearing loss 1 mutation; onset after 10 months of age.
- Model of rheumatoid arthritis: immunization with type II collagen leads to the development of severe polyarthritis mediated by an autoimmune response.
- Intermediate susceptibility to development of atherosclerotic aortic lesions on an atherogenic diet.
- In response to challenge, develop immune-mediated nephritis (Xie *et al.,* 2004).
- Resistant to most DBA/2 transplantable tumors.
- Mammary tumors in about 75% of breeding females over 1 year of age and in some virgins after 18 months (Hoag, 1963).
- Extremely susceptible to audiogenic seizures.
- Calcareous heart deposits in almost all retired breeders.
- Susceptible to inoculated TB.
- Extreme intolerance to alcohol, morphine and related opioids.
- Research applications: general purpose, immunology and inflammation, arthritis, neurobiology, sensorineural, dermatology.

Technician notes:

- Mice jumpy; can be difficult to handle.
- Mice squeal when picked up.
- Young mice can be very jumpy; hyperactive at 2–3 weeks old. Weanlings make a lot of noise when picked up.
- Weanling coat color sometimes appears silver gray.
- Sometimes get diarrhea.
- Females are sexually mature sometimes as early as 25 days of age.
- Good breeders. Lots of missing and "born dead" pups.
- At wean age, large number of pups have short tails.
- Some litters appear runted.

DBA/1LacJ — Stock No. 001140

Common name: D1Lac; **Generation:** F92 (03-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Dilute brown; related genotype: *a/a Tyrp1^{b}/Tyrp1^{b} Myo5a^{d}/Myo5a^{d}*

H2 haplotype: *q*

Genes/alleles: *Cdh23^{ahl}*: cadherin 23 (otocadherin); age related hearing loss 1
common names: Cdh23^{753A}, mdfw

Strain origin: DBA/1LcJ, DBA/1J, and DBA/2J have a common origin. JAX to Laboratory Animal Center (Lac), Carshalton, UK (1955). Recovered from Laboratory Animal Center, Carshalton, UK Frozen Embryo Bank (1981) to Les at JAX.

Source: To Mider and Wooley at Sloan-Kettering (1938), Mider to Heston at NCI, Heston to JAX (1948) at F26. Les to JAX by hysterectomy derivation and fostering on C57BL/6J.

Characteristics and uses:

- Homozygous for *Cdh23^{ahl}*, age-related hearing loss 1 mutation; onset after 10 months of age.
- Genetic monitoring at JAX of 11 genetic markers indicates no difference between DBA/1J and DAB/1LacJ (Les, 1990).
- Low susceptibility to development of atherosclerotic aortic lesions when on an atherogenic diet.
- Analysis of 35 additional loci of DBA/1LacJ mice reported in the literature also indicate that DBA/1J and DAB/1LacJ do not differ.
- Immunization with type II collagen (CII) induces an autoimmune arthritis in susceptible DABG/1LacJH and DBA/1J mice (Joosten *et al.,* 1994; Walter *et al.,* 1996).
- Research applications: general purpose, immunology and inflammation, arthritis, neurobiology, sensorineural.

Technician notes:

- Jumpy, particularly at 3–4 weeks of age; curious when young.
- Diarrhea in the pups at wean age.
- Good parents.
- Urine burn on breeders occasionally.

DBA/2J — Stock No. 000671

Common name: D2; D2J; **Generation:** F223p (03-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Dilute brown; related genotype: *a/a Tyrp1^{b}/Tyrp1^{b} Myo5a^{d}/Myo5a^{d}*

H2 haplotype: *d*

Genes/alleles:
Cdh23^{ahl}: cadherin 23 (otocadherin); age related hearing loss 1;
common names: Cdh23^{753A}, mdfw

Gpnmb^{R150X}: glycoprotein (transmembrane) nmb; iris pigment dispersion;
common names: GPnmb^{ipd}

Hc^{0}: hemolytic complement; deficient
common names: C5-, C5-d, C5-def, C5-deficient, hc^{o}

Tyrp1^{isa}: tyrosinase-related protein 1; iris stromal atrophy
common names: isa

Strain origin: DBA/1J and DBA/2J have a common origin (see DBA/1J).

Source: To Mider and Wooley at Sloan-Kettering (1938), Mider to Heston at NCI, Heston to JAX (1948) at F26.

Characteristics and uses:
- Homozygous for *Cdh23^{ahl}*, age-related hearing loss 1 mutation; severe by 3 months of age.
- Low susceptibility to development of atherosclerotic aortic lesions when on an atherogenic diet.
- Aging mice develop progressive eye abnormalities that closely mimic human hereditary glaucoma.
- Resistant to most DBA/1 tumors.
- Tumor S91 grows in both strains.
- A few mammary tumors in old breeding females; frequency has decreased since 1955.
- C5 deficient, unlike DBA/1J.
- Audiogenic seizures: 100% of mice at 35 days; 5% after 55 days.
- High mortality in males exposed to chloroform fumes.
- High incidence of calcareous heart deposits.
- Extreme intolerance to alcohol, morphine and related opioids (Phillips *et al.,* 1994), morphine (Berrettini *et al.,* 1994; Belknap *et al.,* 1995).
- Characteristics often contrasted with C57BL/6J because of their genetic disparity.
- Research areas: general purpose, cardiovascular, developmental biology, neurobiology, sensorineural, immunology and inflammation, dermatology.

Technician notes:
- Jumpy; easily frightened by loud noises. Squeal when picked up.
- Wean coat color sometimes appears silver gray.
- Difficult to sex at weaning.
- Females are sexually mature very early (approximately 25 days).
- Some litters may appear runted.
- Very dirty; diarrhea more common in this strain.

FVB/NJ — Stock No. 001800

Common name: FVB; Friend Virus B NIH Jackson; **Generation:** F99 (03-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Albino; related genotype: *A/A Tyr^{c}/Tyr^{c}*

H2 haplotype: *q*

Genes/alleles::
Hc^{0}: hemolytic complement; deficient
common names: C5-, C5-d, C5-def, C5-deficient, hc^{o}

Pde6b^{rd1}: phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide; retinal degeneration 1
common names: rd, rd-1, rd1, rodless retina

Fv1^{b}, Friend virus susceptibility 1; susceptibility
common names: Fv-1, Rv-1, Rv1

Strain origin: NIH to Taketo (1987) to Mobraaten (1988). Outbred Swiss mice N:NIH established in the early 1940s. In 1966 mice selected from this colony for histamine resistance (HSFR/N). Homozygous *Fv1^{b}* mice from the HSFS/N line were inbred as strain FVB without further selection (Taketo *et al*., 1991).

Source: Mobraaten to JAX (1990).

Characteristics and uses:

- Homozygous for the retinal degeneration allele *Pde6b^{rd1}*.
- Has not been found to develop spontaneous tumors, but is highly susceptible to chemically-induced squamous cell carcinomas with a high rate of malignant conversion from papilloma to carcinoma.
- Resistant to collagen-induced arthritis.
- Higher than average activity, anxiety, and basal body temperature, and low stress-induced hyperthermia.
- Prone to allergen induced, asthma-like, bronchial hyperresponsiveness.
- Pronuclei of zygotes are large and easily identified, thus facilitating efficient production of transgenics (Taketo *et al.,* 1991).
- FVB/N mice are good breeders; have consistently large litters with a mean of 9.5 pups/litter.
- Sex ratio: 53% females, 47% males.
- Research applications: general purpose, sensorineural, immunology and inflammation.

Technician notes:

- Calm, clean mice.
- Big eaters.
- Good mothers.
- Large litters.
- Aggressive males; we wean males 10 per weaning cage. Retired breeder males housed 1 per box (or they will fight).

HRS/J — Stock No. 000673

Common name: Hairless **Generation:** F93 (date unknown)

Former name: n/a

Strain type: Inbred strain, mutant strain, spontaneous mutation

Appearance: Unpigmented, without hair; related genotype: *Tyrp1^{b}/Tyrp1^{b} Tyr^{c}/Tyr^{c} Myo5a^{d}/Myo5a^{d} Hr^{hr}/Hr^{hr}*

Albino, unaffected; related genotype: *Tyrp1^{b}/Tyrp1^{b} Tyr^{c}/Tyr^{c} Myo5a^{d}/Myo5a^{d} Hr^{hr}/+*

H2 haplotype: *k*

Genes/alleles: *Hr^{hr}*: hairless; hairless
common name: hr

Strain origin: Developed by FAE Crew (Crew *et al.*, 1931) from a pair of mice received from Mr. HC Brooke, a well-known British fancier, captured in an aviary in London (Brooke, 1926). Chase to EL Green (1952), to Les (1956), to MC Green (1959), to Lane.

Source: Lane to JAX (1964) at F24.

Characteristics and uses:

- Albino strain carrying hairless (*hr*) gene. The hairless mutation was caused by a naturally occurring provirus integration (Stoye *et al.,* 1988).
- Homozygous hairless start to lose their hair at about 10 days. Eventually hair loss is total. Some *hr*/+ females have significant hair loss after 4 months of age.
- Carries a Type 1B *M.m. musculus* Chr Y (Tucker *et al.,* 1992).
- Leukemia frequency (C-type MuLV) related to mutant gene dose: 1% in +/+ HRS/J, 20% in heterozygotes, 45% in hairless by 8–10 months, virtually all by 28 months (Meier *et al.,* 1969; Heiniger *et al.,* 1976).
- Strain maintained by breeding haired (*hr/+*) female x hairless (*hr/hr*) male.
- Research applications: cancer, cardiovascular, dermatology, immunology and inflammation, dermatology.

Technician notes:

- Can be jumpy.
- Weanlings squeak a lot when handled.
- Mice not real dirty but not overly clean.
- Big eaters.
- Hair flies when changing litters at 2–3 weeks of age.

I/LnJ — Stock No. 000674

Common name	I Lyon; **Generation:** F144 (22-NOV-06)
Former name	n/a
Strain type	Inbred strain
Appearance	Pink-eyed dilute brown, piebald (spotted); related genotype: *a/a Tyrp1^{b}/Tyrp1^{b} Oca2^{p}/Oca2^{p} Myo5a^{d}/Myo5a^{d} Ednrb^{s}/Ednrb^{s}*
H2 haplotype	*j*
Genes/alleles	*Ednrb^{s}*: endothelin receptor type B; piebald common name: s *Hc^{0}*: hemolytic complement; deficient common names: C5-, C5-d, C5-def, C5-deficient, hc^{o}
Strain origin	Strong (1926) from unpedigreed mice (Strong, 1942).
Source	Fenton, Brown University to JB Lyon, Emory University (1954), to ES Russell at JAX (1969).
Characteristics and uses	• Carries the Oca2^{p} allele at the *Phk* locus. • Phosphorylase kinase activity virtually absent in muscle and reduced in the brain, kidney, and heart. • Spontaneous occurrence of absent corpus callosum (Livy and Wahlsten, 1991; Gruber *et al.,* 1991). • Carries the allele for long incubation period at the *Prn-i* (*Sinc*) locus, determining incubation period of the scrapie agent. • Carries a Chr Y of Asian *M.m. musculus* origin (Tucker *et al.,* 1992). • Research applications: neurobiology, reproductive biology, sensorineural, dermatology, developmental biology, immunology and inflammation.
Technician notes	• Very docile. • Poor breeders.

JF1/Ms — Stock No. 003720

Common name: Japanese Fancy Mouse 1 **Generation:** F26+6+18 (24-MAY-08)

Former name: JF1/Msf (changed: 26-OCT-05)

Strain type: Wild-derived inbred strain (species: *M.m. molossinus*, Japan)

Appearance: Black-spotted white coat, black eyes; related genotype: *a/a Ednrb^{s}/Ednrb^{s}*

H2 haplotype: n/a

Genes/alleles: n/a

Development Mice of a stock identified as a Japanese Fancy Mouse were purchased from a market in Denmark in 1987 and maintained by sibling inbreeding at the National Institute of Genetics, Mishima, Japan. In 1993, at generation F20, the established inbred strain was named JF1 (Japanese Fancy Mouse 1). Genetic analysis of multiple markers led to the conclusion that JF1 is of the species *Mus musculus molossinus* (Koide *et al.,* 1998). Mice of this strain were imported by JAX in May 2000 from Dr. Toshihiko Shiroishi.

Characteristics and uses:
- Research applications: genetics research (evolution and systematics, gene mapping [numerous polymorphisms]).

Technician notes:
- These mice are very difficult to handle.

KK/HlJ — Stock No. 002106

Common name: Kasukabe **Generation:** F107+7 (24-MAY-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Albino; related genotype: *a/a Tyr^{c}/Tyr^{c}*

H2 haplotype: *b*

Genes/alleles: *Cdh23^{ahl}*: cadherin 23 (otocadherin); age related hearing loss 1
common names: Cdh23^{753A}; mdfw

Development: The KK inbred strain was inbred by K Kondo in 1944. Many of the KK stock carry the yellow (*A^{y}*) allele. The KK/HlJ substrain, wild-type for the agouti gene, was provided by Dr. Leiselotte Herberg, Diabetes Research Institute, Dusseldorf, Germany.

Characteristics and uses:
- Males exhibit diabetic symptoms that include hyperglycemia, hyperinsulinemia, insulin resistance.
- Reports indicate that the KK strain is hemolytic complement deficient (*Hc^{0}*); however our strains have not been tested to confirm this allele (Cinader *et al.,* 1964).
- Serves as a model of noninsulin-dependent, type 2 diabetes.
- Research applications: diabetes and obesity, neurobiology, sensorineural.

Technician notes: n/a

LP/J — Stock No. 000676

Common name: n/a **Generation:** F194 (03-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: White-bellied agouti, piebald; related genotype: A^{w}/A^{w} $Ednrb^{s}/Ednrb^{s}$

H2 haplotype: *bc* (Fischer Lindahl K 1997)

Genes/alleles:
$Cdh23^{ahl}$: cadherin 23 (otocadherin); age related hearing loss 1
common names: $Cdh23^{753A}$, mdfw

$Disc1^{del}$; disrupted in schizophrenia 1; deletion
common name: $Disc1^{129S6}$

$Ednrb^{s}$; endothelin receptor type B; piebald
common name: s

Strain origin: Dunn (1928) from a chinchilla stock from Castle and some coat color stocks from English fanciers. To Scott at JAX, to Dickie at JAX (1947) at F30.

Source: Dickie to JAX (1949) at F33.

Characteristics and uses:
- Homozygous for $Cdh23^{ahl}$, age-related hearing loss 1 mutation; onset after 10 months of age.
- Fairly high incidence of tumors that develop later in life, including mammary tumors, lymphoma, lung and soft-tissue sarcomas.
- Carries a Chr Y of Asian *M.m. musculus* origin (Tucker *et al.,* 1992).
- Varied types of late occurring tumors; very long lived.
- Develops bony lesions that are grossly and histologically similar to the lesions of human otosclerosis.
- Research applications: cancer, neurobiology, sensorineural, dermatology, developmental biology.

Technician notes:
- Active mice. Nervous. "Crawly." Don't like to stay in uncovered cages. Jumpy.
- Poor breeders. Poor mothers; like to kill first litter.
- Active nest builders. We use Nestlets®.
- Pile up shavings under water bottle, resulting in wet boxes.

MA/MyJ — Stock No. 000677

Common name: Marsh Murray — **Generation:** F180+18 (23-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Albino; related genotype: Tyr^{c}/Tyr^{c}

H2 haplotype: *k*

Genes/alleles: $Cdh23^{ahl}$: cadherin 23 (otocadherin); age related hearing loss 1
common names: $Cdh23^{753A}$; mdfw
hf: hepatic fusion; hepatic fusion

Strain origin: Marsh from Lathrop-Loeb colony (1903–1915). (MA = Marsh's albino). Murray and Warner separated this strain from MA after 37 generations.

Source: Warner to JAX (1948) at F24.

Characteristics and uses:
- Homozygous for $Cdh23^{ahl}$, age-related hearing loss 1 mutation; onset prior to 3 months of age.
- Spontaneous mutation of *hf* results in varying degrees of fusion in the hepatic lobes.
- Very low spontaneous mammary tumor frequency.
- Diabetes-resistant strain (Leiter, 1989).
- Polyuria and polydipsia marked in breeding females over 6 months (Bernstein, 1966).
- Carries a Type 4 Chr Y of U.S. *M.m. domesticus* origin (Tucker *et al.,* 1992).
- Cysts in neural and intermediate lobes of pituitary in over 50% of the mice.
- Susceptible to skin lesions.
- High systolic blood pressure (128 mm Hg).
- Short breeding life.
- Research applications: neurobiology, sensorineural.

Technician notes:
- Easy mice to work with.
- Poor breeders; may not have first litter until 2 months after mating.
- Chewed tails occasionally.
- Small litter size; missing litters frequent.

MOLF/EiJ — Stock No. 000550

Common name: n/a — **Generation:** ?+F2 (21-NOV-06)

Former name: n/a

Strain type: Wild-derived inbred strain (species: *M.m. molossinus*; Fukuoka, Kyushu, Japan)

Appearance: White-bellied agouti; related genotype: A^{w}/A^{w}

H2 haplotype: n/a

Genes/alleles: $Pde6b^{rd1}$: phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide; retinal degeneration 1; common names: rd, rd-1, rd1, rodless retina

Development: MOLC, MOLD, and MOLF were all independently inbred from single pairings of related *M.m. molossinus* mice held by M Potter at NIH. Sent to TH Roderick and EM Eicher at JAX in 1969.

Characteristics and uses:
- Homozygous for the retinal degeneration allele $Pde6b^{rd1}$.
- Research applications: sensorineural, genetics (evolution and systematics, gene mapping [numerous polymorphisms]).

Technician notes:
- These mice are very difficult to handle.

MRL/MpJ — Stock No. 000486

Common name: n/a **Generation:** F137 (03-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Albino, unaffected; related genotype: *a/a Tyr^{c}/Tyr^{c} Fas^{+}/Fas^{+}*

H2 haplotype: *k*

Genes/alleles: *n/a*

Strain origin: Murphy at JAX from a series of crosses involving strains C57BL/6J, C3H/HeDi, AKR/J and LG/J, followed by inbreeding. *Fas^{lpr}* (lymphoproliferation) mutation found at F12.

Source: Murphy to barrier facility at JAX (1980) by hysterectomy derivation; fostered on C57BL/6J at F37.

Characteristics and uses:

- Homozygous for the normal allele of *Fas^{lpr}*; this strain serves as a homozygous normal control for MRL/MpJ-*Fas^{lpr}*/J (000485).
- Estimated to have derived 75% of genome from LG/J.
- Necrotizing arteritis is common.
- Antinuclear antibodies appear at 10 months in most mice.
- Females die at 73 weeks, males at 93 weeks, with chronic glomerulonephritis.
- One sixth of mice autopsied have reticulum cell neoplasia.
- Females heal faster and more completely than males (Blankenhorn *et al.,* 2003).
- Used as animal model for rheumatoid arthritis (Axford *et al.,* 1994; Hackshaw *et al.,* 1994), Sjogren's Syndrome (Sato and Sullivan, 1994; Hayashi *et al.,* 1995) and systemic lupus erythematosis (SLE) (Granholm and Cavallo, 1994; Green *et al.*, 1995).
- Research applications: immunology and inflammation, internal/organ, neurobiology, sensorineural.

Technician notes:

- Large body size. Docile.
- Large litter size.

MSM/Ms — Stock No. 003719

Common name: M.MOL-MSM **Generation:** F67+16 (24-MAY-08)

Former name: n/a

Strain type: Wild-derived inbred strain (species: *M. m. molossinus*; Mishima City, Shizuoka Prefecture, Japan)

Appearance: White-bellied agouti; related genotype: *A^{w}/A^{w}*

H2 haplotype: n/a

Genes/alleles: n/a

Development: The MSM inbred strain was derived from *M. m. molossinus* mice trapped in Mishima Prefecture, Japan, April 1978, and maintained by Dr. Kazuo Moriwaki (Okumoto *et al.,* 1995). Mice were imported to JAX May 2000 from Dr Moriwaki's colleague, Professor Toshihiko Shiroishi.

Characteristics and uses:

- Resistant to development of lymphoma, due to at least 2 loci identified in crosses involving strain SL/Kh (Pataer *et al.,* 1996).
- C3HxMSM F1 hybrids treated with MNU develop squamous cell carcinomas of the forestomach, with about 20% and 15% having mutation in *H-ras* and *p53* genes, respectively (Masui *et al.,* 1997).
- Research applications: cancer, genetics (evolution and systematics; gene mapping [numerous polymorphisms])

Technician notes:

- These mice are very difficult to handle.

NOD.CB17-*Prkdc*^{scid}/J — Stock No. 001303

Common name:	NOD SCID; NOD *scid* — **Generation:** N10F62 (03-JAN-08)
Former name:	n/a
Strain type:	Mutant strain, spontaneous mutation
Appearance:	Albino; related genotype: *A*/*A* *Tyr*^{c}/*Tyr*^{c}
H2 haplotype:	g^{7}
Genes/alleles:	*Prkdc*^{scid}: protein kinase, DNA activated, catalytic polypeptide; severe combined immunodeficiency common name: scid *Hc*^{0}: hemolytic complement; deficient common names: C5-, C5-d, C5-def, C5-deficient, hc^{o}
Strain origin:	*Prkdc*^{scid} occurred spontaneously in a colony of BALB/c-*Igh*^{b} (C.B-17) mice maintained at the Institute for Cancer Research in Philadelphia, PA. The *Prkdc*^{scid} mutation was backcrossed onto the NOD/ShiLt background as follows: an NOD/ShiLt female was bred to a C.B-17-*Prkdc*^{scid} male; male *Prkdc*^{scid}/+ offspring of the F1/N1 and subsequent generations were mated to NOD/ShiLt females for a total of 10 crosses to NOD/ShiLt. At generation N10, *Prkdc*^{scid} was made homozygous by sister–brother inbreeding.
Characteristics and uses:	• Severe combined immunodeficiency; must be maintained in SPF conditions. • Development of serum antibody, "leakiness," is lower on the NOD genetic background than on other genetic backgrounds. • Under SPF conditions, have a high incidence of thymic lymphomas, which limits the lifespan to about 8.5 months. • Low NK cell activity. • Hypogammaglobulinemia (no detectable IgM, IgG1, IgG2a, IgG2b, IgG3, or IGA). • No hemolytic complement activity. • Insulitis- and diabetes-free throughout life; serves as diabetes-free control for NOD/ShiLtJ mice (001976). • Excellent hosts for xenografts; may be useful for delineation of the role of T cell subsets in autoimmune diabetes; can serve as a source for NOD insulitis-free islets. • Research applications: cancer, diabetes and obesity, immunology and inflammation, internal/organ, virology.
Technician notes:	• Very active; wean are jumpy. • Sometimes mice have tails that are abnormal—kinky, set to one side, higher than normal. • Pair mating is recommended. • Breed very well; large litters. • Strain-related illness sets in as mice reach retiring age; often die just before retiring.

NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ — Stock No. 005557

Common name: NOD *scid* gamma; NSG; NOD-*scid* IL2Rgamma^{null}; NOD-*scid* IL2Rg^{null}; **Generation:** N8F?+4pF2 (03-JAN-08)

Former name: n/a

Strain type: Congenic, mutant strain, spontaneous mutation, targeted mutation

Appearance: Albino; related genotype: *A*/*A* *Tyr*^{c}/*Tyr*^{c}

H2 haplotype: g^{7}

Genes/alleles: *Il2rg*^{tm1Wjl}: interleukin 2 receptor, gamma chain; targeted mutation 1, Warren J Leonard
common names: [KO]gammac, CD132^{-}, gammac^{-}, IL2Rgamma^{null}

Prkdc^{scid}: protein kinase, DNA activated, catalytic polypeptide; severe combined immunodeficiency
common names: scid

Development: These double mutant mice were produced by breeding female NOD.CB17-*Prkdc*^{scid}/J (001303) mice with male mice bearing the X-linked B6.129S4-*Il2rg*^{tm1Wjl}/J allele (003174). The resulting male mice, heterozygous for the *Prkdc*^{scid} allele and hemizygous for the *Il2rg*^{tm1Wjl} allele, were crossed to female NOD.CB17-*Prkdc*^{scid}/J (001303) mice for 8 generations. Heterozygotes were interbred to produce mice homozygous for the *Prkdc*^{scid} allele and homozygous (females) or hemizygous (males) for the *Il2rg*^{tm1Wjl} allele. Donating investigator: Leonard Shultz (JAX).

Characteristics and uses:

- Superior human hematopoietic engraftment.
- Significant human lymphoid expansion.
- Significantly diminished NK activity; improves quality and duration of xenografts.
- Lacks mature lymphocytes (B and T cells) without leakiness.
- Newborn recipients do not require IL-7 for thymopoiesis.
- Does not produce detectable serum immunoglobulin.
- Resistance to lymphoma leads to longer lifespan (>16 months) than that of NOD.Cg-*Prkdc*^{scid} mice.
- Supports adoptive transfer of diabetic T cells without irradiation.
- Superior for HIV and other infectious disease research because of improved lymphoid expansion.
- Research applications: cancer, immunology and inflammation, internal/organ, virology.

Technician notes:

- Because of severe immunodeficiency, mice shold be handled with smooth-tipped forceps that have been soaked in a disenfectant solution.
- Handlers should dip gloved hands into disinfectant between touching the outside of the cage and handling the mice.
- Even with severe immunodeficiency, mice often survive past 9–10 months in SPF colonies.
- Great breeders; rarely lose litters.
- Improved productivity when mice are first bred at 5–6 weeks.
- Change in food or environment can have pronounced effects on productivity.
- Mice love to burrow and nest; we supply nesting material as environmental enrichment.
- 5–10% of breeders die before 7 months.

NOD/ShiLtJ — Stock No. 001976

Common name: Non-obese Diabetic **Generation:** F120 (03-JAN-08)

Former name: NOD/LtJ (changed: 23-FEB-07)

Strain type: Inbred strain

Appearance: Albino; related genotype: *A/A Tyr^{c}/Tyr^{c}*

H2 haplotype: *g7*

Genes/alleles: *Cdh23^{ahl}*: cadherin 23 (otocadherin); age related hearing loss 1
common names: Cdh23^{753A}, mdfw

Hc^{0}: hemolytic complement; deficient;
common names: C5-, C5-d, C5-def, C5-deficient, hc^{o}

Development: NOD inbred mice originated early in the inbreeding of the Cataract Shionogi (CTS) strain. Originally, the mice were outbred Jcl:ICR mice. At F6, the progenitors of the future NOD/Shi mice were inbred on the basis of an elevated fasting blood glucose level in cataract-free mice. At F13, the NOD progenitors were separated from what is now the NON/Shi strain. High fasting blood glucose levels continued to be the basis for selection of the latter strain, while the NOD progenitors at F13 and later were selected on the basis of normal fasting blood glucose level. In 1974, at F20, a female in the "normoglycemic" line spontaneously developed overt insulin-dependent diabetes mellitus with insulitis (IDDM). Selective breeding of the progeny of this diabetic female produced the nonobese diabetic (NOD) strain. Originally restricted to distribution in Japan, NOD substrains were distributed during the early 1980s to Australia and the United States. NOD and NON strains were imported from a colony in Kyoto, Japan, by Dr. M. Hattori to the Joslin Diabetes Center in Boston in 1984. Breeder pairs from Joslin Diabetes Center were sent to Dr. E. Leiter at JAX and are the source of the production strains NOD/ShiLtJ and NON/ShiLtJ (002423).

Characteristics and uses:

- Polygenic model for type 1 diabetes, characterized by insulitis, a leukocytic infiltration of the pancreatic islets. Marked decreases in pancreatic insulin content occur in females at about 12 weeks of age; in males, several weeks later. Consequently, plasma glucose levels increase to greater than 250 mg/dl.
- Females develop diabetes at an incidence of 90–100% by 30 weeks of age; males at 40–60% by 30–40 weeks.
- Exhibit pancreatic beta cell destruction.
- Major component of diabetes susceptibility in NOD mice is the unique MHC haplotype (*H2^{g7}* = K^{d}, Aa^{d}, Ab^{g7}, E^{null}, D^{b}).
- Homozygous for *Cdh23^{ah1}*, the age-related hearing loss 1 mutation; mice are deaf by 3 months of age.
- Aberrant immunophenotypes include defective antigen-presenting cell functions, defects in regulation of the T lymphocyte repertoire, defective NK cell function, defective cytokine production from macrophages, impaired wound healing, C5 complement deficiency.
- Susceptibility to diabetes influenced by environmental factors, including housing conditions, health status, microbiologic status of the colony, and diet.
- Research applications: developmental biology, diabetes, immunology and inflammation, internal/organ, neurobiology, sensorineural.

Technician notes:

- Mice are hyperactive, jumpy, nervous at 4 weeks and older. Forceps and noise seem to make it worse.
- Mice may be aggressive toward humans.
- Mice are large and have large litters; pair mating is recommended.
- Have strong odor when they turn positive for diabetes.
- Mice are injected with Complete Freund's Adjuvant (FAC) and delayed mated. They turn positive for diabetes less often, so a normal rotation has been made possible for the most part.

NON/ShiLtJ — Stock No. 002423

Common name: Non-obese Non-diabetic **Generation:** F124 (05-DEC-07)

Former name: NON/LtJ (changed: 23-FEB-07)

Strain type: Inbred strain

Appearance: Albino; related genotype: *A/A Tyr^{c}/Tyr^{c}*

H2 haplotype: *nb1*

Genes/alleles: *Pde6b^{rd1}*: phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide; retinal degeneration 1 common names: rd, rd-1, rd1, rodless retina

Strain origin: The NON inbred strain arose out of a colony originally developed by selection for high fasting blood glucose early in the inbreeding of the Cataract Shionogi (CTS) strain. These mice were originally outbred Jc1:ICR mice A cataract-free substrain was initiated at F6, with selection made on the basis of a high fasting blood glucose. This line was separated from the future NOD/Shi line at F13, and originally designated NON for nonobese, nondiabetic.

Characteristics and uses:

- Homozygous for the retinal degeneration allele *Pde6b^{rd1}*.
- Although closely related to NOD mice, NON mice contain a diabetes resistant MHC haplotype (*H2^{nb1}* = K^{b}, A^{nb1}, E^{k}, D^{b}).
- Name derived from "Non-Obese Non-diabetic"; however, NON/ShiLtJ mice should not be considered "normal."
- Although ALS/Lt males share the impaired glucose tolerance phenotype of NON/ShiLt males, the ALS/Lt males differ in developing progressive hyperinsulinemia as they gain weight, whereas NON/ShiLt males fail to hypersecrete insulin as they gain weight as adults, suggesting a potential beta cell response defect.
- Maintains low plasma insulin level, which may account in part why certain loci from NON/ShiLt can enhance development of autoimmune type 1 diabetes in segregating hybrid mice following outcross to NOD/ShiLtJ.
- NON strain-characteristic immunologic abnormalities include perivascular/periductular leukocyte infiltration into the pancreas and submandibular salivary glands, and T lymphocytopenia evident by 20 weeks of age in spleen.
- NON/ShiLt mice clearly harbor genes predisposing to type 2 diabetes, as evidenced by early impaired glucose tolerance in males and females, development of moderate mature-onset obesity in the presence of low plasma insulin levels, and development of glomerulosclerotic kidney lesions.
- Research applications: diabetes and obesity, immunology and inflammation, sensorineural.

Technician notes:

- Aggressive.
- Tend to be non-productive.
- Play with their food; seem to pull it down but not eat it, but rather leave a lot at the bottom of the cage

NONcNZO10/LtJ — Stock No. 004456

Common name: RCS-10 **Generation:** N1F11+8 (16-NOV-05)

Former name: RC-10 (Changed: 15-DEC-04)

Strain type: Recombinant congenic strain

Appearance: Albino; related genotype: *A/A Tyr^{c}/Tyr^{c}*

H2 haplotype: *nb1*

Genes/alleles: n/a

Development: A recombinant congenic developed by introgressing 6 known diabesity QTLs from the type 2 diabetes-prone and obese NZO/HlLt inbred strain into the nominally nonobese and diabetes-resistant NON/ShiLt strain background. NZO-derived QTLs include those marked by D1Mit411, D5Mit7, D11Mit261/D11Mit41, D12Mit231 and D15Mit159. Donating investigator: Edward Leiter (JAX).

Characteristics and uses:

- Polygenic model of human metabolic syndrome and type 2 diabetes.
- Type 2 diabetes in males results from polygenic interactions, producing a moderate obesity rather than the massive obesity elicited by the single locus diabesity mutant models, such as the BKS.V- *Lep^{ob}*/J (000696) and the BKS.Cg-*m* +/+ *Lepr^{db}*/J (000642) strains.
- Progressively increasing plasma glucose (280–600 mg/dl) with age.
- Moderately increased serum and leptin.
- Elevated serum triglycerides.
- Unlike mice with monogenic obesity syndromes, males do not exhibit hypercorticism, are not hyperphagic, and show no obvious thermoregulatory defects.
- Males weaned onto chow with 10–11% fat (wt/wt) develop visceral obesity, maturity-onset hyperglycemia, dyslipidemia, moderate liver steatosis, and pancreatic islet atrophy.
- Differentially sensitive to adverse hepatic side effects of thiazolidinediones; may be useful for pharmacogenetic analysis.
- Research applications: diabetes and obesity.

Technician notes: n/a

NZB/BlNJ — Stock No. 000684

Common name: New Zealand Black **Generation:** F206 (03-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Black; related genotype: *a/a*

H2 haplotype: *d2* (Fischer Lindahl K, 1997)

Genes/alleles: Hc^{0}: hemolytic complement; deficient;
common names: C5-, C5-d, C5-def, C5-deficient, hc^{o}
$Pctp^{R120H}$: phosphatidylcholine transfer protein; R120H

Strain origin: Outbred mice from Imperial Cancer Research Fund, London, to University of Otago Medical School, New Zealand (1930). Inbred by Bielschowsky (1948).

Source: NIH to ES Russell to JAX (1969).

Characteristics and uses:

- Displays a number of autoimmune abnormalities, including hemolytic anemia, elevated levels of immunoglobulin, anti-DNA antibodies, anti-thymocyte antibodies, and circulating immune complexes causing glomerulonephritis.
- Develops autoimmune hemolytic anemia of the Coombs positive type and a nephropathy (Bielschowsky *et al.,* 1959).
- Carries a Type 1 B *M.m. musculus* origin Chr Y (Tucker *et al*., 1992).
- NZB/BlNJ mice fed an atherogenic diet fail to develop atherosclerotic aortic lesions.
- Poor reproductive performance.
- High frequency of chronic gastroduodenal ulceration.
- F1 hybrids of NZB/BlNJ and NZW/LacJ (NZBWF1/J [100008]) are widely used as a model for autoimmune disease resembling human systemic lupus erythematosus.
- In hybrids with C57BL there is late-appearing positive direct systemic lupus erythematosis (Hayes and Greiner, 1992).
- Research applications: cardiovascular, developmental biology, hematological, immunology and inflammation.

Technician notes:

- Appear “dumb.”
- Aggressive with each other. For males, we house only littermates together.
- Barbering in breeders and weanlings.
- Older breeders have a brownish tint to coat color.
- Many missing litters.
- High incidence of non-productive.
- Females get chubby with age.
- Build nests under water bottle, resulting in wet nests.
- We use Nestlets® for breeders.

NZW/LacJ	**Stock No. 001058**
Common name:	New Zealand White **Generation:** F84 (03-JAN-08)
Former name:	n/a
Strain type:	Inbred strain
Appearance:	Albino; related genotype: *Tyrp1^{b}/Tyrp1^{b} Oca2^{p} Tyr^{c}/Oca2^{p} Tyr^{c}*
H2 haplotype:	*z*
Genes/alleles:	*Pctp^{R120H}*: phosphatidylcholine transfer protein; R120H
Strain origin:	From same outbred stock as NZB but inbred and selected for white color by Hall (1952).
Source:	Recovered from Laboratory Animal Center, Carshalton, UK Frozen Embryo Bank (1981) to S Bernstein at JAX, to JAX by hysterectomy derivation and fostering on C57BL/6J.
Characteristics and uses:	• Normal lifespan, but develop anti-DNA antibodies, high serum levels of retroviral gp70 antigen, and nephritis later in life. • Express glomerulonephritis beginning at 13 months. • High frequency of exencephaly, predominantly in female mice (Vogelweid *et al.,* 1993). • F1 hybrids of NZB/BlNJ and NZW/LacJ (NZBWF1/J [100008]) are widely used as a model for autoimmune disease resembling human systemic lupus erythematosus. • Research applications: hematological, immunology and inflammation.
Technician notes:	• Calm. Curious. • Small litters. • Fair to good breeders. Good parents.

P/J — Stock No. 000679

Common name: n/a **Generation:** F231 (21-NOV-06)

Former name: n/a

Strain type: Inbred strain

Appearance: Pink-eyed fawn, short ear; related genotype: *a/a*, *Tyrp1^{b}/Tyrp1^{b}*, *Oca2^{p}/Oca2^{p}*, *Bmp5^{se} Myo5a^{d}/Bmp5^{se} Myo5a^{d}*

H2 haplotype: *p*

Genes/alleles: *Cdh23^{ahl}*: cadherin 23 (otocadherin); age related hearing loss 1
common names: Cdh23^{753A}; mdfw

Pde6b^{rd1}: phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide; retinal degeneration 1
common names: rd, rd-1, rd1, rodless retina

Strain origin: Snell, extracted following outcross of strain BDP, which was developed by Gates. Sent to McNutt at Wisconsin at F35.

Source: McNutt to JAX (1948) at F39.

Characteristics and uses:

- Homozygous for *Cdh23^{ahl}*, age-related hearing loss 1 mutation; onset after 10 months of age.
- Homozygous for the retinal degeneration allele *Pde6b^{rd1}* (Sidman and Green, 1965).
- High incidence of lymphatic leukemia.
- Carries an Asian *M.m. musculus* Chr Y (Tucker *et al.,* 1992).
- High resistance to chronic whole-body X-irradiation.
- Highly susceptibility to audiogenic and electroconvulsive seizures (Deckard *et al.,* 1976).
- Susceptible to the induction of colon carcinogenesis (Rosenberg and Liu, 1995).
- Research applications: cancer, dermatology, developmental biology, neurobiology, sensorineural.

Technician notes:

- Small litters; many born dead.
- Occasional kinky tails.

PERA/EiJ — Stock No. 000930

Common name: n/a **Generation:** F101 (23-JAN-08)

Former name: n/a

Strain type: Wild-derived inbred strain (species: *M.m. domesticus,* Rimac Valley, Peru)

Appearance: Agouti; related genotype: *A/A*

H2 haplotype: *k*

Genes/alleles: *Lith9$^{PERA/EiJ}$*: lithogenic gene 9; PERA/EiJ

Development: In 1961, founders were trapped for ME Wallace, University of Cambridge, by O Atteck in a Peruvian yard that was used to dry maize cobs in Nana Village, Rimac Valley, Peru. These mice were held in a small closed colony by Wallace. In 1971, at approximately F25, members of this colony were sent to EM Eicher and TH Roderick at JAX, where inbreeding of mice continued.

Characteristics and uses:

- Research applications: genetics (evolution and systematics, gene mapping [numerous polymorphisms]).

Technician notes:

- These mice are very difficult to handle.

PL/J — Stock No. 000680

Common name: n/a **Generation:** F209 (03-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Albino; related genotype: *Tyr^{c}/Tyr^{c}*

H2 haplotype: *u*

Genes/alleles: *Pde6b^{rd1}*: phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide; retinal degeneration 1 common names: rd, rd-1, rd1, rodless retina

Strain origin: From noninbred Princeton stock started in 1922 from 200 mice purchased from a dealer. Inbred by Lynch, Rockefeller Institute, giving rise to a high leukemia strain (PL) and a second strain (PLA) with lower frequency.

Source: Lynch to JAX (1951) at F22.

Characteristics and uses:

- Homozygous for the retinal degeneration allele *Pde6b^{rd1}* (Sidman and Green, 1965).
- Develops clinical and histological experimental allergic encephalomyelitis (EAE) (Zamvil *et al.,* 1987), with high mortality.
- Reports of leukemia incidence vary from 50% in females and 19% in males to 80–90%.
- Low percentage of mammary tumors.
- Carries a Type 4 U.S. *M.m. domesticus* origin Chr Y (Tucker *et al.,* 1992).
- In addition to low threshold to electroconvulsive seizures, mice are susceptible to handling and rhythmic tossing-induced seizures (Kitami T *et al.,* 2004).
- Abnormal diploid/binucleated sperm cells found in this strain (Burkhart and Malling, 1989).
- Research applications: cancer, immunology and inflammation, neurobiology, sensorineural.

Technician notes:

- Gentle but active mice.
- Eyes sometimes different sizes.
- Fair breeders.
- Older breeders may have seizures.
- Most weanlings have bent tails.
- Weanlings often spin.

PWK/PhJ — Stock No. 003715

Common name: n/a **Generation:** F69+3+16 (24-MAY-08)

Former name: n/a

Strain type: Wild-derived inbred strain (species: *M.m. musculus,* Prague; Lhotka, Czech Republic)

Appearance: Agouti; related genotype: *A/A*

H2 haplotype: n/a

Genes/alleles: n/a

Development: PWK/Ph is descended by sibling mating from a single pair of mice of the subspecies *Mus m. musculus* caught in 1974 in Lhotka, Czech Republic (Von Deimling *et al.,* 1988). The strain was imported into JAX in May 2000 by Dr. Jiri Forejt, Institute of Molecular Genetics, Prague.

Characteristics and uses:
- Research areas: reproductive biology, genetics (evolution and systematics; gene mapping [numerous polymorphisms]).

Technician notes:
- These mice are very difficult to handle.

RBF/DnJ — Stock No. 000726

Common name: POSF **Generation:** F108 (1995)

Former name: n/a

Strain type: Inbred strain, chromosomal aberration (Robertsonian, translocation)

Appearance: Albino; related genotype: Tyr^{c}/Tyr^{c} $Mc1r^{E\text{-}tob}/Mc1r^{E\text{-}tob}$

H2 haplotype: n/a

Genes/alleles: $Mc1r^{E\text{-}tob}$: melanocortin 1 receptor; tobacco darkening
common name: E^{tob}

$Rd3^{rd3}$: retinal degeneration 3

Rb(9.14)6Bnr, Robertsonian translocation, Chr 9 and 14; Chr 14; Universitat Bonn/Rein 6

Rb(1.3)1Bnr, Robertsonian translocation, Chr 1 and 3; Chr 1; Universitat Bonn/Rhein 1

Rb(8.12)5Bnr, Robertsonian translocation, Chr 8 and 12; Chr 12; Universitat Bonn/Rein 5

Strain origin: RBF strain originated with inbred sister–brother from F1 hybrids between wild-derived *Mus musculus poschiavinus* (*domesticus*) mice, captured in Valle de Poschiavo in S.E. Switzerland, and Swiss NMRI/Han (by A. Gropp, Lubéck, Germany.

Source: Gropp to Roderick (1969), to Davisson (1981), to barrier facility at JAX (1984) via hysterectomy derivation and fostering on C57BL/6J.

Characteristics and uses:
- Homozygous for Robertsonian translocations Rb(1.3)1Bnr, Rb(8.12)5Bnr, and Rb(9.14)6Bnr.
- Homozygous for the $Mc1r^{E\text{-}tob}$ locus as well as albino.
- Homozygous for retinal degeneration 3, *Rd3*, (Chang *et al.,* 1993).
- Used to facilitate selection of immunoglobulin-competent hybridomas (Taggert, 1983).
- Research applications: neurobiology, sensorineural, endocrine deficiency, genetics.

Technician notes:
- Active.
- Weanlings are especially active.
- Chubby; clean and white coats.
- Poor breeders.

RF/J		**Stock No. 000682**
Common name:	n/a	**Generation:** F196 (22-NOV-06)
Former name:	n/a	
Strain type:	Inbred strain	
Appearance:	Albino; related genotype: *a/a Tyr^{c}/Tyr^{c}*	
H2 haplotype:	*k*	
Genes/alleles:	*Hc^{0}*: hemolytic complement; deficient common names: C5-, C5-d, C5-def, C5-deficient, hc^{o} *Rmcf^{s}*: resistance to MCF virus; MCF sensitive	
Strain origin:	Furth (1928) from unknown stock at Rockefeller Institute. Stock transferred to Oak Ridge.	
Source:	From Upton at Oak Ridge to JAX (1954) at F14 (since stocks arrival at Oak Ridge).	
Characteristics and uses:	• Expresses about 40% frequency of leukemia (Buchberg *et al*., 1986), and approximately 50% reticulum cell sarcomas. • Carries a Type 5 Chr Y of U.S. *M.m. domesticus* origin (Tucker *et al.,* 1992).	• High incidence of spontaneous glomerular hyalinisation and glomerulosclerosis developing between 8–20 months. • Research applications: cancer, neurobiology, sensorineural, immunology and inflammation.
Technician notes:	• Poor breeders. • Average litter size.	• Breeders get fat with age.

RIIIS/J — Stock No. 000683

Common name: R3; **Generation:** F135 (22-NOV-06)

Former name: RIII/2J

Strain type: Inbred strain

Appearance: Albino; related genotype: *Tyr^{c}/Tyr^{c}*

H2 haplotype: *r*

Genes/alleles: n/a

Strain origin: Derived from RIII and SEC/1Re. RIII from Dobrovolskaia-Zavadskaia, Paris (1928). To Wood, Curtis and Dunning, Columbia University. Wood to Eisen to JAX (1948) (RIII/J). Curtis and Dunning to Andervont, NCI, to JAX (1953) at F23 (RIII/AnJ). SEC/1ReJ from SEC/1Gn derived by Green from cross of NB x BALB/c (1941).

Source: In 1967 RIIIS/J was derived from RIII/AnJ x SEC/1ReJF1 backcrossed to RIII/AnJ, then crossed to RIII/J. Originally called RIII/2J, corrected to RIIIS/J in 1979 (Mobraaten). 1/8 of RIIIS/J genes can be expected to be derived from SEC/1ReJ. RIIIS/J mice are a model for von Willebrand disease (Sweeney *et al.,* 1990); the disease is caused by a defect outside of von Willebrand factor gene (Nichols *et al.,* 1994).

Characteristics and uses:

- Prolonged bleeding times with normal platelet activity and low levels of factor VIII:C and plasma von Willebrand factor antigen, making it a good animal model for human von Willebrand disease.
- Low antibody response to several bacterial polysaccharide antigens; reported to be resistant to collagen induced arthritis.
- Despite a B cell immunodeficiency, develops severe experimental autoimmune myasthenia gravis (EAMG) (Tüzün *et al.,* 2004).
- Carries no detectable endogenous ecotropic MuLV DNA sequences (Jenkins *et al.,* 1982).
- Largest known deletion of the T cell receptor V beta genes—13 of 21 known V beta genes (Haqqi *et al.,* 1989).
- Carries a Type 1A Asian origin *M.m. musculus* Chr Y (Tucker *et al.,* 1992).
- High incidence of mammary tumors and ovarian tumors.
- Carries Mtv8, and Mtv14, but high incidence of tumors has not been reported. In fact, some studies indicate a resistance to chemically induced tumors. RIIIS/J mice have been reported to develop far fewer lung tumors than A/J or SWR/J mice subsequent to urethane treatment. BALB/c x RIII F1 males are also highly resistant to diethylstilbesterol-cholesterol induced testicular tumors even though BALB/c is highly susceptible.
- Carries a spontaneous mutation, *Hsf4^{ldis1}*, that leads to a partial or complete disruption of the lens and cataracts (Jablonski M, *et al.,* 2004).
- Research applications: cancer, hematological, immunology and inflammation, sensorineural.

Technician notes:

- Poor breeders.
- Litters often born dead or missing.

SEA/GnJ	**Stock No. 000644**
Common name	sea Green **Generation:** F194+22 (10-DEC-07)
Former name	n/a
Strain type	Inbred strain
Appearance	Light brown agouti, short ears; related genotype: *A/A Tyrp1^{b}/Tyrp1^{b} Bmp5^{se} Myo5a^{d}/Bmp5^{se} Myo5a^{d}* Light brown agouti, normal ears; related genotype: *A/A Tyrp1^{b}/Tyrp1^{b} Bmp5^{se} Myo5a^{d}/+ Myo5a^{d}*
H2 haplotype	*d*
Genes/Alleles	*Bmp5^{se}*: bone morphogenetic protein 5; short ear common name: se^{GnJ} *Myo5a^{d}*: myosin Va; dilute common names: d, d^{v}, maltese dilution *Rmcf^{s}*: resistance to MCF virus; MCF sensitive
Strain origin	n/a
Source	n/a
Characteristics and uses	• Research applications: dermatology, development biology, metabolism.
Technician Notes	n/a

SJL/J — Stock No. 000686

Common name: Swiss Jim Lambert; **Generation:** F145 (1995)

Former name: n/a

Strain type: Inbred strain

Appearance: Albino; related genotype: *Oca2^{p} Tyr^{c}/Oca2^{p} Tyr^{c}*

H2 haplotype: *s2* (Fischer Lindahl K ,1997; Shen *et al.*, 1982)

Genes/alleles:
Dysf^{im}: dysferlin; inflammatory myopathy
common names: SJL-Dysf

Pde6b^{rd1}: phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide; retinal degeneration 1
common names: rd, rd-1, rd1, rodless retina

Rmcf^{s}: resistance to MCF virus; MCF sensitive

Strain origin: Developed by James Lambert at JAX (1955) from Swiss Webster stock from 3 sources imported between 1938 and 1943 (SJL = Swiss Jim Lambert). Pen bred until 1955, when inbreeding started in Foundation Stocks.

Source: Reintroduced to Foundation Stocks by hysterectomy derivation and fostering on C57BL/6J (1974) at F69.

Characteristics and uses:

- Homozygous for the retinal degeneration allele *Pde6b^{rd1}* (Caffee *et al.,* 1993), resulting in blindness after 2 weeks of age.
- Develops multicellular reticulum cell sarcomas resembling Hodgkin's disease in 91% of virgin females at 13.3 months, 91% of males at 12.5 months (Murphy, 1963, 1969).
- Predominant tumor type is pre-B-cell lymphoma (Mucenski *et al.,* 1988).
- Carries a Type 3 Chr Y of Western European *M.m. domesticus* origin (Tucker *et al.,* 1992).
- Develops a spontaneous myopathy resulting from a splice-site mutation in the Dysferlin gene. This *Dysf^{im}* allele has been shown to result in decreased levels of dysferlin protein in SJL/J mice and makes this strain a good model for limb girdle muscular dystrophy 2B (Bittner *et al.,* 1999).
- Susceptible to experimental autoimmune encephalomyelitis (EAE), making mice useful for multiple sclerosis research.
- Increased rate of muscle regeneration after injury when compared to BALB/c mice.
- Fed an atherogenic diet, fails to develop atherosclerotic aortic lesions.
- Useful as a control strain for studying immune defects in NOD/ShiLtJ mice (001976), a model for type 1 diabetes (IDDM). Both NOD and SJL/J are derived from Swiss mice; SJL are immunocompetent but have elevated levels of circulating T cells.
- Research applications: cancer, cardiovascular, diabetes and obesity, neurobiology, sensorineural.

Technician notes:

- Dirty; smelly.
- Males are very aggressive; get mad easy and fast; fight a lot; may kill cage mates.
- High level of infant mortality due to male aggression. Do not handle litters until pups are at least 6 days old.
- Females are extremely aggressive, too.
- Fair breeders.
- Weanlings are small in size.
- Weanlings and female breeders have bite wounds. Pups easily frightened when in with the breeders. Wean have missing fur from bites, mostly on the head.
- Provide nesting material. Remove male breeder the week before pups are born.

SM/J	**Stock No. 000687**
Common name:	small; **Generation:** F192 (14-DEC-06)
Former name:	n/a
Strain type:	Inbred strain
Appearance:	White-bellied agouti: related genotype: A^w/a Black: related genotype, *a/a*
H2 haplotype:	*v*
Genes/Alleles:	*Neu1*^{a}: neuraminidase 1; a variant common name: Neu-1^s
Strain origin:	MacArthur (1939) crossed 7 stocks (including DBA) selected for small body size in a genetically heterogeneous population. Transferred to Runner at JAX (1948).
Source:	Runner to JAX (1954) at F14.
Characteristics and uses:	• Low tumor frequency. • Both sexes live for 570–600 days. • Hyperresponsiveness to B cell mitogens (Clark *et al.,* 1981; Engel *et al.,* 1981). • Point mutation in *Neu1* is responsible for partial deficiency of lysosomal neuraminadase; may explain altered immune response (Rottier *et al.,* 1998). • Small in size at birth through weaning, but attain a normal body weight as they age. • Carries a number of rare polymorphic alleles; often matched to LG/J (000675), A/J (000646) or NZB/BlNJ (000684) for quantitative trait locus (QTL) analysis. • Susceptible to diet-induced atherosclerosis (Nishina *et al.*, 1993) and cholelithiasis (gallstones); carries the atherosclerosis 8, *Ath8*, gene (Paigen, 1995). • Research applications: general purpose as well as cardiovascular, developmental biology, diabetes and obesity, immunology and inflammation.
Technician notes:	• Calm. Easy to work with. • Small body size. • Delicate appearing. • Good parents; produce consistently.

SPRET/EiJ	**Stock No. 001146**
Common name:	n/a **Generation:** F75 (01-DEC-06)
Former name:	n/a
Strain type:	Wild-derived inbred strain (species: *M. spretus*; Puerto Real, Cadiz Province, Spain)
Appearance:	White-bellied agouti; related genotype: A^w/A^w
H2 haplotype:	n/a
Genes/alleles:	n/a
Strain origin:	n/a
Source:	n/a
Characteristics and uses:	• Resistant to high doses of tumor necrosis factor alpha (TNFa) (Staelens *et al.,* 2002). • Mice from a C57BL/6J x SPRET/Ei F1 cross were protected from TNFa-induced arthritis and partially protected against induced allergic asthma (Staelens *et al.,* 2004). • Often used in crosses with common inbred strains to create highly polymorphic panels for genetic mapping. • Research applications: immunology and inflammation, reproductive biology, genetics (evolution and systematics, gene mapping [numerous polymorphisms]).
Technician notes:	• These mice are very difficult to handle.

ST/bJ — Stock No. 000688

Common name: n/a **Generation:** F164p

Former name: n/a

Strain type: Inbred strain

Appearance: Albino; related genotype: *a/a Tyrp1^{b}/Tyrp1^{b} Tyr^{c}/Tyr^{c}*

H2 haplotype: *k*

Genes/alleles:
Hc^{0}: hemolytic complement; deficient
common names: C5-, C5-d, C5-def, C5-deficient, hc^{o}

Pde6b^{rd1}: phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide; retinal degeneration 1
common names: rd, rd-1, rd1, rodless retina

Rmcf^{s}: resistance to MCF virus; MCF sensitive

Strain origin: From a closed colony of Danish white mice from a Mrs. Street (1932) to Engelbreth-Holm, Copenhagen (1940), to Heston, NCI at F23 (1947).

Source: Heston to JAX (1948) at F25.

Characteristics and uses:
- Homozygous for the retinal degeneration allele *Pde6b^{rd1}*.
- 1–2% leukemia, including some plasma cell leukemias; about 3% other tumors, principally pulmonary adenomas and mammary carcinomas; some polydypsia and polyuria; high resistance to chronic whole-body X-irradiation.
- Resistant to the induction of experimental allergic encephalomyelitis (Levine and Sowinski, 1973).
- Of 19 inbred strains assessed for nicotine-induced seizure sensitivity, ST/bJ mice were the most sensitive to i.v. and i.p. injection of nicotine (Miner and Collins, 1989).
- Research applications: sensorineural, immunology and inflammation.

Technician notes:
- Poor breeders.
- Small litter size (2–3 pups).
- Breeders get fat with age.

SWR/J — Stock No. 000689

Common name: swiss; SW **Generation:** F229 (03-JAN-08)

Former name: n/a

Strain type: Inbred strain

Appearance: Albino; related genotype: *A/A Tyr^{c}/Tyr^{c}*

H2 haplotype: *q2* (Fischer Lindahl K, 1997; Shen *et al.*, 1982)

Genes/alleles: *Hc^{0}*: hemolytic complement; deficient
common names: C5-, C5-d, C5-def, C5-deficient, hc^{o}

Pde6b^{rd1}: phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide; retinal degeneration 1
common names: rd, rd-1, rd1, rodless retina

Strain origin: Swiss mice from A de Coulon of Lausanne, inbred by Clara Lynch, Rockefeller Institute; to R Parker, University of Toronto.

Source: Parker to JAX (1947) at F28+.

Characteristics and uses:

- Homozygous for the retinal degeneration allele *Pde6b^{rd1}*.
- Aging mice exhibit high incidence of lung and mammary gland tumors; also develop extreme polydipsia and polyuria (nephrogenic diabetes insipidus) with increasing age.
- Females develop polydipsia at 6–10 months (Kutscher and Miller 1974).
- Highly susceptible to experimental allergic encephalomyelitis (EAE).
- Germline deletion of about 50% of T-cell receptor V beta-chain gene segments and a T-cell receptor V alpha polymorphism are responsible for resistance to collagen type II-induced arthritis.
- Intermediate susceptibility to developing atherosclerotic aortic lesions on atherogenic diet.
- Recommended for generation and propagation of transgenic mice because they are high responders to exogenous hormones, have large and prominent pronuclei with good resistance to lysis following microinjection, and are genetically well defined.
- Appear to be the only inbred strain carrying the allele *Soa^{a}* (Taster), characterized by avoidance of sucrose octaacetate solutions at low concentrations (< 10-3M).
- Highest blood pressure of 19 JAX® mice tested (Schlager and Weibust, 1967).
- Spontaneous ovarian granulose cell tumors (Beamer *et al.,* 1985; 1993).
- About half have primary lung tumors (Mirvish and Kaufman, 1970).
- Arteriosclerosis common (Russell and Meier, 1966).
- Low frequency of mammary tumors in breeders and virgins.
- High mortality in males exposed to ethylene oxide oxidation products.
- May be used for comparison to the autoimmune diabetic NOD/ShiLtJ mice (001976), especially for experiments examining the aberrant immune functions of NOD/ShiLtJ mice. Both NOD and SWR/J mice are derived from Swiss mice. SWR/J are in some cases more suitable than random bred Swiss ICR mice because of their genetic uniformity. Unlike NOD/ShiLtJ mice they are not immunocompromised, and they are genetically very different from NOD.
- Research applications: general purpose as well as cancer, cardiovascular, developmental biology, diabetes and obesity, immunology and inflammation, sensorineural.

Technician notes:

- Wide variation in reports from technicians on behavior and breeding.
- Cages appear dirty.
- Jumpy. Quiet mice; easy to handle.
- Aggressive toward people. Good idea to have same tech changing this strain.
- Breeding performance may be variable. Poor to good breeders; good parents.

WSB/EiJ	**Stock No. 001145**
Common name:	Watkin Star line B **Generation:** ?+F1 (05-DEC-07)
Former name:	n/a
Strain type:	Wild-derived inbred strain
Appearance:	Agouti with head blaze, grayish coat; related genotype: *A/A* *Note*: This strain is homozygous for a recessive pigment mutation that contributes a signature head blaze (very high penetrance) and variable belly spotting. In addition, slight coat dilution gives the illusion of the mouse being A^w (white bellied agouti). The pigment mutation is being tested for allellism with recessive spotting, *rs*.
H2 haplotype:	n/a
Genes/alleles:	n/a
Strain origin:	Geographic origin: Centreville, Queen Anne City, Eastern Shore, Maryland
Source:	n/a
Characteristics and uses:	• Research areas: dermatology, genetics (evolution and systematics, genetics [gene mapping])
Technician notes:	• These mice are very difficult to handle.

4.B. Reproductive performance

Table 4.1 contains breeding data collected in the production colonies (pedigreed expansion stocks) from which JAX® Mice are shipped. Keep in mind that all mouse colonies, even within a facility, are different and that breeding performance can vary among them. Thus, the values in Table 4.1 should be used as guidelines to indicate the breeding potential of the strain and to help identify potential breeding problems. Values do not represent absolute performance standards.

Table 4.1. Reproductive performance for selected strains of JAX® Mice.

Strain of JAX® Mice (stock number)	Number of breed-ing pairs	Number of pups weaned per female (mean)	Number of litters per female* (mean)	Maternal age (days)		Litter size (pups)		Percent wean: born‡	Percent females weaned	Maternal age (months) when last litter weaned§ (mean, range)	Dates of data gen-eration (mo/yr)
				At pairing (range)	Birth of first litter (mean)	At birth (mean)	At wean† (mean)				
129P3/J (000690)	48	13.4	3.2	18-30	80	4.6	4.2	86	50	6.1 (2.9-10.5)	12/06-5/08
129S1/SvImJ (002448)	47	22.5	4.6	17-30	67	5.3	4.9	89	51	7.4 (3.8-11.3)	12/05-2/08
129X1/SvJ (000691)	50	20.7	4.5	19-50	77	5.1	4.6	89	49	7.4 (3.2-9.8)	1/06-2/08
A/J (000646)	50	21.4	4.3	17-35	74	5.3	5.0	88	51	7.3 (5.2-9.2)	12/05-1/07
AKR/J (000648)	49	18.6	3.4	20-33	65	5.5	5.4	96	47	5.7 (3.6-7.6)	12/05-11/07
B6(Cg)-*Tyr*$^{c-2J}$/J (000058)	19	21.6	3.4	29-62	70	6.6	6.3	92	50	6.3 (2.9-8.9)	6/07-6/08
B6.129P2-*Apoe*^{tm1Unc}/J (002052)	50	17.3	3.9	25-31	74	5.2	4.5	83	48	7.2 (5.0-9.5)	12/05-8/07
BALB/cByJ (001026)	50	19.8	3.8	18-62	84	5.4	5.2	96	57	7.8 (5.4-10.3)	12/05-9/07
BALB/cJ (000651)	50	21.1	4.1	18-26	92	5.5	5.4	98	50	7.7 (5.9-9.1)	3/06-7/07
C3H/HeJ (000659)	50	17.3	3.5	18-41	73	5.2	5.0	92	47	5.9 (3.7-7.2)	12/05-6/07
C3H/HeOuJ (000635)	50	20.1	4.3	19-25	72	5.0	4.7	93	46	6.1 (2.6-7.7)	12/05-7/07
C3HeB/FeJ (000658)	34	34.1	5.3	17-26	65	6.6	6.4	96	49	7.2 (4.6-9.3)	12/05-5/08
C57BL/10J (000665)	50	18.3	3.9	24-33	78	5.1	4.7	91	54	7.2 (4.6-9.8)	1//06-3/08
C57BL/6J (000664)	50	29.5	5.4	24-31	67	5.9	5.5	92	51	8.3 (5.7-11.2)	12/05-7/07
C57BLKS/J (000662)	45	14.6	3.1	21-35	83	5.3	4.7	86	47	7.2 (3.4-9.7)	12/05-6/08
C57L/J (000668)	42	16.5	3.5	25-31	88	4.9	4.7	90	44	7.7 (3.2-11.4)	12/05-3/08
CAST/EiJ (000928)	49	13.8	3.7	23-38	109	4.4	3.7	81	51	7.9 (4.8-9.9)	5/07-4/08
CBA/CaJ (000654)	49	22.7	4.2	17-33	71	5.7	5.5	96	49	6.6 (3.9-8.2)	12/05-1/08

Table continued on next page.

Table 4.1. Reproductive performance for selected strains of JAX® Mice. (continued)

Strain of JAX® Mice (stock number)	Number of breeding pairs	Number of pups weaned per female (mean)	Number of litters per female* (mean)	Maternal age (days)		Litter size (pups)		Percent wean: born‡	Percent females weaned	Maternal age (months) when last litter weaned§ (mean, range)	Dates of data generation (mo/yr)
				At pairing (range)	Birth of first litter (mean)	At birth (mean)	At wean† (mean)				
CBA/J (000656)	49	21.7	5.4	18-26	79	4.3	4.0	90	51	7.5 (6.2-9.0)	12/05-7/07
DBA/1J (000670)	50	20.3	5.2	19-30	71	4.1	3.9	89	44	7.6 (5.3-9.4)	1/06-12/07
DBA/1LacJ (001140)	49	22.8	5.2	19-28	68	4.9	4.4	84	50	7.6 (4.4-9.3)	1/06-1/08
DBA/2J (000671)	50	24.8	5.3	18-47	73	4.8	4.6	92	50	6.9 (5.9-9.6)	12/05-8/07
FVB/NJ (001800)	50	35.5	4.9	19-29	68	7.4	7.3	98	51	7.1 (5.6-11.5)	1/06-9/07
KK/HlJ (002106)	25	14.9	3.2	25-31	72	5.5	4.7	83	52	6.0 (2.1-8.1)	12/05-10/06
LP/J (000676)	50	12.7	3.9	18-29	85	4.0	3.3	77	55	7.2 (3.9-8.9)	12/05-4/08
MRL/MpJ (000486)	50	18.0	3.4	19-27	72	5.6	5.4	95	51	6.4 (3.7-8.1)	12/05-2/08
NOD.CB17-*Prkdc*^{scid}/J (001303)	50	23.7	4.1	18-32	81	6.1	5.8	94	51	6.1 (4.0-8.4)	1/06-8/07
NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (005557)											
(pair-mated)	25	25.2	4.0	19-65	69	6.7	6.4	96	48	6.1 (3.0-10.3)	10/06-10/07
(trio-mated)	10 trios	20.4	3.2	21-65	77	6.5	6.6	98	53	6.7 (5.2-10.5)	
NOD/ShiLtJ (001976)	50	27.0	3.5	19-35	83	8.4	7.7	92	51	6.0 (4.2-8.8)	1/06-8/07
NZB/BlNJ (000684)	50	7.7	2.6	25-30	89	4.3	3.0	58	50	5.7 (3.6-8.5)	12/05-11/07
NZW/LacJ (001058)	50	15.8	3.9	21-31	76	4.8	4.0	81	45	6.4 (3.8-9.1)	12/05-11/07
PL/J (000680)	49	19.1	4.5	19-32	91	4.6	4.3	89	47	7.2 (3.9-10.7)	1/06-3/08
SJL/J (000686)	50	32.3	5.2	19-27	68	6.6	6.3	92	51	7.7 (4.5-9.7)	12/05-8/07
SM/J (000687)	50	11.8	2.9	21-33	80	4.7	4.1	79	49	6.2 (3.6-9.5)	12/05-3/08
SWR/J (000689)	50	21.8	3.3	18-27	77	6.8	6.6	98	49	6.2 (3.1-10.1)	2/06-5/08

*The number of litters produced during the optimal breeding period (see § below), not the total number of litters a breeding pair *can* produce. (Includes litters for which no pups were weaned.)

†Includes litters from which no pups survived (weaned litter size = 0) and litters with more pups weaned than "born," which occurred occasionally due to undercounting litter size at birth.

‡Excludes litters with more pups weaned than "born" to provide a more accurate estimate of neonatal mortality.

§A breeding pair was retired when the caretaker judged that the period of optimal breeding performance had ended for that breeding pair, based on experience and general guidelines for the strain.

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Chapter 5: Choosing a Mouse Strain for Research—Considerations and Resources

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The choice of mouse strain and controls influences both the potential and limitations of a research program. This choice is complicated by the massive amounts of information and the quantity of mouse strains available.

Our objective for this chapter is to help researchers with the selection process by providing 1) lists of resources that can supply the detail necessary to help refine and finalize a strain choice for specific research areas, and 2) some universal guidelines and "words to the wise" about choosing a strain and controls.

The chapter is organized as follows:

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5.A. General sources of information about mouse strains

Following is a list of resources—at The Jackson Laboratory and elsewhere—that are useful for a strain search. We also provide an example of how one resource, the Mouse Phenome Database (MPD) can help refine a search.

For the most widely-used strains of JAX® Mice, detailed tabular information can be found within this book in Chapter 4, "Characteristics of Popular Strains of JAX® Mice, Including Reproductive Performance," and in Appendix D, "Commonly-Used Inbred Strains and Substrains of JAX® Mice—Genes and Research Applications."

5.A.1. Resources at The Jackson Laboratory website: www.jax.org

Besides providing details on more than 4,000 strains of JAX® Mice, The Jackson Laboratory website also provides access to several databases and other information related to research that can help refine a choice of mouse strain. Table 5.1 will help you get started accessing our online information. Because our site is updated on a regular basis, new resources will become available; therefore, be sure to check www.jax.org frequently for new information.

Table 5.1. The Jackson Laboratory website (www.jax.org): information about mouse strains and research.

Resource	Web address
JAX® Mice strain database: Phenotypic and genotypic characteristics and husbandry information for each strain of JAX® Mice. Search on strain name, stock number, gene, allele, research area, disease term, etc.	www.jax.org/jaxmice/query
JAX® Mice strain lists, organized as follows: • Most popular • Strain type (categories such as inbred, congenic, etc.) • New JAX® Mice strains • Strains under development • JAX® Mice by research area (including mice used as research tools, for example, with Cre-lox Systems and fluorescent proteins)	www.jax.org/jaxmice/findmice/browse
Mouse Genome Informatics (MGI) website: Data on the genetics, genomics, mutant phenotypes, and biology of the laboratory mouse, including • Links to databases such as – Mouse Phenome Database (MPD) – Mouse Tumor Biology (MTB) database – International Mouse Strain Resource (IMSR) database • Online books – *Biology of the Laboratory Mouse*. 1996. Green E. – *Mouse Genetics*. 1995. Silver, LM. – *The Anatomy of the Laboratory Mouse*. 1965. Cook MJ. – *The Coat Colors of Mice*. 1979. Silvers WK. – *Origins of Inbred Mice*. 1978. Morse, HC III.	www.informatics.jax.org www.jax.org/phenome http://tumor.informatics.jax.org/mtbwi/index.do www.informatics.jax.org/imsr www.informatics.jax.org (More Resources menu option)
Assistance with locating or creating a novel mouse strain	www.jax.org/jaxservices/straincreation
Information about transgenic and targeted mutant mice	www.jax.org/imr

Table continued on next page

Table 5.1. The Jackson Laboratory website (www.jax.org): information about mouse strains and research. (continued)

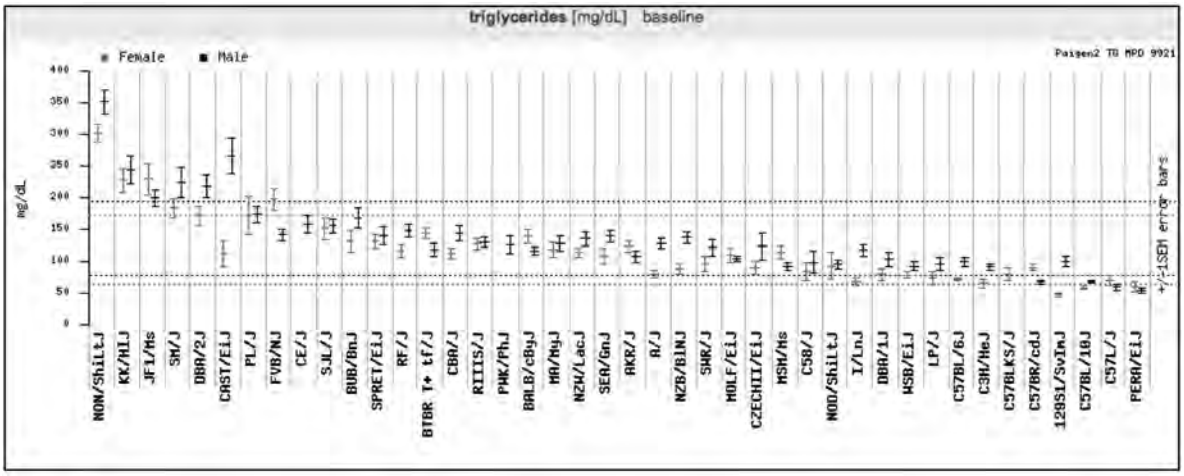
Resource	Web address
Information about mice carrying spontaneous mutations	http://mousemutant.jax.org/index
Information about recombinant inbred (RI) and chromosome substitution (CS) strains	www.jax.org/smsr
Research areas and faculty website: Links to information about research being conducted at The Jackson Laboratory	www.jax.org/research/faculty
Courses and education website	www.jax.org/courseseducation
Technical support and literature website: • Requests for JAX® Mice Literature (including the JAX® Mice Catalog, resource manuals, mouse model lists, price list, JAX® NOTES) • Technical support on any research issues involving strains of JAX® Mice and related services	www.jax.org/jaxmice/support
Festing's characteristics of inbred mice and rats	www.informatics.jax.org/external/festing/search_form.cgi

5.A.2. An example of a strain characteristic comparison from the Mouse Phenome Database (MPD): www.jax.org/phenome

The Mouse Phenome Database (MPD), which is maintained at The Jackson Laboratory, is an international, collaborative initiative to establish a comprehensive collection of phenotypic data for mouse strain comparisons. The MPD is a very powerful way to compare well-characterized strains of laboratory mice. (For an overview of the MPD, see 6.C.2, "Other databases.")

For our example, we searched for mouse models with which to study heart disease risk factors. A search of the MPD for triglyceride data displayed a strain comparison of circulating triglycerides (Svenson *et al*., 2007) (Figure 5.1). This strain comparison, comprising 42 strains, allowed us to very quickly narrow our search to the strain subset with high values. Using additional MPD functionality, we could have linked directly to additional information about the strains. For strains of JAX® Mice, we could have linked directly to the strain datasheet.

Figure 5.1. Example of Mouse Phenome Database (MPD) strain comparison for triglycerides.



5.A.3. Other websites

Table 5.2 provides a list of other websites that provide information related to mouse strains. This list represents sites that our researchers and technical support personnel have found particularly valuable.

Table 5.2. Other public websites: information about mouse strains.

Organization	Web address
National Institutes of Health (NIH)	www.nih.gov
Trans-NIH Mouse Initiatives	www.nih.gov/science/models/mouse/
European mutant mouse pathology database	www.pathbase.net
Organizations related to specific research areas, for example	
• National Cancer Institute	www.cancer.gov
• American Society of Hematology	www.hematology.org
Literature search engines, for example, the PubMed service provided by the National Library of Medicine and the National Institutes of Health	www.pubmed.gov
Institute for Laboratory Animal Research (ILAR), U.S. National Academy of Sciences	http://dels.nas.edu/ilar
Deltagen Mouse Histology Atlas	www.deltagen.com/target/histologyatlas/HistologyAtlas.html
Ensemble Genome Browser	www.ensembl.org
University of California, Davis (UCDavis) Mouse Biology Program	http://mouse.ucdavis.edu

5.A.4. Books

- *Biology of the Laboratory Mouse,* Second Edition. 1966. Green E, ed.
- *Guide for the Care and Use of Laboratory Animals* (often referred to simply as *The Guide*). 1996. National Research Council.
- *Inbred Strains in Biomedical Research*. 1979. Festing MFW.
- *The Laboratory Mouse*. 2001. Suckow, *et al.*
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- *The Mouse in Biomedical Research,* Second Edition. 2007. Fox JG, *et al.*, eds.
- *NOD Mice and Related Strains: Research Applications in Diabetes, AIDS, Cancer, and Other Diseases*. In *Medical Intelligence Unit.* 1998. Leiter E, Atkinson M (eds.). RG Landes Co. Austin TX.

5.B. Seven considerations for selecting a mouse strain

1. Confirm that the strain is appropriate for the study.

Strain characteristics that can affect a study include variations in anatomy, development, behavior, survivability, reproduction, and onset and variability of disease. For all strains of JAX® Mice, comprehensive information is conveniently provided in the strain datasheet (see sidebar). For the most popular JAX® Mice, an overview is provided in Chapter 4, "Characteristics of Popular Strains of JAX® Mice, Including Reproductive Performance"; a brief summary of genes and applications in a tabular format is provided in Appendix D, "Commonly-Used Inbred Strains and Substrains of JAX® Mice—Genes and Research Applications." Following are a few examples of the types of things to watch for:

- Mice from strains homozygous for the *Pde6b*^{rd1} allele (for example, C3H/HeJ [000659]) are blind by 4–5 weeks of age. These strains would be inappropriate for behavioral studies that rely on vision.
- A/J (000646) mice are homozygous for the progressive muscle dystrophy allele of the dysferlin gene (*Dysf*^{prmd}). Thus, while they may be good models for the influence of dysferlin on muscle maintenance, they could be poor models for the study of general influences (such as exercise) on muscle development.
- AKR/J (000648) mice express the ecotropic retrovirus AKV from birth in all tissues, leading to a high incidence of thymic lymphoma beginning around 4–6 months of age. Thus, while they might be appropriate for studies of normal function before the lymphoma develops, they would be inappropriate for studies of normal function in mature adults.

The JAX® Mice datasheet: the most up-do-date, detailed information for any strain of JAX® Mice

Each strain of JAX® Mice has an associated datasheet that is updated on a regular basis. Information includes

- Full description of the strain: full name and nomenclature, stock number, common and former names, genetic and allelic details, phenotypes, coat color, *H2* haplotype, mating scheme, current generation number
- Strain origin and history
- Related strains and control information
- Husbandry information, including diet
- Links to health reports
- Research applications, including links to references
- Price and availability
- Links to relevant databases, customer service, tech support, JAX® Services, and other web-based information.

Access strain datasheets at www.jax.org/jaxmice/query.

2. Understand that the strain background may affect the phenotypic expression of a mutation.

A phenotype in a mutant strain is the result of both the genetic mutation, spontaneous or induced, and the strain background on which it is maintained. Even an allele that is named for the phenotype it produces in one strain may not produce that phenotype on other genetic backgrounds. Table 5.3 shows the effects of strain background on two diabetogenic mutations, *Lepr*^{db} and *Lep*^{ob}. The appropriate choice of model depends on which phenotype is of interest.

Table 5.3. Effects of strain background on the *Lep*^{ob} and *Lepr*^{db} mutations.

The combination of this allele…	…on this background…	…creates this strain…	…with this phenotype.
Obese mutation (*Lep*^{ob})	C57BL/6J (B6) (000664)	B6.V- *Lep*^{ob}/J (000632)	*Transient* hyperglycemia with obesity
	C57BLKS/J (BKS) (000662)	BKS.V- *Lep*^{ob}/J (000696)	*Overt* diabetes with obesity
Diabetes mutation (*Lepr*^{db})	C57BL/6J (B6) (000664)	B6.BKS(D)-*Lepr*^{db}/J (000697)	*Transient* hyperglycemia with obesity
	C57BLKS/J (BKS) (000662)	BKS.Cg-*m* +/+ *Lepr*^{db}/J (000642)	*Overt* diabetes with obesity

When transferring a mutation onto another background strain to create a congenic, we recommend that the phenotype be evaluated at backcross generation N5 or N6. At this point more than 95% of the genome typically is of the host genotype, and it is by this stage that background effects will become noticeable. (See 3.C.2.a.1, "Congenic strains.")

3. Do not assume that substrains have identical phenotypes because they have the same strain of origin or a similar name.

Most substrains have been separated by at least 20 generations—often many more, over a span of many years—and they are often further apart genetically than one might think. (See 3.B.1.a.2, "Substrains," and Appendix C, "Origins and Relationships among Common Strains and Substrains of Laboratory Mice.") Examples of substrain differences include the following:

- C57BL/6J (000664) and C57BL/10J (000665). These substrains have been separated since the mid 1930s, and they differ at approximately 140 loci (calculation based on formulas from Bailey [1978]), including a minor histocompatibility locus (*H9*), an immunoglobulin heavy chain locus (*Igh2*), and a locus that regulates heme metabolism (*Lv*).
- A muscular dystrophy mutation ($Dysf^{prmd}$) arose in A/J (000646) mice as a result of a retrotransposon insertion in the dysferlin (*Dysf*) gene. Because evidence of the mutation was not present in the AXB and BXA recombinant inbred (RI) lines, which were constructed in 1975, this mutation occurred and became fixed in The Jackson Laboratory A/J foundation stock sometime after 1975. The mutation does not exist in the A/HeJ (000645) substrain, which was separated from Strong's progenitor A strain in 1938. (Ho *et al*., 2004.)

4. Consider using strains that are the founders of recombinant strain panels.

Strains that have been used as founders for recombinant strain panels offer a distinct advantage. As an example, assume that you investigate a phenotype in a founder strain, find a difference from published values for other strains (including the other founder strain for a panel), and then determine that the difference is reproducible in your own lab. By evaluating that phenotype in the strains of the recombinant panel, you can readily study the genetic regulation of the phenotype and its relationship to other phenotypes without genotyping or further phenotyping. Thus, studying just one founder strain opens the door to the powerful mapping opportunities provided by the entire recombinant strain panel. An additional benefit of studying a founder strain is that the availability of information on other characteristics for strains of the panel provides the opportunity to look for mechanistic relationships with the phenotype you originally evaluated.

One caveat in comparing a strain panel founder to the derived strains of the panel is that the founder strain genotype is not completely reproduced in the strains of the panel; genetic drift can influence the genotypes in both. The example given above (Consideration 3) for substrain divergence also illustrates this point. The dysferlin mutation in A/J (000646) is not present in any of the strains in the AXB or BXA RI panels derived in the 1970s (Ho *et al*., 2004).

Strain panels as research models: A strategy to combine genetic homogeneity with diversity

No single inbred strain represents laboratory mice any more than a single person represents *Homo sapiens*. Thus, to incorporate diversity into a study, one research approach is to use a genetically mixed population of mice. But individual genotypes in a population of this type cannot be reliably reproduced.

An alternate approach is to use a panel of inbred strains of mice. Strain panels combine genetic diversity with all the advantages of inbred strains—including reproducibility.

An example: In The Jackson Aging Center, a 32-strain panel of inbred mice was screened to study genetic influences on patterns of aging and age-related disease. Because these strains have already been densely mapped (SNP markers), genetic analysis of aging phenotypes can proceed without genotyping any mice. For details, refer to our aging center website at www.agingmice.org.

Another application of strain panels is for drug studies. By treating a panel of well-characterized inbred strains or a panel of their F1 hybrids, pharmacology researchers may observe a range of responses. This strategy avoids the generalization of results based on an idiosyncratic response of a single strain or F1 hybrid. In addition, the use of an inbred strain panel can establish a foundation for studies on the genetic basis of the response differences. The common use of standard outbred mice for this type of research is a poor substitute because outbred stocks typically are incompletely inbred and genetically uncharacterized.

5. Ensure that your facilities can accommodate all the requirements for the strain and the study.

Some disease models require special husbandry conditions and animal care such as isolator caging, a specific pathogen free (SPF) environment, a warmer temperature, or special feeding strategies. For example, immunodeficient mice need an SPF environment. Also, the health of hypothyroid mice, such as the Snell dwarf strain (DW/J *Mlph*^{ln} *Pou1f1*^{dw}/J [000643]), may be compromised even at temperatures just below 22 C (72 F). (K. Flurkey, personal observation).

Some mice may require single housing. For example, SJL/J (00686) and BALB/cJ (000651) males can usually be weaned in groups. Over time, however, they often become so aggressive that they may kill cage mates.

Phenotypes may vary depending on the microbial environment of the mouse room. Ensure that your colony conditions match the needs of your model and the phenotype you intend to study. For example, almost all inbred strains have greater lifespans in SPF colonies than in conventional colonies where pathogens are not controlled (Russell, 1966), indicating that old mice are particularly sensitive to subclinical infections that are common in conventional colonies. Therefore, SPF conditions are necessary to study normal aging without complication from age-related sensitivity to infectious disease. For other strain-dependent phenotypes, however, a very clean mouse room might result in the loss of a phenotype. As an example, gallstone development in mice requires non-pathogenic bacteria *Helicobacter* in the environment (Maurer *et al.*, 2006). This issue has become increasingly relevant since the late 1990s, when numerous colony managers began testing for and eliminating *Helicobacter*.

6. For transplantation studies, be sure that histocompatibility and tissue rejection do not interfere with the study requirements.

One major advantage of inbred mice is that, within individuals of a strain, tissues can be transplanted from experimentally treated mice to normal hosts or vice versa to compare the functions of that tissue in different internal environments. For example, by transplanting liver from an old mouse to a young mouse, one can study whether age-related impairments in liver are due to intrinsic aging or general aging of the individual. Recognize, however, that although inbred strains are considered genetically homogeneous, one cannot be assured that they are homozygous for all of the 100+ histocompatibility loci until they have been inbred for more than 60 generations.

Non-inbred stocks in particular are inappropriate for use in tissue transplantation studies. Even if only minor histocompatibility differences exist, the resulting gradual tissue rejection can complicate interpretation of results of a transplantation study.

Also, keep in mind that, because of differences in innate immunity, F1 hybrids are not completely histocompatible with their parental strains. This phenomenon, called hybrid resistance, results from innate immunity rather than acquired immunity. This issue is especially relevant for hematopoietic cells. Lethal irradiation (with hematopoietic reconstitution) and anti-NK cell treatment may alleviate this problem. (For details, see 3.B.3, “F1 and F2 hybrids.”) For tables listing histocompatibility genotypes, see Appendix F, “Histocompatibility Haplotypes and Loci.”

It is not just the histocompatibility loci that affect tissue rejection. Antigenic differences are important as well. For example, injecting syngeneic B lymphocytes into B6.129S2-*Igh-6*^{tm1Cgn}/J (002288) recipients leads to rejection because the mice have never seen mature B lymphocytes and therefore are not tolerant to B cells.

7. Solicit input from animal caretakers and technicians about their experience with specific strains.

When considering a new mouse strain, check to see if the caretakers and technicians in your facility have had experience with it. Often, they possess a wealth of information about specific models that is not reflected in the technical literature. This knowledge is particularly important regarding characteristics that can vary among different animal facilities, such as breeding performance, behavior (especially aggression), and health. Involving your caretakers and technicians in the discussion of a mouse strain has an additional advantage: If they are aware of the strain characteristics and phenotypes of interest in your mice, they are much more likely to be attuned to anything unexpected that might appear when your program is in process.

5.C. Guidelines for selecting and planning for control mice

Sometimes, choosing the most appropriate control is a challenging component of a research program. Several considerations and examples follow. For details on choosing controls for specific of JAX® Mice, refer to the strain datasheet, available at www.jax.org/jaxmice/query.

5.C.1. Selecting control mice

5.C.1.a. Controls for inbred strains

- *For an inbred strain model of a specific condition or disease (e.g., NOD/ShiLtJ [001976] for diabetes research)*, if no condition-free or disease-free control with the same genetic background exists, an appropriate control is a closely related strain or substrain that does not exhibit the phenotype of interest. For example, for NOD/ShiLtJ (001976) mice, which get type 1 diabetes, NOR/LtJ (002050) mice can be used as a control strain because they are MHC identical and 88% of their genome is NOD. NOR/LtJ mice exhibit no insulitis and no diabetes.

  Another appropriate control for studies of basal measurements could be mice that have been treated experimentally or genetically to prevent disease expression. For example, NOD.CB17-*Prkdc*^{scid}/J (001303) mice, which have no functional T cells and are both insulitis- and diabetes-free throughout life, serve as a diabetes-free control for comparison to NOD/ShiLtJ mice. NOD.CB17-*Prkdc*^{scid}/J can be used as treatment controls to identify effects of a treatment for diabetes that are independent of the effects on the disease. And, they are ideal as donors of NOD islets that are free of diabetogenic T cells.

- *For an inbred strain being used to test the effect of an experimental condition*, if possible, always use littermate controls. The use of untreated littermate controls assures that all mice have been subjected to the same maternal and environmental conditions except for those produced by the experimental treatment. To avoid the confusion of litter effects with treatment effects, do not assign all mice from one litter to the same treatment or control group. Rather, randomly assign littermates to treatment and control groups.

5.C.1.b. Controls for mutant strains on an inbred background, including segregating inbred strains, congenic and coisogenic strains

- *When possible, the best controls are gender-matched, unaffected littermates*, which are produced when the mutation is maintained heterozygously. If the mutation is dominant, one parent must be a homozygous wild-type. If the mutation is recessive, one parent must be a homozygous mutant. (With a recessive mutation, the symbol +/? identifies these controls, indicating that genotyping has not been performed and that they may be either +/+ or +/*mutant*.)
- *When littermates cannot be used as controls—for example, when the mutation is maintained homozygously—the background inbred strain can be used as controls.* Note that the degree of similarity between the background inbred strain and the mutant strain depends on two variables: 1) the amount of residual heterozygosity in the background strain when the separation of the mutant strain occurred, and 2) the number of generations that separates the strains. (For a detailed discussion on substrain divergence, refer to 3.B.1.a.2, "Substrains.")

  If the genetic difference between the background strain and the mutant strain is a concern, an alternate strategy is to outcross the mutant with an inbred mouse of the desired genetic background and intercross the F1 hybrids to produce F2 hybrids. In this F2 generation, the phenotype will segregate to produce mutants and controls.

  If the exact background strain is not available, select the most closely related substrain. But keep in mind that, in some cases, substrains with dissimilar names are genetically closer than those with more similar names. For example, for CBA/CaJ (000654) mice, CBA/CaH-T(14:15)6Ca/J (000655) mice are more appropriate controls than CBA/J (000656) mice. (For charts that show the relationship between many common substrains, see Appendix C, "Origins and Relationships among Common Strains and Substrains of Laboratory Mice.")

5.C.1.c. Controls for mutant strains on a mixed background

If a mutation is maintained heterozygously on a mixed background, unaffected littermates will be available and can be used as controls. If the mutation is maintained homozygously on a mixed background, you may need a breeding colony of approximate control mice. One example is targeted gene knockouts that have been made in embryonic stem (ES) lines derived from a 129 strain. The mutated ES cells are usually injected into C57BL/6J (B6; 000664) blastocysts, which results in chimeras that contain cells of B6 and 129 origin. Often, these chimeras are then crossed with a B6 mouse. If the targeted mutation has gone germline, the progeny are hybrids of B6 and 129 strains. These hybrids are then intercrossed to maintain the mutation. Approximate physiological controls can be provided from a B6129F2 colony. Because of the allelic segregation and assortment from F1 hybrid parents, the genetic background of the F2 generation will vary in composition similarly to that of the targeted mutant. (The Jackson Laboratory offers two F2 hybrids—B6129PF2/J [100903] and B6129SF2/J [101045]). B6129F1 hybrids are a less appropriate control because all mice of the F1 generation are genotypically identical.

5.C.2. Purchasing and housing control mice

To minimize variation due to substrain differences, obtain controls and research mice from the same supplier. To minimize variation due to environment or health status, house treated mice and controls in conditions as similar as possible. If treated and control mice can be unequivocally distinguished, house them in the same cages. Otherwise, make every effort to house them as close to each other as possible.

5.D. Guidelines for selecting a supplier of mice

Expect the following from a supplier of laboratory mice:

- Animals of high genetic quality that are free from common infectious diseases and pathogens.
- Quality control procedures to ensure genetic consistency—so that the mice of a strain they ship three years from now will be as genetically identical as possible to those they ship this year.
- Publicly available health reports on the mice.
- Trained technical support personnel who can assist with any issues you may encounter.

Unless absolutely necessary, avoid switching from one supplier to another during a research program. Even though the strain names might be the same from one supplier to another, it is likely that the mice are different enough genetically to confound research results.

5.E. Guidelines for alternatives to maintaining live mice

For some research it may be cost effective to use biological materials other than live mice.

- Studies involving analysis of extracted DNA, RNA or protein; frozen tissues or organs; or histology may not require live mice at your site. An option is to purchase this material instead.
- Some institutions may not have the specific facilities or expertise to house, breed, maintain, or treat specific mouse models or to perform specific procedures for *in vivo* studies. One option is to outsource specific tasks. Another option is to contract for someone else to run the study.

To learn more about these alternatives, or for information about how these strategies might meet the needs of your research program, call our technical information scientists at 1-800-422-6423 (North America) or 1-207-288-5845 (international). Also, refer to Chapter 18, "JAX® Services."

5.F. Sources of information related to selected research areas

Tables 5.4 through 5.13 provide lists of resources for selected research areas. They supplement the more general information provided in 5.A, "General sources of information about mouse strains."

Table 5.4. Information resources: aging.

Resource	Web address
The Jackson Laboratory:	
• Jackson Aging Center, including the Nathan Shock Center	www.agingmice.org
• The Mouse Phenome Database (MPD)	www.jax.org/phenome
• Information about our aging research	www.jax.org/research/faculty
• Lifespan data on some common strains of JAX® Mice and hybrids from those mice	www.jax.org/research/faculty/harrison/ger1vi_Data2
National Institute on Aging (NIA)	www.nia.nih.gov
Biology of Aging Program (BAP) of the NIA	www.nia.nih.gov/ResearchInformation/ExtramuralPrograms/BiologyOfAging/BAPScientificPrograms
The Aging Program of The Ellison Medical Foundation	www.ellisonfoundation.org/adsp.jsp?key=10aging_about

Table 5.5. Information resources: autoimmunity, including type 1 diabetes.

Resource	Web address
The Jackson Laboratory:	
• Type 1 Diabetes Resource and repository	http://type1diabetes.jax.org/index.html
• Diabetes incidence studies for JAX® Mice most commonly used to study autoimmunity	www.jax.org/t1dr/gqc_incidence_studies
• Lists of research models	www.jax.org/jaxmice/research/diabetes/type1.html
Center on Immunological Tolerance in Type-1 Diabetes (Harvard Medical School)	www.citdh.org/mock_new2_content.html
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)	www2.niddk.nih.gov
National Institute of Allergy and Infectious Diseases (NIAID of the NIH	www3.niaid.nih.gov
Juvenile Diabetes Research Foundation International	www.jdrf.org

Table 5.6. Information resources: cancer.

Resource	Web address
The Jackson Laboratory:	
• The Jackson Laboratory Cancer Center website	www.jax.org/research/cancer
• Our cancer resource, with links to – lists of research models – the Cancer Research Manual – the cancer research databases (Mouse Tumor Biology [MTB] and the Mouse Phenome Database [MPD])	www.jax.org/jaxmice/research/cancer
• Information about our cancer research	www.jax.org/research/faculty
American Cancer Society (ACS)	www.cancer.org
• Cancer statistics	www.cancer.org/docroot/stt/stt_0.asp
National Cancer Institute	www.cancer.gov
• Cancer Models Database	https://cancermodels.nci.nih.gov/camod/login.do
• The Mouse Models of Human Cancers Consortium (MMHCC)	http://emice.nci.nih.gov/emice
American Association of Cancer Research	www.aacr.org

Table 5.7. Information resources: cardiovascular biology.

Resource	Web address
The Jackson Laboratory:	
• Heart, Lung, Blood and Sleep Disorders Center website	www.jax.org/pga
• Lists of research models	www.jax.org/jaxmice/research
• Cardiovascular Research Manual	www.jax.org/jaxmice/manual
• Information about our research	www.jax.org/research/faculty
National Heart Lung and Blood Institute of the National Institutes of Health	www.nhlbi.nih.gov
CardioGenomics, a program for genomic applications sponsored by the National Heart, Lung & Blood Institute (NHLBI)	http://cardiogenomics.med.harvard.edu/
American Heart Association	www.americanheart.org

Table 5.8. Information resources: hematology.

Resource	Web address
The Jackson Laboratory:	
• Hematological web resource, with links to – lists of research models – Mouse Heart, Lung, Blood and Sleep Disorders (HLBS) Center website – information about our research	www.jax.org/jaxmice/research/hematology.html
National Heart Lung and Blood Institute of the National Institutes of Health	www.nhlbi.nih.gov

Table 5.9. Information resources: immunology, immunodeficiency and infectious disease.

Resource	Web address
The Jackson Laboratory: • Immunology and inflammation web resource, with links to – lists of research models – the immunodeficient poster – the Infectious Disease Resource Manual – information about our research	www.jax.org/jaxmice/research/immunology
National Institute of Allergy and Infectious Diseases (NIAID) of the NIH	www.niaid.nih.gov

Table 5.10. Information resources: metabolism.

Resource	Web address
The Jackson Laboratory: • Metabolism web resource, with links to – lists of research models – information about our research	www.jax.org/jaxmice/research/metabolism
National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health	www.nigms.nih.gov

Table 5.11. Information resources: neurobiology, including neuromuscular and sensorineural biology.

Resource	Web address
The Jackson Laboratory:	
• Information about our research	www.jax.org/research/faculty
• Alzheimer's disease web resource, with links to – lists of research models – information about the Alzheimer's Disease Mouse Model Resource (ADMMR) – the Neurobiology Resource Manual – other relevant information	www.jax.org/jaxmice/research/neurobiology/alzheimers
• Cranofacial mutant web resource, with links to – lists of research models – other relevant information	http://craniofacial.jax.org/index
• Cytogenic models web resource, with links to – lists of research models – protocols – other relevant information	www.jax.org/cyto
• Neural tube mutant web resource, with links to – lists of research models – other relevant information	www.jax.org/ntd
• Neurobiology web resource, with links to – lists of research models – the Neurobiology Resource Manual – other websites related to neurobiology research	www.jax.org/jaxmice/research/neurobiology

Table continued on next page

Table 5.11. Information resources: neurobiology, including neuromuscular and sensorineural biology (continued).

Resource	Web address
• Parkinson's disease web resource, with links to – lists of research models – other relevant information	www.jax.org/jaxmice/research/neurobiology/parkinsons
• Sensorineural web resource, with links to – lists of research models – information about our research – a web resource about hereditary hearing impairment in mice	www.jax.org/jaxmice/research/sensorineural
Neuromice	www.neuromice.org
The Mouse Brain Library	www.mbl.org
The Gene Expression Nervous System Atlas (GENSAT) project	www.ncbi.nlm.nih.gov/projects/gensat
The Neuroscience Gateway	www.neuroscience-gateway.org
The Allen Institute for Brain Science	www.alleninstitute.org
The Allen Brain Atlas	www.brain-map.org
The Alzheimer Research Forum	www.alzforum.org
The ALS (amyotrophic lateral sclerosis) Association	www.alsa.org
The National Ataxia Foundation	www.ataxia.org
The National Institutes of Health:	
National Institute of Alcohol Abuse and Alcoholism	www.niaaa.nih.gov
National Institute of Deafness and Other Communication Disorders	www.nidcd.nih.gov
National Institute of Drug abuse	www.nida.nih.gov
National Eye Institute	www.nei.nih.gov
National Institute of Mental Health	www.nimh.nih.gov
National Institute of Neurological Disorders and Stroke	www.ninds.nih.gov

Table 5.12. Information resources: pharmacology.

Resource	Web address
The Jackson Laboratory:	
• Phenotyping and efficacy testing services (JAX® Services)	www.jax.org/jaxmice/services/phenotyping-and-efficacy-testing
National Institute on Alcohol Abuse and Alcoholism (NIAAA) of the National Institutes of Health	www.niaaa.nih.gov
National Institute on Drug Abuse (NIDA) of the National Institutes of Health	www.nida.nih.gov
European Medicines Agency (EMEA)	www.emea.europa.eu

Table 5.13. Information resources: reproductive biology.

Resource	Web address
The Jackson Laboratory:	
• Information about our research	www.jax.org/research/faculty
• Reproductive Genomics: Mutant Mouse Models of Infertility website, with links to – lists of research models – other information related to reproductive biology	http://reproductivegenomics.jax.org/lists.html
National Institute of Child Health & Human Development (NICHD), National Institutes of Health	http://nichd.nih.gov
GermOnline	www.germonline.org

Table 5.14. Information resources: type 2 diabetes, obesity, and metabolic syndrome.

Resource	Web address
The Jackson Laboratory:	
• Diet and obesity web resource, with links to – lists of research models – additional resources – the Type 2 Diabetes and Obesity Resource Manual	www.jax.org/jaxmice/research/diabetes/type2.html
• Diet-Induced Obesity (DIO) Services (JAX® Services)	www.jax.org/jaxmice/services/dio
• Genome-wide scan profiles for all 10 NONcNZO strains	www.jax.org/research/faculty/leiter/type2_genomics
American Diabetes Association	www.diabetes.org
Animal Models of Diabetic Complications Consortium (AMDCC)	www.amdcc.org
Joslin Diabetes Center	www.joslinresearch.org
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)	www2.niddk.nih.gov
Diabetes Genome Anatomy Project (DGAP)	www.diabetesgenome.org
Obesity Gene Map Database	http://obesitygene.pbrc.edu

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Chapter 6: Bioinformatics Resources at Mouse Genome Informatics (MGI) and The Jackson Laboratory

Joanne M. Currrer

Throughout the history of The Jackson Laboratory, a consistent goal has been to enhance biomedical research around the world. One way we do this is by freely sharing information. Currently, with both public and private funding, and in collaboration with other institutions, our staff provides investigators worldwide with access to the most up-to-date information about the genetics of the laboratory mouse and available research models. Today, The Mouse Genome Informatics (MGI) website and The Jackson Laboratory website are recognized as preeminent repositories of public databases and resources related to laboratory mouse genetics.

Our objective for this chapter is to introduce the online resources available to the public from MGI and The Jackson Laboratory and to show how to get started using them. The chapter is organized as follows:

***Acknowledgments**: We would like to thank Karen Fancher (Technical Information Services) and Janan Eppig, David Shaw and Susan McClatchy (all from Mouse Genome Informatics) for their valuable input to this chapter.*

6.A. What is bioinformatics?

The term bioinformatics refers to computer access, integration, and analysis of collections of biological data. Uses of bioinformatics can be as simple as a search for information about a mouse gene in one database or as complex as the generation of a novel discovery through analyses of multiple databases.

The rapid advancements in genetic research since the late 1970s would not have been possible without bioinformatics. For example, suppose in 1986 that a researcher was searching for the gene responsible for a mutant phenotype in a mouse. Even if the phenotype was mapped to a 10–20 cM region, it might have taken several years—and considerable luck—to narrow the search down to a few reasonable candidate genes. To actually identify the gene would have taken several more years. Also, because so many genes had not yet been identified or annotated, the candidate list would have been incomplete, and might not have included the gene. Today, however, the mouse genome is sequenced and most of the genes are identified. Thus, within a mapped region of the same size of 10–20 cM, a researcher could conduct a similar search and identify several candidate genes within minutes.

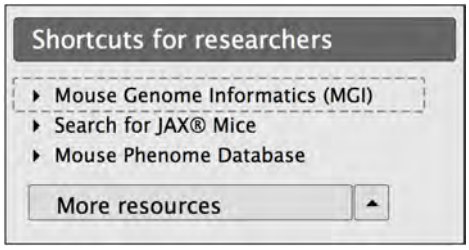
Taking full advantage of the power of bioinformatics presents several challenges: First is locating and accessing the specific databases that can help resolve a research issue. Second is finding ways to integrate relevant—but disparate—databases to exploit the strengths of each. Third is understanding the use of the computational tools that "crunch" and analyze the data. MGI and The Jackson Laboratory assist researchers with these issues by maintaining several databases accessible to the public, by providing links to—and integration with—databases maintained by others, and by providing computational resources for analysis of this data.

6.B. Introduction to bioinformatics resources at the Mouse Genome Informatics (MGI) website

The Mouse Genome Informatics (MGI) website offers seamless queries of multiple databases from a single search interface, with integration of all results in one display, and links to other databases and resources from search results.

6.B.1. How to access MGI and get help

To access the MGI website, link to Mouse Genome Informatics (MGI) in the "Shortcuts for researchers" text box, which is displayed on The Jackson Laboratory homepage (www.jax.org) and on many other pages of The Jackson Laboratory website. Alternately, go directly www.informatics.jax.org.



For help using the MGI website, from the MGI homepage, choose the "Tour the MGI website" button. Also look for frequently asked questions (FAQs) and links to context-sensitive help information throughout the site.

For additional help, contact the MGI staff using one of the following methods:

- In the menu bar on each MGI webpage, click the "Contact Us" link and complete the online form.
- Email mgi-help@informatics.jax.org.
- Call 1-207-288-6445.
- Fax 1-207-288-6132.

6.B.2. What you can do from the MGI website (www.informatics.jax.org)

The MGI homepage has three basic functional areas:

- Quick Search, for single searches on individual MGI ID numbers, gene symbols, or gene names.
- Explore MGI, for access to resources and help documents organized by functionality.
- A menu bar, for quick central access to MGI features.

Following is an overview of these three functional areas.

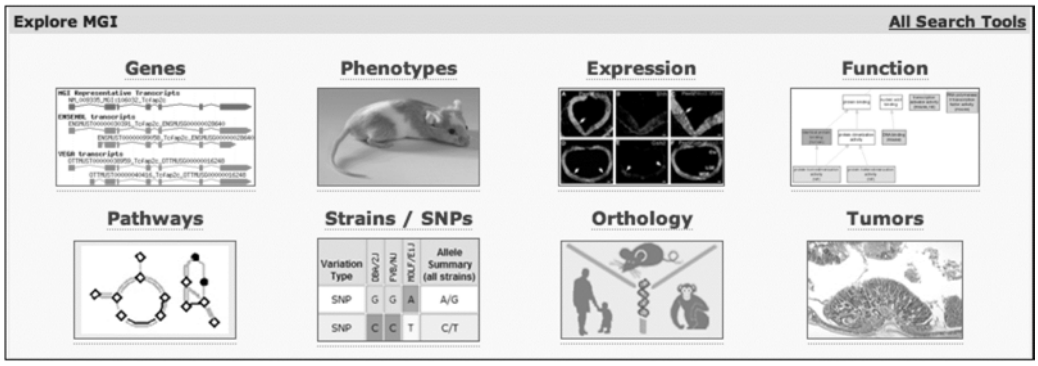
6.B.2.a. Quick Search

The "Quick Search" area of the MGI website allows users to quickly conduct a search on an ID number, gene symbol, or gene name directly from the upper right-hand corner of most MGI webpages. (The ID number is the MGI accession number that is assigned sequentially to data objects as they are added to the database. An example of an ID number is MGI:75909.)

To conduct a Quick Search, simply enter the search argument and click the Quick Search button.

6.B.2.b. Explore MGI

The "Explore MGI" area of the MGI website is organized in eight functional areas, identified by title and icons.



Following is a task-based overview of each functional area. It is important to note that, because these areas are organized by function, they generally do not represent a one-to-one relationship with a specific database. Rather, some databases are accessible from multiple areas, as tasks require. For reference information about the most commonly used databases, see 6.C, "Reference information: bioinformatics resources available at MGI and The Jackson Laboratory," later in this chapter.

6.B.2.b. Explore MGI (continued)

Genes (Genes, Genome Features & Maps)

- Search for
  - genes and genome features by symbol, name, location, gene ontology classification or phenotype,
  - sequences by mouse strain or biological attribute,
  - mouse genes and genome features using nucleotide or protein sequences (MouseBLAST).
- Display
  - genetic maps,
  - specific regions of the mouse genome,
  - nomenclature guidelines.
- Download
  - results of batch searches (multiple arguments),
  - plain text files of any genes and markers query result.
- Submit a new gene or genome feature to receive official nomenclature and an MGI accession identifier.

Also find FAQs, information about collaborators, and links to help text and other related sites.

Phenotypes (Phenotypes, Alleles & Disease Models)

- Search for mutations or QTLs based on
  - phenotype, allele, disease model,
  - mammalian phenotype (MP) ontology terms,
  - human disease (Online Mendelian Inheritance in Man® [OMIM®]) terminology,
  - mutation type,
  - gene,
  - genome location.
- Display nomenclature guidelines for naming alleles and mutations.
- Download results of batch searches (multiple arguments).
- Submit
  - a new allele, mutation or transgene to receive official nomenclature and an MGI accession identifier,
  - a description of a spontaneous, induced or genetically-engineered mutation that is already registered in MGI.

Also find FAQs, information about collaborators, and links to help documents and other related sites

Expression (Gene Expression Database [GXD])

- Search for
  - detailed gene expression assay results,
  - references on gene expression during development,
  - genes expressed in some anatomical structures and/or developmental stages but not in others,
  - anatomical structures, including links to associated expression results,
  - anatomical terms and associated expression data in the Edinburgh Mouse Atlas,
  - expression information based on tissue or cell-line origin of cDNAs.
- Download a tool to manage your own expression data and submit them.

Also find FAQs, information about collaborators (such as the Mouse Gene Expression Information Resource [GEIR]), the Gene Expression Database (GXD), and links to help documents and other relevant resources.

Function (Functional Annotation Using the Gene Ontology [GO])

- Browse the Gene Ontology (GO) and mouse annotations in MGI.
- Search for genes using GO terms and other gene attributes.
- Access GO annotations for genes associated with OMIM®.
- Download sets of IDs or symbols for use in GO tools.
- Use GO annotations to discover what gene sets might have in common.

Also find FAQs; information about GO resources, annotation projects and literature; and hints for interpreting annotations.

Pathways (Biochemical [metabolic] Pathways)

- Search the MouseCyc database.
- Compare metabolic pathways across species.
- Browse the MouseCyc
  - pathway ontology,
  - all pathways, genes, proteins and compounds represented in MouseCyc.
- Download gene names for any pathway.
- Overlay experimental data onto the Pathway Tools Omics Viewer.
- Generate a mouse metabolic map.

Also find FAQs, information about MouseCyc and collaborators, and links to help documents and other relevant websites.

Strains/SNPs (Strains, SNPs & Polymorphisms)

- Search for
  - SNPs by strain, attributes, genomic position or associated genes,
  - RFLP or PCR based on polymorphisms by strain, locus symbol or map position,
- Search through the listing of *Inbred Strains of Mice and Rats*, by M. Festing and an MGI report of official strain names
- View
  - a chart depicting the origins and relationships of inbred mouse strains,
  - nomenclature and naming guidelines.
- Register a new strain and receive official nomenclature and an MGI accession identifier.

Also find FAQs; information about strains, SNPS and collaborators; and links to help documents, the International Mouse Strain Resource (IMSR) database, and other relevant websites.

Orthology (Mammalian Orthology)

- Search for
  - orthologs between 2 species using marker symbol or name, accession ID, chromosomal location, author or reference ID,
  - gene family data using the Protein Information Resource SuperFamily (PIRSF) browser.
- Build a comparative map using the Linkage Map tool.
- View the mouse-human and mouse-rat orthology maps.
- Download a complete set of rat, human, dog or chimpanzee orthologs for mouse genes.

Also find FAQs, information about orthology, and links to help documents, other relevant websites, and reports generated nightly by MGI.

Tumors (Mouse Tumor Biology [MTB] Database)

Link to the Mouse Tumor Biology (MTB) database and

- Search for
  - tumor types,
  - tumor incidence and latency,
  - tumor pathology reports and images,
  - genetic factors associated with tumors and tumor development.
- Compare cancer profiles of different strains of mice.
- Review the patterns of mutations in specific cancers; identify genes commonly mutated across a spectrum of cancers.

Also find information on, and links to, related online resources.

6.B.2.c. The menu bar

The menu bar on the MGI website is available from almost every MGI webpage.

The menu bar includes the following functionality:

- Search: Access to MGI search functionality from a single location.
- Download: Access to MGI download functionality from a single location.
- More Resources: Links to online resources such as research community email lists, online books, nomenclature information, online databases, other relevant websites, and software developer tools.
- Submit Data: Links to web resources for submitting and registering gene, allele, and strain names and for contributing data.
- Find Mice (IMSR): Link to the International Mouse Strain Resource (IMSR) database.
- Contact Us: Send an email message directly to the MGI staff.

6.B.3. Examples: using MGI to find a mouse model associated with a disease and to find phenotypic information for a gene

Following are two examples of using MGI: How to find a mouse model associated with cystic fibrosis, and how to find information about phenotypes related to the *Cftr* mouse gene.

Keep in mind that MGI functionality is *very* diverse and *very* flexible. Each example represents just one way to complete a task. We encourage you to explore ways that might suit you better.

Table 6.1. Example: How to find mouse models associated with cystic fibrosis.

Action	Result
1. From the "Explore MGI" area of the MGI homepage, select the Phenotypes option.	Phenotypes, Alleles & Disease Models page.
2. Select the "Human Disease (OMIM) Browser" link.	Human Disease Vocabulary Browser.
3. In the "Search the vocabulary" field, type "cystic fibrosis" and click the Search button.	A list of OMIM® IDs and descriptions with numbers of available mouse models.
4. In the "Human Disease" column, select a cystic fibrosis link that indicates a quantity of available mouse models.	Human Disease and Mouse Model Detail page.
5. In the Mouse Models section of the page, select the mouse model with the allelic composition of interest.	Phenotypic Allele Detail page.
6. In the Allele details section of the page, select the link to the International Mouse Strain Resource (IMSR) or the Mouse Models of Human Diseases.	Information about mouse models.

Table 6.2. Example: How to find phenotypes associated with the *Cftr* (cystic fibrosis transmembrane conductance regulator homolog) gene.

Action	Result
1. From the "Explore MGI" area of the MGI homepage, select the Genes option.	Genes, Genome Features & Maps page.
2. Select the "Genes and Markers Query" link.	Genes and Markers Query Form.
3. In the "Gene/Marker Symbol/Name" field, type "cftr" and click the Search button.	Genes and Markers, Query Results – Summary.
4. In the "Symbol, Name" column, select the "Cftr" link.	Gene Detail page
5. In the Phenotypes section of the page, select the link of interest.	Information about the gene with links to more detailed information.

6.C. Reference information: bioinformatics resources at MGI and The Jackson Laboratory

Following is reference information about the most commonly used online resources accessible through the MGI and The Jackson Laboratory websites. Most often, you will access these databases via links from other webpages or search interfaces. We provide the web addresses of the databases, however, should you want to access them directly.

6.C.1. Databases that are part of MGI

The MGI database system comprises integrated databases that collectively provide access to data on the genetics, genomics, and biology of the laboratory mouse. The databases listed below are those that are accessed from the functional areas of the "Explore MGI" area and menu bar of the MGI website (www.informatics.jax.org).

Mouse Genome Database (MGD)			
Description:	The international community database for the laboratory mouse, providing a current, integrated representation of mouse genetic, genomic, and biological information. The MGD forms the core of MGI.		
Contents:	• genes and genomic features • allelic variants • phenotype descriptions	• orthologous gene relationships and comparative sequence map	• functional gene classifications • molecular reagent data • SNP data
Functions:	• Search, download, and display data in a variety of tabular, graphical, and map formats. • Link from "mouse" data to relevant related data in a variety of other data resources.		
Address:	www.informatics.jax.org		

Gene Expression Database (GXD)			
Description:	A community resource of endogenous embryonic gene expression data for the laboratory mouse; integrated with MGI and hosted by The Jackson Laboratory.		
Contents:	Integration of multiple types of expression data, including		
	• RNA *in situ* hybridization • immunohistochemistry	• northern and western blot • RT-PCR	• RNAase protection • cDNA source data
Functions:	• Access data in formats appropriate for comprehensive analysis. • Integrate with the MGD for combined analysis of genotype, expression, and phenotype information. • Interconnect with sequence databases, databases for other species, and other on-line resources.		
Address:	www.informatics.jax.org/mgihome/GXD/aboutGXD.shtml		

6.C.1. Databases that are part of MGI (continued)

International Mouse Strain Resource (IMSR) database			
Description:	An online database of mouse strains and stocks—including inbred, mutant, and genetically-engineered—available worldwide. The goal of the IMSR is to assist the international scientific community in locating and obtaining mouse resources for research.		
Contents:	• holder of the strain	• state of the strain (e.g., live, frozen, etc.)	• type of mutation (i.e., targeted or transgene)
Functions:	• Search for mice by strain, status, mutation, chromosome. • Link to information about specific strains, alleles, and the holder of the strain. • From MGI allele detail pages, automatically search the IMSR for relevant models.		
Address:	www.informatics.jax.org/imsr		

Mouse Tumor Biology (MTB) Database			
Description:	A community resource for integrated information on tumor genetics and pathology in genetically defined mice. The MTB allows cancer models to be evaluated as they apply to humans, presents mutation patterns for specific mouse cancers, and allows comparison across cancer models. The MTB is the most comprehensive database available on cancer characteristics of different strains of mice.		
Contents:	Integration of multiple types of expression data, including • tumor types • tumor incidence and latency data in various mouse strains	• tumor pathology reports and images • genetic factors associated with tumors and tumor development	• information on, and links to, related online resources
Functions:	• Choose experimental models • Compare the cancer profiles of different strains of mice	• Review the patterns of mutations in specific cancers. • Identify genes that are commonly mutated across a spectrum of cancers.	• Search for tumor information based on parameters such as mouse strain, tumor type, gene name, and organ name.
Address:	http://tumor.informatics.jax.org/mtbwi/index.do		

MouseCyc Database			
Description:	A genome database of metabolic pathways for the mouse that is integrated with MGI's well-curated information on phenotypes, gene expression data, functional annotations, and mammalian homology for mouse genes. Emphasis is on genes with direct human counterparts.		
Contents:	• proteins • pathways	• reactions • compound genes	• RNAs
Functions:	• Analyze mouse genetic and genomic data in the context of biochemical and metabolic processes. • Compare human and mouse pathways based on curated orthologous genes and conserved synteny relationships.		
Address:	http://mousecyc.jax.org		

6.C.2. Other databases

The Jackson Laboratory provides access to other databases that play an integral role in mouse genetics. Following are descriptions of two of the most widely used. For access to other online genetics resources, visit www.jax.org/research/resources.

Mouse Phenome Database (MPD)	
Description:	Database of information gathered by the Mouse Phenome Project, an international, collaborative initiative to establish a comprehensive collection of phenotypic data for mouse strain comparisons. The MPD also includes data generated from a focused effort to comprehensively evaluate multiple phenotypes in a set of 36 "priority" strains under uniform conditions. An objective of the MPD is to facilitate the study and use of laboratory mice in researching human health issues. The MPD is maintained at The Jackson Laboratory.
Contents:	Information on mouse phenotypes, including • cancer susceptibility • neurological and behavioral disorders • sensory function defects • atherosclerosis • gallstones • blood disorders • infectious disease susceptibility • lung function • hypertension • osteoporosis • obesity • body weight • blood chemistry values • images
Functions:	• Assists investigators in identifying mouse strains for experiments. • Makes phenotype-genotype association predictions. • Identifies and determines the function of genes that participate in normal and disease pathways. • Graphically compares data across—or between—strains.
Address:	www.jax.org/phenome

JAX® Mice Database	
Description:	An online database providing direct access to comprehensive information about the biology and genetics of mouse strains maintained at The Jackson Laboratory.
Contents:	Strain-specific information, including • complete strain name, common name and nomenclature • strain description and source • phenotypes and genotypes • full and common names of relevant genes and alleles, including haplotype and coat color • links to references • husbandry conditions (including room number and diet) • animal care tips
Functions:	• Provides a complete picture of all strains of mice maintained or cryopreserved at The Jackson Laboratory. • Provides search functionality on Mammalian Phenotype Ontology and human disease (Online Mendelian Inheritance in Man® [OMIM®]) terminology. • Feeds current strain information to several databases at The Jackson Laboratory, avoiding duplication and assuring accuracy.
Address:	www.jax.org/jaxmice/query

6.C.3. Online books available from MGI

MGI provides public online access to five important books related to mouse genetics. The *Biology of the Laboratory Mouse* is available in limited quantities (see sidebar for ordering information). The other four books are out of print.

- *Biology of the Laboratory Mouse*. 1968. Green EL (ed). Dover Publications, Inc., NY.
- *Mouse Genetics, Concepts and Applications*. 1995. Silver L. Oxford University Press.
- *The Anatomy of the Laboratory Mouse*. Cook M. 1965. Academic Press. M.R.C. Laboratory Animals Centre, Carshalton, Surrey, England.
- *The Coat Colors of Mice, A Model for Mammalian Gene Action and Interaction*. 1979. Silvers WK. Springer Verlag.
- *Origins of Inbred Mice*. 1978. Morse HC III (ed). Academic Press. National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

To order a copy of the *Biology of the Laboratory Mouse* …

1. Go to the online version of the book. (Use the procedure below left or go directly to www.informatics.jax.org/greenbook.)
2. At the bottom of the book cover webpage, select the link to purchase the book from The Jackson Laboratory.

To access an online book:

1. From the MGI menu bar, select More Resources.
2. From the More Resources drop down menu, select Online Books. Then, from the flyout menu, select the book.

6.C.4. Additional resources

- www.jax.org/jaxmice/support
  Technical support and literature on JAX® Mice, including the JAX® Mice Catalog, resource manuals, model lists, price list, JAX® NOTES, the JAX® Mice News newsletter
- Appendix N: Sources of Information about Laboratory Mice

6.D. Literature

6.D.1. Literature related to MGI

Begley DA, Krupke DM, Vincent MJ, Sundberg JP, Bult CJ, Eppig JT. 2007. Mouse Tumor Biology Database (MTB): status, update, and future directions. *Nucleic Acids Res*. 35:638–642.

Bult CJ, Eppig JT, Kadin JA, Richardson JE, Blake JA, Mouse Genome Database Group. 2008. The Mouse Genome Database (MGD): mouse biology and model systems. *Nucleic Acids Res.* 36:724–728.

Eppig JT, Blake JA, Bult CJ, Richardson JE, Kadin JA, Ringwald M, The MGI staff. 2007. Mouse genome informatics (MGI) resources for pathology and toxicology. *Toxicol Pathol*. 35:456–457.

Eppig JT, Blake JA, Bult CJ, Kadin JA, Richardson JE, Mouse Genome Database Group. 2007. The mouse genome database (MGD): new features facilitating a model system. *Nucleic Acids Res*. 35:630–637.

Eppig JT, Bult CJ, Kadin JA, Richardson JE, Blake JA, Mouse Genome Database Group. 2005. The Mouse Genome Database (MGD): from genes to mice, a community resource for mouse biology. *Nucleic Acids Res*. 33:471–475.

Gene Ontology Consortium, Blake JA, Bult CJ, Eppig JT, Ringwald M. 2008. The Gene Ontology project in 2008. *Nucleic Acids Res*. 36: D440–444.

Krupke D, Näf D, Vincent M. Allio T, Mikaelian I, Sundberg J, Bult C, Eppig J. 2005. The Mouse Tumor Biology Database: integrated access to mouse cancer biology data. *Exp Lung Res*. 31:259–270.

Smith CL, Goldsmith CA, Eppig JT. 2005. The Mammalian Phenotype Ontology as a tool for annotating, analyzing and comparing phenotypic information. *Genome Biol*. 6:7.

6.D.2. Literature related to other databases

Grubb SC, Churchill GA, Bogue MA. 2004. A collaborative database of inbred mouse strain characteristics. *Bioinformatics*. 20:2857–2859.

McKusick VA. 2007. Mendelian Inheritance in Man and Its Online Version, OMIM. *Am J Hum Genet*. 80:588–604.

Section IV: Colony Management

Colony management in its broadest sense involves your animals, your facility, your staff, and your administration. Colony management issues range in scope from simple tasks performed day after day (checking water bottles and changing cages) to complex tasks that you hope you never have to perform (recovering from microbial contamination or a natural disaster). Colony management has one purpose: to produce and maintain healthy, genetically consistent animals, suitable for biomedical research.

In this section, we will address issues that almost every colony manager and institution must address. Our objective is to present guidelines and choices, anticipate questions you might have, and explain where to get more information. We'll also explain what we do at The Jackson Laboratory. This information is especially relevant because it details the conditions under which JAX® Mice are bred and housed.

This section is organized as follows:

Chapter 7: Animal Health—Preventing, Identifying, and Eradicating Microbial Contamination

Peggy Danneman, Dorcas Corrow, Joanne M. Currer, Kevin Flurkey

If you manage a production or research colony, maintaining animal health is one of your most critical responsibilities. This issue includes preventing and identifying microbial contamination as well as ridding your colony of a contamination, should one occur. Whether you purchase mice or acquire them from other research colonies, your concern also includes the possibility of introducing contaminated animals into your colony.

The objective of this chapter is to describe considerations for developing an animal health plan and managing a microbial contamination. We also provide an overview of our animal health plans at The Jackson Laboratory.

The chapter is organized as follows:

7.A. Developing an animal health plan

There is no universal agreement on the desired health status of mice used in research. Individual scientists must avoid microbial contaminants that could impair the performance of mice in their research programs, either directly, by causing clinical disease, or indirectly, by causing physical or physiological changes that could alter or confound data. Facility managers and veterinarians would prefer to exclude any microorganism that could have a negative impact on any of the animals or research projects in their facility. Thus, there is widespread, if not universal, interest in excluding pathogenic murine viruses from research facilities. However, there is no consensus on the importance of excluding organisms such as pinworms, *Helicobacter*, *Pneumocystis murina*, *Staphylococcus*, and *Pseudomonas*. The strict barrier operations needed to maintain mouse colonies free from these organisms are costly and labor intensive, and researchers often find that working under the restrictions imposed by these operations is cumbersome. If these costs are perceived as being out of proportion to any potential negative impact of a particular microorganism, there may be little enthusiasm for attempting to exclude it.

In fact, in some situations, microorganisms that are viewed as unacceptable by some are actually considered desirable by others. There are many examples where the desired phenotype of a valued mouse model was lost following rederivation and transfer to an ultra-clean barrier environment. For example, in most mouse models of inflammatory bowel disease (IBD), a complex enteric flora is required to produce gut pathology. Accordingly, 100% of IL-10 knockouts (e.g., B6.129P2-*Il10*^{tm1Cgn}/J [002251]) maintained under conventional conditions will develop enterocolitis by three months of age, whereas most rederived knockouts housed under specific pathogen free (SPF) conditions show no signs of enterocolitis by 12 months, and germfree knockouts never develop enterocolitis (Kuhn *et al*., 1993; Kullberg *et al*., 1998; Sellon *et al*., 1998). In some cases, multi-level SPF facilities within an institution may be necessary to accommodate different research applications and requirements.

Thus, it is up to you and your institution to set suitable standards for your environment and develop an animal health program to meet those standards. Most programs comprise four components:

- The exclusion list
- Preventive measures
- Monitoring procedures
- Containment and eradication procedures

Notification procedures, in the event of a break, are critical for commercial suppliers and should be considered for any institution that distributes mice to collaborators or other researchers.

7.A.1. The exclusion list—microbial agents that are unacceptable in your colonies

Realistically, you cannot exclude all agents that might cause disease or interfere with research in your mouse colonies. Only you can decide which agents you can and cannot live with based on the needs of your research program or institution.

When determining your exclusion list, consider the ability or willingness of your institution to exclude an organism and to eradicate if it is found. Unless you can and *will* implement appropriate measures to exclude and eradicate an agent, there is no point in including it on your list. Considerations include the following: What are the potential consequences of the presence of the agent on animal health? Can your institution live with the agent in all colonies? Some colonies? How disruptive will an infection be to research? Can the agent affect your technicians adversely? How costly would an infection be in terms of lost animals or compromised research?

For an example of the organisms you might want to consider excluding, refer to Table 7.1, "Infectious agents monitored at The Jackson Laboratory," later in this chapter. This table lists the organisms on our exclusion list at The Jackson Laboratory. This list is quite comprehensive, and it may not be practical or economically feasible to exclude all of these organisms from your animal colonies. Also, note that not all organisms are excluded from all animal colonies at The Jackson Laboratory; some opportunistic organisms (*Helicobacter* spp. and *Pasteurella pneumotropica*) are currently tolerated in some research colonies, and as noted above, are essential for pathology development in IBD models. These opportunistic organisms are not tolerated in our production colonies.

7.A.2. Preventive measures to keep unacceptable agents out of your colonies

Your pathogen protection goals are to keep undesirable agents out of your facility, your mouse rooms, and your cages. The best protection is provided by the use of multiple strategies, including the following:

- Set up physical barriers to prevent pathogens from entering your facility. Define strict procedures for anything and anyone entering a barrier facility. Develop detailed procedures for any animal work conducted within a room. Thoroughly document all procedures and requirements, distribute the procedures to all personnel who enter the rooms, train personnel in the procedures, and post the procedures in the rooms.
- For equipment and supplies, set up traffic routes so that contact between clean, incoming material and dirty, outgoing material is eliminated or minimized. For employees, set up procedures to minimize movement from one colony to another.
- Develop detailed procedures for introducing mice into your facility.
  - Thoroughly disinfect the outside of shipping containers and check their integrity before opening them. Briefly check each mouse as you unpack it.
  - Determine whether mice should go directly to the colony room, be quarantined, or be rederived. The greatest risk is with the first option. However, if you limit this practice to vendor mice from an SPF facility, the risk is comparatively low. Quarantine is more expensive because it requires separate, dedicated space. A good quarantine program also involves thorough follow-up testing. If necessary, animals in quarantine may be made available to researchers if appropriate considerations are made to avoid the potential for contamination. For example, mice that must be used in research soon after arrival may be received into a separate quarantine area and manipulated using strict biocontainment procedures. Rederivation is the safest option—and also the most expensive. It requires a separate facility and technicians with expertise in assisted reproductive techniques (ARTs). Also, all rederivation procedures require a source of highly-monitored SPF foster or recipient mothers.
  - It is worth noting that any violation of quarantine and import procedures opens the colony to a wide range of pathogens that can affect the health, performance and value of the mice. Thus, it is essential to ensure full cooperation of all members of a research staff in adhering strictly to institutional policies.

Can a colony be completely germ free? Would this be a good idea?

It is possible to have completely germ free—axenic—colonies, but the extreme effort required is warranted only for very specific purposes, such as microbiological studies involving reconstruction of gut bacteria populations with single organisms. For most research, however, axenic colonies are undesirable. Some bacteria, such as those in the gastrointestinal tract, are necessary for normal, biological processes. Ridding colony animals of these agents may result in animals inappropriate for most research.

7.A.3. Monitoring procedures to identify the presence of unacceptable agents

The goal of health monitoring is to find at least one animal that is infected—or was previously infected—with an agent on your exclusion list that has gained access to your colony. Of course, you cannot test every animal. Thus, you must conduct routine monitoring at a stringency to guarantee that you will meet or exceed the level of detection acceptable to your institution. If you have a break or are recovering from one, more stringent monitoring will be necessary. Following are considerations for effective health monitoring.

7.A.3.a. Sampling considerations

All monitoring programs are based on the concept of prevalence—the proportion of mice in the colony that are infected. Many formulas for the calculation of the sample size needed to detect a given prevalence are based on the simplifying assumption that any infection is randomly distributed through the colony. For example, if the prevalence is 10%, the chance that any given animal in the colony is infected is 10%. To find organisms that are present at lower prevalence, more mice must be tested. If you are able to tolerate the presence of a contaminant until it is present at a higher prevalence, you can test fewer mice.

Everyone should be on the monitoring team…

Every person who works with your mice should be vigilant for any overt sign of "sick" mice that could indicate a breach in the pathogen barrier. Such signs include unusual newborn mortality, diarrhea, wasting, unresponsiveness to cage disturbance, or unusual behavior.

Animal care technicians, who observe your mice on a daily basis and who are familiar with their normal characteristics, are valuable stewards of the health of your animals. They, and anyone else who is in regular contact with your mice, should be made aware of symptoms of ill health and should be encouraged to freely communicate any concerns they might have.

Of course, to take full advantage of well-trained and involved staff, researchers must be willing to submit unanticipated "sick" mice to the veterinary health monitoring program.

The assumption of random distribution of an infection complicates the interpretation of results. In fact, infections are rarely randomly distributed through a colony. Thus, assignment of mice to be sampled should take into account a number of non-random factors. For example, if several strains of mice are present in the room, or if mice from several different investigators are present in the room, it is desirable to test mice from every strain and/or investigator. In general, test animals of both sexes and various ages. Note also that practices designed to prevent the spread of disease (i.e., microisolator caging and microisolator technique) will also complicate health monitoring efforts, as these practices will inhibit or prevent the random distribution of contaminants within a colony. Once you control for variables such as these, however, in the absence of some specific information that would help pinpoint which animals are most likely to be contaminated, the best approach is to test randomly selected animals.

7.A.3.b. Frequency of monitoring

The frequency of monitoring for organisms on your exclusion list may vary for different agents based on the likelihood of infection and the potential impact of the infection. See 7.B.3.a. for information about the frequency of our monitoring at The Jackson Laboratory. In general, it is advisable to monitor more frequently for organisms that are more prevalent in laboratory mice or are more likely to cause disease or interfere with research (e.g., MPV or MHV). Costs can be reduced by monitoring less frequently for organisms that are less likely to be present or to interfere with research (e.g., K virus, Polyoma virus).

7.A.3.c. Choice of test animals

Appropriate test animals are those that are at least as likely as other members of the colony to become infected with the organism(s) of interest, and if infected, to show a positive response on the test being used to identify the infecting organism.

Choose colony animals or sentinels on the basis of your needs and availability of animals. Colony animals are especially useful because they have the same genetic background and have been treated the same (including experimental manipulations) as the rest of the colony. Sentinel animals, which are brought into the colony specifically to detect contamination, are an option when valuable colony animals cannot be sacrificed or for serologic monitoring of immunodeficient animals or those that are prone to autoimmune disorders. (Immunodeficient animals have impaired antibody response to pathogens. Autoimmune animals produce a high level of antibodies that could interfere with a serologic test.)

Sentinels are of two types: Dirty bedding sentinels are routinely exposed to dirty bedding from multiple cages of colony animals. Cage contact sentinels are housed in the same pen with colony animals. Sentinels are less preferable than colony animals because they may have a different genetic background and history. Furthermore, although dirty bedding sentinels in theory permit you to survey a large portion of the colony, they have additional disadvantages. They are notoriously poor indicators of colony infections by organisms that are not spread by the fecal-oral route (e.g., Sendai virus). And, even with organisms that spread readily by the fecal-oral route (e.g., MHV and MPV), spread to dirty bedding sentinels may be slow. Therefore, exposure time of dirty bedding sentinels to potentially contaminated material should be prolonged (a minimum of four weeks), which may be an issue when this approach is used to monitor animals during quarantine. Also, if sentinels do not originate from a frequently monitored colony of known high health status, they can actually introduce contaminants. This is a particular concern with cage contact sentinels.

7.A.3.d. Types of tests

No single category of tests is sufficient. An effective monitoring strategy incorporates the following:

- Serology, for detection of antibodies that indicate exposure to viruses, mycoplasma, and some bacteria. Note that false positives are more common with ELISA (enzyme-linked immunosorbent assay) than with IFA (indirect fluorescent-antibody) assays, but may occur with either.
- Microbiologic culture, for specific, but more time-consuming, detection of most bacteria.
- Parasitology (fecal flotation, direct microscopy, tape test, other).
- Polymerase chain reaction (PCR), for direct identification of the DNA or RNA of viruses, bacteria, fungi and parasites. Note that PCR can result in false positives *or* false negatives.
- Histopathology and gross pathology.
- General appearance, including fur loss, skin lesions, poor grooming, and emaciation, as well as high neonatal mortality and runted weanlings.

7.A.3.e. Interpretation of monitoring results

It is worth noting that a monitoring program is designed only to detect the presence of an organism, not its prevalence within the colony. It is also important to understand that no monitoring program is foolproof. False positives and false negative results can occur, and even if all results are accurate, there is always a potential for failing to detect infectious agents that are present at low prevalence within the colony.

There is some art to interpreting test results. If you don't trust negative results, retest. If you don't trust a positive result, retest using the same *and* different tests. (For example, if you tested originally using ELISA, retest using ELISA plus an indirect fluorescent-antibody [IFA] test and/or PCR.) If the result is reconfirmed, you may want to test more animals. Base your final interpretation on all test results and your experience—whether the results make sense to you.

When an initial positive result is confirmed and makes sense, it is time to implement your containment and eradication plan.

7.A.4. Containment and eradication procedures to prevent the spread of an infection and to eliminate it from your colony

Although your hope is to never need your containment and eradication plan, a wise strategy is to assume that a break will happen. A comprehensive plan, developed by those who know your mouse room operations the best, will ensure a swift, efficient, and effective response when the break occurs.

Once you confirm an infection, your goal is to prevent further spread—both within the room or area of origin and to other animal rooms—while working to eradicate it. Strategies for eradication include depopulation, rederivation, "test and cull," and, perhaps, burnout (see sidebar below right for important caveats regarding this often deceptively appealing alternative). In most institutions, this means a significant deviation from "business as usual." Thus, it is important to identify decision makers and detailed procedures—and to get the endorsement of administrators—before a break occurs.

Your plan should include written documentation of the decision makers and their areas of responsibility, the criteria for implementation of the plan, the definition of the containment and eradication strategies, and criteria for resumption of research in the affected area. Once your plan is approved, you should rehearse it on a regular basis to make sure that everyone is familiar with their roles in the plan and to identify modifications that may be required as a result of changes in research, policies, physical plant, etc.

A final note: As absolute insurance against loss of an irreplaceable stock of animals due to a contamination event, it is wise to back up the stock via cryopreservation. For details about cryopreservation and its use as a backup strategy, refer to Appendix J, "Cryopreservation."

Is "burnout" a reasonable option to handle contamination in your colony?

Burnout refers to the strategy of letting the infection "run its course." Burnout may be an option when the pathogen is one that spreads rapidly through a susceptible population, causes only transitory infections, and results in lifelong immunity (e.g., MHV). However, it is risky, especially when dealing with immunodeficient mice or genetically modified animals, which may develop more prolonged infections and/or fail to produce an effective immune response.

Researchers may pressure colony managers to use burnout so they can complete long-term studies or for economic reasons. But the agreement of all those potentially affected (for example, researchers with colonies in adjacent rooms) must be obtained before proceeding with a burnout strategy.

If an infection is allowed to progress in one colony, extreme measures must be taken to avoid spreading the infection to other SPF colonies. For example, the flow of supplies and mice, as well as caretaker and researcher traffic, must be explicitly stated. It is critical to cease introduction of new mice into a room during burnout; this includes cessation of breeding. The affected room should not be considered free of contamination until 1) all animals in the room when the infection was identified test *positive* for antibodies to the organism (indicating that they have recovered from the infection), and 2) for newly added mice, at least one mouse from every cage tests *negative* for antibodies to the organism for at least three consecutive months.

7.B. Our animal health plan at The Jackson Laboratory

Following is a summary of the animal health plan at The Jackson Laboratory. For up-to-date details, including monitoring procedures and health status reports, visit our "Animal health & genetic quality" website at www.jax.org/jaxmice/geneticheal th.

7.B.1. Our exclusion list

At The Jackson Laboratory, we screen mice for a wide range of viruses, bacteria, fungi, protozoa, and parasites. It should be noted that many of the organisms listed are unlikely to cause overt disease. We include some, such as mouse parvovirus, primarily because of their potential for interference with experimental results. We include others, such as *Pneumocystis murina*, because they are opportunistic pathogens that seldom or never cause problems in normal, healthy animals but may cause disease in severely immunodeficient or otherwise compromised mice. We include trichomonads on the list because some users of JAX® Mice prefer to exclude these protozoa from their barrier mice even though they are nonpathogenic and have never been shown to interfere with research.

Table 7.1 lists the agents we monitor as of the printing of this book. For a current list, visit www.jax.org/jaxmice/health/agents_list.

Table 7.1. Infectious agents monitored at The Jackson Laboratory.

Viruses	
• Ectromelia virus (agent causing mouse pox)	• Mouse hepatitis virus (MHV)
• GDVII (Theiler's mouse encephalomyelitis) virus	• Mouse parvovirus (MPV)
• Hantaan virus (Hantavirus)*†	• Mouse norovirus (MNV)
• K virus	• Mouse thymic virus (MTV)
• Lactic dehydrogenase elevating virus (LDV)	• Pneumonia virus of mice (PVM)
• Lymphocytic choriomeningitis (LCMV)†	• Polyoma virus
• Mouse minute virus (MMV)	• Reovirus 3 (REO 3)
• Mouse adenovirus (MAV)	• Rotavirus (Epizootic diarrhea of infant mice [EDIM])
• Mouse cytomegalovirus (MCMV)	• Sendai virus
Bacteria, mycoplasma, and fungi.	
• *Bordetella bronchiseptica*	• *Mycoplasma pulmonis*
• CAR *bacillus*	• *Pasteurella pneumotropica*
• *Citrobacter rodentium (Citrobacter freundii* 4280)	• *Pneumocystis murina*‡
• *Clostridium piliforme*	• *Pseudomonas* spp.
• *Cornebacterium kutsheri*	• *Salmonella* spp.†
• *Helicobacter* spp.	• *Staphylococcus aureus*
• *Klebsiella* spp.	• *Streptobacillus moniliformis*†
	• *Streptococcus* spp.
Parasites and protozoa	
• *Encephalitozoon cuniculi*	• Roundworms and other helminthes
• Fleas, fur mites, lice	• Tapeworms
• Follicle mites	• Opportunistic protozoa (e.g., *Giardia, Spironucleus*)
• Pinworms	• Nonpathogenic protozoa (e.g., trichomonads)

* Neither wild nor laboratory mice of the genus *Mus* are natural hosts for hanta viruses, but they may become infected if exposed.

† Zoonotic agent that may cause disease in humans.

‡ *Pneumocystis murina* monitoring is conducted in severely immunodeficient mice only.

7.B.2. Our preventive measures

Our health maintenance plan is based on physical barriers and strict adherence to barrier procedures. Under no circumstances do we move mice from research colonies to production colonies without rederivation. Everyone on our campus follows detailed standard operating procedures (SOPs) for moving mice, supplies, and themselves into, out of, and around our mouse colonies, and for activities within the animal room and operational support areas. New staff members and new research technicians and students are fully briefed as to the required measures for animal health protection to which they must fully subscribe.

7.B.2.a. Maintenance of mice

7.B.2.a.1. Barrier levels of our mouse rooms

We operate all of our animal rooms as barriers. The barrier level varies from low to maximum depending on the location and use of the room. All levels have several characteristics in common, including 100% fresh, HEPA-filtered air (under positive pressure in most areas), and pre-defined traffic patterns that keep clean and dirty supplies from crossing paths. We rederive all mice that come into The Jackson Laboratory from any outside source. For current information about our barrier levels, visit www.jax.org/jaxmice/health.

7.B.2.a.2. Mouse room activities

We have detailed SOPs for entry into and exit from our mouse rooms, as well as for any tasks that must be done within a room. SOPs cover a wide range of tasks, including how to remove a mouse box from a shelf, how to add food and a new water bottle, and how to physically move the mice from dirty to clean cages, as well as procedures and schedules for cleaning all parts of the room.

7.B.2.b. Quarantine and importation of mice

At The Jackson Laboratory, our quarantine and importation facilities are located in a building separate from research and production facilities. All new stocks of mice arriving at our campus must enter through the quarantine facility. This facility is operated at negative pressure, as are the isolators and individual ventilated caging (IVC) systems used for quarantine housing. We generally rederive strains via embryo transfer or *in vitro* fertilization; however, we also use hysterectomy derivation and, on rare occasions, ovarian transplantation. (For details on these procedures, see 13.E.2, "Assisted reproductive techniques [ARTs].") All material and equipment leaving the quarantine facility are autoclaved out.

What exactly do we mean by specific pathogen free (SPF) colonies?

By definition a specific pathogen free (SPF) colony is one from which specified pathogenic microorganisms are excluded. Because the excluded microbes can differ from colony to colony, SPF colonies are not necessarily microbiologically equivalent to each other. At The Jackson Laboratory, the abbreviation SPF is also used to denote animals with a defined aerobic flora. Currently, JAX® Mice from our "SPF" colony harbor only the following aerobic bacteria: *Enterococcus* spp., *Lactobacillus* spp., and coagulase-negative *Staphylococcus* spp. These mice are used as foster mothers for rederivation of imported mice and for embryos recovered from cryopreservation. They are also deployed as sentinels in a few animal rooms in which we rely in part on sentinels for health monitoring.

Our importation facility also includes an SPF barrier facility that contains several strains of mice with an aerobically defined flora. We use females from this colony as foster mothers and as recipients of ovarian and embryo transplants. All material that enters this maximum barrier facility must be sterilized.

Importing and distributing new mutant mice: our responsibilities as a publicly funded national repository

One of the most important roles The Jackson Laboratory plays is to distribute mutant mice developed by researchers throughout the world—including here at The Jackson Laboratory. We import these mice via rederivation to assure their status as pathogen-free mice. Currently, our repository staff manages more than 2800 mutant strains. We import new strains at the rate of about 600 per year.

We receive federal funding to provide this repository service as well as to collect, summarize, and publish information about these specialized mice.

For further information about our repository and how research scientists can take advantage of our services, visit our Repository website at www.jax.org/jaxmice/findmice/repository.

Technicians who work in our quarantine and importation facilities are not allowed to enter other mouse rooms on our campus.

Regardless of the technique used for rederivation, at weaning we send *all* foster mothers and recipient females to our diagnostic laboratory for health monitoring. We release litters only if we receive negative results for all organisms other than the allowed SPF flora.

7.B.3. Our monitoring procedures

7.B.3.a. Frequency and types of monitoring

At The Jackson Laboratory, we base our health monitoring on the mouse room, not the mouse strain. We perform health monitoring in all animal rooms at least quarterly. In some special areas, such as those that house our foundation stocks, pedigree expansion stocks, and foster mother colonies, we monitor monthly. We screen for most organisms listed on the health report at each monitoring interval. However, we make exceptions for some organisms that are uncommonly found in modern laboratory mouse colonies: We screen semi-annually for lymphocytic choriomeningitis virus, mouse thymic virus, and reovirus 3. We screen annually for K virus, lactic dehydrogenase elevating virus, and polyoma virus. Current health reports are available to the public at www.jax.org/jaxmice/health.

During daily welfare checks and as cages are changed, trained technicians search specifically for mice with conditions abnormal for a specific strain. Animals with injuries or abnormalities that are not clearly caused by disease or a genetic mutation are culled. Those showing abnormalities that might be caused by an infectious agent are sent to the diagnostic laboratory. If the abnormality is clearly not related to a microbial contamination, mice are set aside to see if any researchers are interested in the phenotype. (See sidebar in 3.C.1.a.1 for information on our deviant search program.) Some of the conditions that warrant diagnostic testing include diarrhea, abnormal discharge from a body opening, visible masses on or under the skin, abnormal swelling of a body part, poor physical condition, sores or other skin lesions with or without loss of fur, head tilt or circling to one side, or runting of an entire litter.

7.B.3.b. Sample categories and sizes

When testing for pathogens at The Jackson Laboratory, we use colony or sentinel animals or a combination of both, depending on the characteristics and use of the colony. When sentinels are needed (e.g., for colonies of immunodeficient mice), we prefer to use SPF cage contact sentinels. However, in some instances, we use dirty bedding sentinels as a supplement to colony animals or cage contact sentinels.

The typical cage contact sentinel is a castrated male weanling of a strain that can be readily infected with, and will mount a strong immunological response to**,** a wide range of microorganisms. We most often use BALB/cJ (000651) and DBA/2J (000671) mice.

In our research facility, we test randomly-selected animals from every animal room on a quarterly basis. If a particular room houses animals owned by more than one investigator, all investigators are asked to submit animals for monitoring. The sample size for each mouse room is calculated based on a prevalence of 18% for all organisms being monitored. Weanling, young adult, and retired breeder mice are included in the surveillance program.

A different surveillance paradigm was developed for our production colonies. In this system, mice from randomly selected sample cages are pooled 10 to a cage for a total of 60 mice in six cages. The pooled mice are co-housed for six weeks, after which at least one mouse from every cage is sent to our diagnostic laboratory for testing. If immunodeficient mice are housed in the room, immunodeficient mice are pooled with immunocompetent mice. Only immunocompetent mice are tested for viruses and other organisms that are detected serologically, and only immunodeficient mice are tested for *Pneumocystis murina*. Both immunocompetent and immunodeficient mice may be tested for all other organisms (bacteria, parasites, and protozoa). At least one mouse from each strain housed in the room is incorporated into the pooled monitoring sample for that room at least twice per year (quarterly in most cases). In addition to the pooled mice, we test 15 retired breeders for serologic evidence of viral infection; this is done at every monitoring interval (quarterly or monthly, depending on the room). Fecal samples are collected quarterly from animals in approximately 10% of the cages in each of our maximum barrier rooms; these samples are tested for excluded fecal bacteria, e.g., *Pseudomonas*, *Proteus*, or *Klebsiella*.

The sick and injured animal program at The Jackson Laboratory: technicians and investigators partnering to maintain high quality research

In our production colonies, any technician who finds a sick or injured animal has the authority to deal with it appropriately. But in a research colony, an animal that looks or acts unusual may be "normal" or valuable for the research. Thus, in our research facilities, we have a formal program for reporting sick and injured animals. Any technician who observes an animal that is exhibiting an abnormal phenotype that could indicate illness or injury can initiate a process that, at a minimal level, involves the investigator. If the investigator does not respond within a specific time range (depending on the observation, from 2–24 hours), the technician has the authority to involve our veterinary staff directly.

This program serves several important purposes: It engages the technicians with the animals at a level above routine husbandry. It has a direct impact on the well being of the animals. And, it has a direct effect on research, by providing a means of identifying animals that might be inappropriate for study, and by identifying unanticipated side effects of a treatment that researchers might initially miss.

Our sick and injured animal program provides an effective example of how to utilize the unique knowledge and experience of technicians to both improve the welfare of the mice and the quality of the research.

7.B.4. Our containment and eradication procedures

7.B.4.a. Our plan

Our containment and eradication plan is intended to prevent the further spread of a microbial contaminant, eradicate the contaminant, and notify the public about the contamination. The plan outlines the organization, facilities, and procedures used to protect employees, the public, and the mice in the event of a microbial contamination. Specifically, the plan

- describes actions to be taken in response to an outbreak,
- establishes lines of authority for direction and coordination of activities during an outbreak, and,
- defines facilities, equipment, and communication pathways to be used during an outbreak.

The plan has four phases:

- Phase 1, Confirmation: confirmation that a break has occurred, which requires confirmed positive tests for the organism in question in at least two animals.
- Phase 2, Lockdown: the initiation of containment, which involves
  - severe restrictions on the movement of animals, people, and materials within the institution,
  - special procedures to prevent the possible spread of the contaminant by air or water,
  - special procedures for personnel entry/exit, and
  - special procedures for handling of animals and materials within the room and after leaving the room.

  Notification of customers and other interested parties occurs early during the lockdown phase and continues at frequent intervals until the contaminant is eliminated and shipping of affected stocks resumes.
- Phase 3, Elimination: ridding the contaminant from quarantined areas, either by depopulation or test and cull. We select the method based on the organism in question and the degree of spread within the room. Off-site housing is considered when valuable animals must be relocated for completion of experiments or rederivation. Once mice are moved off-site, we never return them to our campus.
- Phase 4, Room redeployment: sanitization, disinfection, and monitoring, to assure elimination of the contaminant from the room environment prior to restocking of the room or resumption of shipping.

We hold at least four training exercises and drills every year. We update procedures when necessary.

7.B.4.b. Additional details on containment and notification procedures

Actions taken upon confirmation of a contamination depend on the organism found, and in some cases, on the location where it is found.

- Many organisms are rigorously excluded from all facilities at The Jackson Laboratory and, if found, would dictate immediate cessation of shipping and immediate notification of customers. Specific actions taken upon confirmation of the contamination include the following:
  - Immediately suspend shipment of mice involved in the contamination.
  - Implement the containment and eradication plan outlined above.
  - Update the ordering status for new orders to “on hold.”

  - Within two business days, send first class mail or a fax to institutions on the general JAX® Mice mailing list; post notification of the contamination on the health report for the affected room on www.jax.org/jaxmice/health (and retain the posting for six months from the date of notice).
  - Send additional information to our purchasing department, which will contact any institution that received mice from this area within past three months.
- Other organisms are rigorously excluded from only our production colonies. If any of these organisms is found in a production colony, shipping would be stopped and customers notified as described above.

  Detection of these organisms in a research mouse room would trigger an investigation to find and eliminate all infected animals. The findings would be noted on the health report for the room, but research collaborations would not be stopped. It is the responsibility of the individual researcher to notify collaborators of contamination in a research mouse room. However, collaborators may request to be notified when a particular contaminant(s) is found in a room from which they have received (or are scheduled to receive) mice. Researchers who are interested in this option should contact Customer Service at 1-800-422-6423 (North America) or 1-207-288-5845 (International). Or email us at orderquest@jax.org.
- Still other organisms are viewed as undesirable in most, but not all, facilities at The Jackson Laboratory. If found in a room from which they are excluded, an investigation would be undertaken to find and eliminate all infected animals. The findings would be noted on the health report for the room, but shipping would not be stopped. If found in a room in which they are currently tolerated, the finding would be noted on the health report for the room, but no additional effort would be made to identify or eliminate infected animals and shipping would not be stopped. Although we do not routinely notify customers of such contaminants, a customer may request to be notified as described above.

For lists of specific organisms that correspond to the categories described above, visit our website at www.jax.org/jaxmice/health.

7.B.5. Routine health reports

We update all health monitoring reports quarterly for all animal housing areas at The Jackson Laboratory. When we ship JAX® Mice, we include a printed copy of the health report from the area of origin of the mice. The most current reports can be viewed at www.jax.org/jaxmice/health.

7.C. References

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Chapter 8: Genetic Quality Control—Preventing Genetic Contamination and Minimizing Genetic Drift

Joanne M. Currer, Ray Vonder Haar, Dorcas Corrow, Kevin Flurkey

Whether you are a colony manager or researcher, you must be aware of the possibilities of genetic contamination and genetic drift whenever you breed mice. How you manage each concern has a direct effect on the genetic integrity of your mice and the reliability of your data.

In this chapter we highlight issues related to genetic contamination and genetic drift and explain strategies we use at The Jackson Laboratory to prevent genetic contamination, minimize genetic drift, and manage both.

The chapter is organized as follows:

Acknowledgments*: We would like to thank Laura Frye, Cathy Lutz, Rachel Malcolm, and Laura Trepanier for their valuable input to this chapter.*

8.A. Genetic contamination and genetic drift: what they are and how to manage them

8.A.1. Genetic contamination

Genetic contamination is the accidental introduction of alleles from one mouse strain into the genome of a second strain. Genetic contamination occurs quickly. It is probably the greatest source of uncontrolled genetic variation in a research colony. It is due mostly to human errors such as inadvertent outcrossing or mislabling of animals. As serious as genetic contamination is, however, with commitment to proper precautions and procedures, it is relatively straightforward to minimize, detect, and quickly eradicate.

8.A.1.a. Examples of genetic contamination

Following are three examples that illustrate the consequences of genetic contamination:

- The inadvertent outcrossing of C57BL/6J (000664) to DBA/2, and probably to BTBR, resulted in the C57BLKS strain (Petkov *et al*., 2004b), which dramatically changed the carbohydrate metabolism in the animals, making them more susceptible to induction of diabetes (Naggert *et al*., 1995).
- Multiple accidental outcrossings of the 129 strain has complicated ES cell research (Simpson *et al*., 1997; Threadgill *et al*., 1997).
- Genetic contamination (affecting several chromosomes) with either FVB or an FVB-like strain in two NIA contract colonies of C57BL/6 mice required destruction of the colonies (www.nia.nih.gov/ResearchInformation/ScientificResources, 4/5, 2002; National Institute on Aging, National Institutes of Health, 7201 Wisconsin Ave., GW 2C231, Bethesda, MD 20892; 301-496-0181).

8.A.1.b. Preventing genetic contamination

The most effective way to prevent genetic contamination is for all technicians and researchers to be observant and diligent with their recordkeeping and animal care, and to develop breeding strategies that minimize the possibility of errors that could lead to the mating of incorrect animals. Precautions include the following:

- If you must house multiple strains within a colony, ensure that strains housed adjacently are as phenotypically and genotypically different as possible, to increase the probability that contamination can be readily detected.
- Adopt breeding strategies that make it easy to identify a desirable or undesirable genotype via an observable phenotype such as coat color.
- Maintain diligent breeding records; use complete, established nomenclature; vary the color of cage cards among strains.
- Practice animal handling techniques that reduce the chances of an escaped mouse. For example, whenever lifting a cage top, check for mice that might be clinging to it. Return the mice immediately to the cage. Never have two cages open at the same time on the same table. This is especially critical with duplex caging.
- Capture every escaped mouse as soon as possible. Mice can mate across a wire cage barrier surprisingly easily, so you must prevent any escaped mouse from taking advantage of this opportunity. When you capture an escaped mouse, euthanize it immediately.
- Cryopreserve strains as a backup.

8.A.2. Genetic drift

Genetic drift is caused by spontaneous mutations that become fixed in a line. Genetic drift occurs slowly, subtly and surely. It is difficult to detect and control. If you breed mice, it will occur. It is so relentless that after 10 generations, a strain of mice is likely to have undergone some permanent genetic change. Genetic drift in isolated populations is a major cause of inadvertent substrain creation. (For a discussion about genetic drift and the role it plays in creation of substrains, see 3.B.1.d.2, "Genetic drift.")

8.A.2.a. Examples of genetic drift

Following are examples of progressive genetic change that resulted from genetic drift:

- At least 40 C57BL substrains developed between 1930 and 1970. Although some substrains resulted from deliberate outcrossing, most originated as a result of genetic drift, because the subcolonies were maintained separately from the originating colony (Bailey, 1978).
- Histocompatibility variants have been discovered within numerous inbred strain families: A, AKR, BALB/c, CBA, C3H, C57BL, C57L, DBA, and WG strains (Bailey, 1982).
- A mutation of the nicotinamide nucleotide transhydrogenase gene (*Nnt*) was fixed in the C57BL/6J (000664) foundation stock sometime after 1975. The mutation is not found in C57BL/6 substrains that were separated from the C57BL/6J line before 1975 (Freeman *et al*., 2006). While this mutation may alter the initial β-cell secretion of insulin in response to glucose, it does not influence the steady-state clearance of glucose (Berglund *et al*., 2008) or the development of impaired glucose tolerance in diet-induced obesity models (JAX® Services, 2008). Further details are given in the sidebar on the next page.

Genetic drift and residual heterozygosity from a quality control perspective

When inbreeding to create an inbred strain begins, the proportion of loci that are polymorphic (i.e., the residual heterozygosity) may be as high as 30% (Bailey, 1978). In inbred strains, this residual heterozygosity is a potential source of genetic variation over time. It may take as many as 60 generations of sister–brother mating before one can be 99% certain that the level of residual heterozygosity has diminished to the point where extinction of polymorphic alleles is balanced by the appearance of new alleles due to genetic drift (i.e., to the point where heterozygosity in the strain is at a minimal steady-state level).

While colony managers normally do not concern themselves with residual heterozygosity in their inbred mice, the strategy used to minimize effects of genetic drift—strict sister–brother mating—also maximizes the rate at which residual heterozygosity is eliminated. This breeding strategy maximizes the rate of extinction and of fixation of polymorphic alleles, whether they arose spontaneously in a subline or were carried over from the parental line. Other breeding strategies will result not only in a slower elimination of residual heterozygosity, but also in maintenance of a greater, and potentially fluctuating, number of heterozygous loci in the strain.

8.A.2.b. Minimizing the effect of genetic drift

The most effective way to minimize the impact of genetic drift is to periodically replenish breeders with new breeding stock from your supplier rather than breeding your own mice. This one action virtually eliminates any divergence from the standardized stock.

Keep in mind that no inbred strain is 100 percent homozygous: A level of heterozygosity will always exist due to spontaneous mutations. Although you can do nothing about the rate of the occurrence of spontaneous mutations, you *can* affect the steady-state level of heterozygosity by your choice of breeding strategies. To maximize the extinction rate of new mutations, use strict sister–brother mating in a single "foundation" lineage. (Note that sister–brother mating also maximizes the fixation rate of new mutations.) As a consequence, sister–brother mating has another effect: it minimizes the steady-state level of heterozygosity.

A recently developed strategy to minimize genetic drift itself is to cryopreserve a large stock of embryos from the same generation and periodically replenish breeders from that frozen stock. This approach, which greatly lengthens effective generation time, is the only breeding strategy that actually *retards* genetic drift within an inbred strain. (For a specific example of how this strategy is used at The Jackson Laboratory, see 8.B.2.b, "Our Genetic Stability Program.")

8.A.3. Identifying and managing events of genetic contamination and genetic drift

8.A.3.a. Identifying an event

Because genetic contamination involves large portions of multiple chromosomes, it is relatively easy to directly identify. In contrast, identifying genetic drift is extremely difficult. Typically, genetic drift is "assumed" to be the cause when a heritable deviant phenotype in an inbred strain cannot be explained by contamination.

The most effective way to identify genetic contamination is through a program of regular genetic monitoring. Often, screening is done using markers for as few as half the chromosomes. Intuitively, this may seem insufficient; however, within the first few generations following a contamination event, most chromosome pairs will be affected. Therefore, discovery of just two or more aberrant chromosomes is sufficient evidence to indicate that a contamination event occurred. Subsequently, more complete genotyping for selected mice should be carried out to confirm and characterize the contamination.

An additional strategy is to monitor for observable phenotypes that are not characteristic of a strain and for any unexpected or idiosyncratic research results. Following observation of a deviant phenotype, determine if the phenotype has appeared in other mice, particularly in mice that are directly related to the deviant. In addition, set up appropriate breeders to determine if the phenotype is heritable. Confirm any suspected contamination by genotyping. For a heritable deviant phenotype that newly appears in an established inbred strain (filial generation > 60), if genotyping does *not* indicate that contamination occurred, it is probable that the phenotype is a result of genetic drift.

Because the vast majority of genetic drift events will not be identified, a secondary tactic is to minimize the effect of genetic drift on heterogeneity. This is done through strict adherence to sister–brother mating.

An example of the difficulty in positively confirming genetic drift.

Confirmation of a specific instance of genetic drift in C57BL/6J (B6J; 000664) mice illustrates the laborious process required to unambiguously identify the genetic basis for a deviant phenotype that results from genetic drift.

Cox and colleagues (Toye *et al*., 2005; Freeman *et al*., 2006), observed that B6J mice cleared injected glucose more slowly than mice of other standard laboratory strains. These researchers used an F2 hybrid cross between B6J and C3H/HeH mice to map a locus—on Chr 13—that was associated with slower glucose clearance. They sequenced a candidate gene, nicotinamide nucleotide transhydrogenase (*Nnt*), at that locus and identified a 5-exon deletion in the B6J *Nnt* gene.

Analysis of the *Nnt* gene in other strains, including closely related C57BL/6 substrains, indicated that the mutation exists only in the B6J strain, and that it arose at The Jackson Laboratory sometime after 1975. Because glucose tolerance in B6J mice is within the normal range, as indicated by hypoglycemic, euglycemic, and hyperglycemic glucose clamp studies (Berglund *et al*., 2008), the altered phenotype is the relatively diminished glucose stimulation of early phase insulin secretion from pancreatic islets, and *not* impaired glucose tolerance as claimed by Freeman *et al*. (JAX® Services, 2008).

To unequivocally identify this mutation as the cause of the altered phenotype, Freeman *et al*. (2006) created a transgenic for a normal *Nnt* gene on the B6J background. Clearance of injected glucose in the B6J transgenic was comparable to that of C3H/HeH mice, demonstrating that the *Nnt* deletion in B6J mice could account entirely for the altered phenotype. These results illustrate the considerable effort necessary to identify genetic drift as the cause of a "new" phenotype.

8.A.3.b. Managing an event

With either genetic contamination or genetic drift, your goals are to identify the responsible breeders, cull the colony to eradicate the contamination, and take action to prevent another occurrence. Also, you must notify any users of the mice about the contamination.

8.B. What we do at The Jackson Laboratory

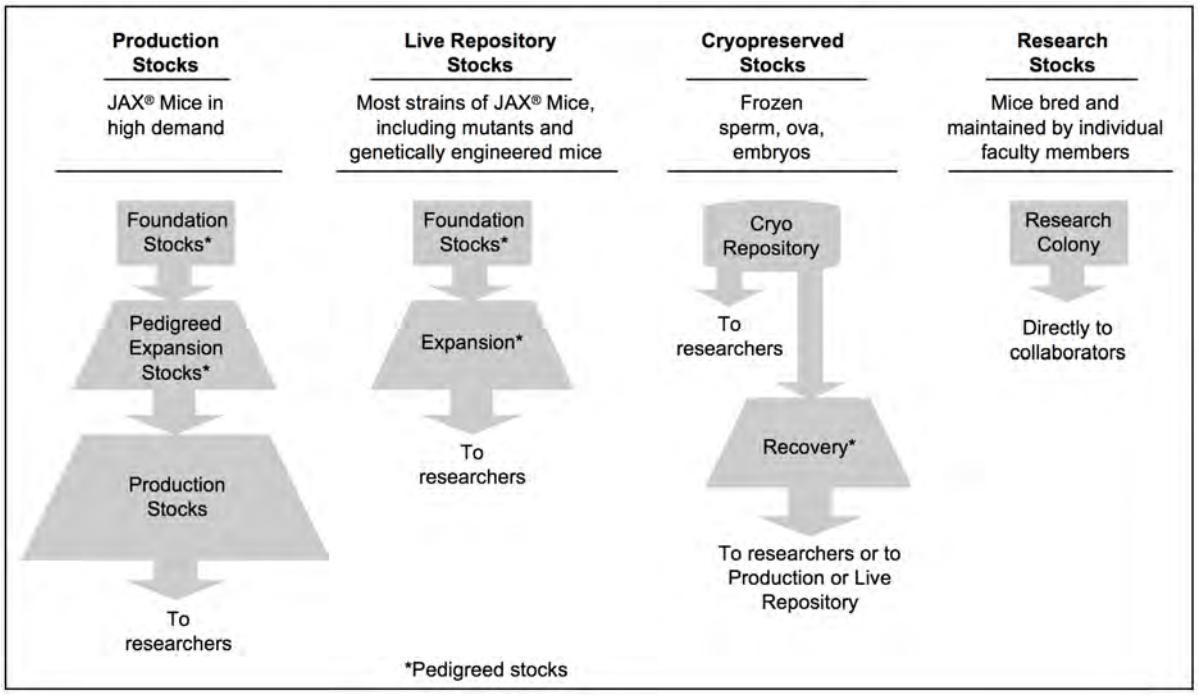
At The Jackson Laboratory, we ensure the genetic integrity of all JAX® Mice at both our Maine and California facilities with protocols that are the result of 75 years of experience in the areas of mouse husbandry, mammalian genetics, and assay development. The success of our programs relies on our rigorous standards as well as our skilled animal caretakers and technicians, who are well trained in basic genetics and deviant recognition.

Following are overviews of the colony structure and genetic quality control processes we employ at The Jackson Laboratory, including overviews of the two programs we have developed to ensure the genetic integrity of JAX® Mice—our Genetic Quality Control Program and Genetic Stability Program. We regularly upgrade our procedures to stay abreast of the latest technologies and advances in mouse biology. For current program details, visit www.jax.org/jaxmice/geneticquality.

8.B.1. Our mouse colony structure: how it helps us maintain genetic quality control

At The Jackson Laboratory, we maintain more than 4,000 strains of inbred mice that we distribute to researchers throughout the world. We have organized our mouse colonies—and the procedures we use to manage them—to meet the varying levels of demand for these mice while adhering to strict genetic quality control guidelines. Figure 8.1 provides an overview of this colony structure.

Figure 8.1. Our mouse colony structure at The Jackson Laboratory.



8.B.1.a. Our production colonies

The purpose of our production colonies at The Jackson Laboratory is to maintain pedigreed stocks of our most popular inbred strains of JAX® Mice and to efficiently breed large quantities of these strains for distribution to researchers. To maximize genetic consistency while minimizing effects of genetic drift, we maintain and expand these stocks using a three-tiered structure of isolated colonies—foundation stocks, pedigreed expansion stocks, and production stocks (Figure 8.1). This structure allows us to track individual animals and their ancestral relationships. It also enables our colony managers to unambiguously identify and remove from the breeding pool any mice thought to carry a genetic change that could compromise the genetic integrity of that inbred strain.

8.B.1.a.1. Foundation stocks (FSs)

Our foundation stocks (FSs) of JAX® Mice are maintained by pedigreed sister–brother breeding pairs. Currently, we maintain foundation stocks for about 175 of our most popular strains.

Normally, we maintain just one family per strain of JAX® Mice in an FS, with a single, parental, sister–brother breeding pair per generation. However, for strains with very high demand, we may maintain two families. We never cross these families, however, and we maintain them so that they are never more than 10 generations away from a single sister–brother breeding pair. This strategy prevents inadvertent development of substrains.

We cryopreserve embryos from foundation stocks to provide a source for rederivation should the stock be lost due to a disaster.

8.B.1.a.2. Pedigreed expansion stocks (PESs)

Our pedigreed expansion stocks (PESs) include pedigreed sister–brother breeding pairs from our foundation stocks. The purpose of our PES colonies is to expand the foundation stock to create breeders for the production stock. Sometimes, for more rapid expansion, we use sibling trio mating (two sisters, one brother), which we define as PED expansion. To minimize genetic drift, we ensure that mice in a PES are never more than five generations removed from their founding FS breeding pair.

8.B.1.a.3. Production stocks (PSs)

Our production stocks (PSs) comprise breeding pairs from the PES colonies and their offspring—the mice that we distribute to researchers throughout the world. We use either sister–brother breeding pairs from the PES or pooled breeders from a group of PES and PED litters. (We call these mice our Pooled Production Stocks [PPSs].) Regardless of the expansion method, we never exceed two generations in a PS. Thus, mice distributed from a PS are never more than seven generations removed from a single FS sister–brother breeding pair.

8.B.1.b. Our Repository

Most strains of JAX® Mice are maintained in our live repository. These mice include standard inbred strains that are in low demand, almost all of our mutant and genetically engineered mice, and all new mice that have been recently imported into The Jackson Laboratory. We currently manage more than 1,500 strains of JAX® Mice in our live repository. For the past several years, we have been introducing new strains of mice into this repository at a rate of 200–300 per year. We cryopreserve embryos for each strain of mice in the live repository.

All JAX® Mice in our live repository are maintained as pedigreed foundation stocks. We maintain each stock with strict sister–brother mating and expand the stock for distribution on demand. Any mice we ship will never be more than two generations removed from the foundation stock.

We maintain stocks in our live repository unless demand is high enough to warrant a move to the production colonies or low enough so that we maintain the stock only as cryopreserved ova, sperm, or embryos in our cryopreservation storage facility.

8.B.1.c. Cryopreserved stocks: embryos, ova and sperm

We maintain supplies of frozen embryos, ova, and sperm in our cryopreservation facilities both on and off campus (at a backup facility). This inventory has two main purposes: (1) as a resource from which to recover any strain maintained by The Jackson Laboratory if, for some reason, the live strain is lost; and, (2) as a way of preserving strains with a very low demand, which are not maintained as live mice. From our cryopreservation repository, we ship frozen material directly to researchers. Or, we recover frozen material and generate live mice for shipment.

8.B.1.d. Research colonies

Research colonies are bred and maintained by individual faculty members for their research programs. These mice are physically and administratively separate from our production and repository colonies. These mice are not JAX® Mice and are available to collaborators only through direct contact with the faculty member who maintains them.

Mice from research colonies are subject to our lab-wide animal health policies (see Chapter 7, "Animal Health: Preventing, Identifying, and Eradicating Microbial Contamination); however, due to the nature of their maintenance and use, they are not subject to the institutional policies for genetic quality control that apply to JAX® Mice.

From a quality control perspective, does it really matter whether JAX® Mice come from our production or repository colonies?

The level of demand determines whether a strain of JAX® Mice is maintained and bred in production colonies or repository colonies. In our production facilities, we maintain and breed large quantities of popular strains of mice. In our repository colonies, we maintain a greater number of strains with smaller demand. We move strains from one type of colony to the other when demand warrants a change in maintenance and breeding strategies. Researchers can be assured that, regardless of where a strain of JAX® Mice is maintained, our quality control standards and practices are consistently stringent.

For information on where a strain of JAX® Mice is maintained, please refer to its strain datasheet, available at www.jax.org/jaxmice/query.

8.B.2. Our genetic integrity programs

At The Jackson Laboratory, we have developed and documented precise protocols for genetic quality control—our Genetic Quality Control (GQC) Program and our Genetic Stability Program (GSP).

8.B.2.a. Our Genetic Quality Control (GQC) Program

At the core of our quality control effort is specific animal husbandry practices designed to prevent genetic contamination and minimize the effects of genetic drift.

8.B.2.a.1. Preventing genetic contamination and minimizing genetic drift

At The Jackson Laboratory, we follow the procedures outlined in 8.A.1.b, "Preventing genetic contamination." In addition, because of the numbers of strains and mice we must accommodate, our rigorous breeding protocols also include the following:

- We isolate foundation, expansion, and production stocks from each other.
- We keep thorough breeding records in accordance with our detailed pedigree numbering SOPs (See 11.A.2.b, "Our pedigree numbering system for JAX® Mice.")
- Wherever possible, we limit the number of generations attained in our expansion stocks so that they are never more than seven generations from the main pedigree line.

8.B.2.a.2. Monitoring for genetic contamination

Regularly scheduled SNP genotyping

At The Jackson Laboratory we use single nucleotide polymorphisms (SNPs) as our major genotyping tool. With a panel of just 27 SNPs, we can cover all 19 autosomes and Chr X (Petkov *et al*., 2004a; 2004b) and differentiate most standard strains of JAX® Mice from each other. Through the use of this panel, when we identify a contamination event, we can usually immediately determine the contaminating strain.

Production colonies:
The vast majority of the JAX® Mice we distribute to researchers are bred in our production colonies. Because of the scale of the expansion process and the exactness required, we have developed and documented precise protocols for genetic quality control.

Currently, we conduct routine SNP genotyping in our colonies of JAX® Mice on the following schedule:

- Pedigreed foundation stocks: We genotype every new breeder pair when we set them up.
- Pedigreed expansion stocks: Every 6 months, we genotype six new breeders of our most popular 100 strains. For all other PES stocks, we genotype four mice annually.
- Production stocks: Every six months, we genotype six mice of our most popular 100 strains. For all other production stocks, we genotype four mice annually. Additionally, for certain mutant strains, we also monitor mice for the heritability and expression of the phenotype and, when a genotype assay exists for the mutation, for the presence of the mutant allele.

Repository colonies:
Our repository colonies require a quality control strategy with a slightly different focus. Because the majority of strains are specific mutant strains, we must validate that the mutation is propagated and that it still produces the associated phenotype:

- When new mice arrive in a repository colony, we characterize the genetic background of each using the 27-SNP genotyping panel.
- We characterize all new mutant or genetically engineered mice for the presence of the mutant allele using a specific genotyping assay.
- For each mutant strain in the live repository, we evaluate every sister–brother breeding pair in the foundation stock for the presence of the mutant allele. Similarly, we type mutant stocks that we recover from the cryo repository for the presence of the mutant allele.
- Once a year, we evaluate each strain for possible contamination using the 27-SNP genotyping panel.
- Periodically, we verify expected phenotypes in selected genetically engineered and mutant mice.

Continual monitoring by technicians: all colonies

Our technicians are trained to be on high alert for any signs of genetic contamination. Phenotypes of interest include any variation in coat color, body size, weight, skeletal structure, behavior, reproductive performance, and occurrence of tumors. If technicians find unexpected traits, they set the mice aside for further investigation.

Upon suspicion of contamination, we troubleshoot to identify the problem. We use SNPs to test the animals to see if the deviant phenotype resulted from genetic contamination. If SNP markers show that the deviant mouse has the correct genetic background profile, we mate it to determine whether the phenotype is genetically transmitted. If the trait is heritable and is not a result of contamination, we assume the deviant phenotype is a result of genetic drift.

8.B.2.a.3. Managing a genetic contamination event

If we confirm any genetic contamination, it is our strict policy to notify anyone who purchased the mice as quickly as possible and to cull the colony as appropriate. Then we re-establish the colony, genotyping new mice to ensure their genetic integrity.

8.B.2.b. Our Genetic Stability Program

We have initiated a Genetic Stability Program to minimize genetic drift in important foundation stocks of JAX® Mice by using cryopreservation. For each foundation stock in the program we freeze enough embryos from a single generation to last 10–25 years. Every five generations, we re-establish foundation stocks from the frozen embryos.

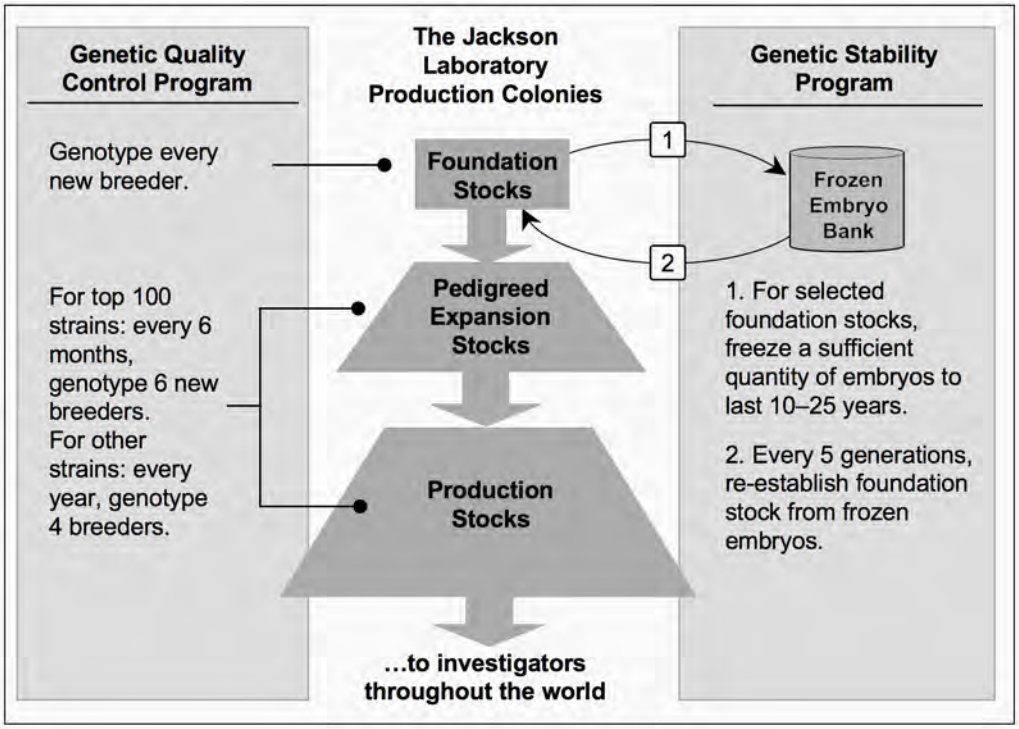
Our program functionally extends the "length" of a single generation to between 10 and 25 years. The result is that, within a 100-year period, a foundation stock will advance no more than 4–10 generations. This is in contrast to even the best colony management production practices, which result in about 3 generations per year—about 300 generations over a 100-year period. This effectively retards the rate of genetic drift by 20- to 50-fold.

We initiated our Genetic Stability Program with our most important strains of JAX® Mice, and we continue to add additional strains on a regular basis. To learn which strains are currently in the program, visit www.jax.org/jaxmice/geneticquality/stability.

8.B.2.c. Summary of our programs

Figure 8.2 illustrates how our genetic integrity programs relate to our production breeding colonies.

Figure 8.2. Steps we take to prevent genetic contamination and minimize genetic drift in our production colonies at The Jackson Laboratory.



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Chapter 9: Animal Husbandry

Joanne M. Currer, Dorcas Corrow, Kevin Flurkey

Animal husbandry involves the physical setup of mouse colonies and the regular activities that keep them running smoothly. Some requirements are federally mandated. Others are specific to your institution and often represent the implementation of your animal health and genetic quality control programs. Some husbandry tasks are simple and repetitive, but this does not imply that they are easy to do well. They often involve adherence to meticulous standards under demanding time constraints. Their objectives are clear: to provide a calm, comfortable environment for your animals so they remain healthy and as free from stress as possible.

Our objective in this chapter is to provide guidelines related directly to the housing and daily care of your animals. We also explain what we do at The Jackson Laboratory, which provides you with critical information about the conditions under which your JAX® Mice were raised.

It is important to note that, at The Jackson Laboratory, we continually evaluate our choices in colony management and animal care by (1) adhering to current criteria specified by the National Research Council (NRC), the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), and the American Association for Laboratory Animal Science (AALAS); (2) maintaining our certification with AAALAC and AALAS; (3) monitoring relevant research publications; and (4) listening to and working with our technicians, who provide a pragmatic perspective on animal care.

The chapter is organized as follows:

9.A. Physical aspects of a mouse room

9.A.1. Caging

Besides providing a method of mouse room organization and feeding and watering, caging systems also protect the mice from pathogens and determine the ease of access to the mice for technicians and researchers. The choice of caging system involves tradeoffs among cost, convenience, and effectiveness.

9.A.1.a. Types of caging systems

Table 9.1 briefly describes the characteristics of three common types of caging systems, their advantages, and their disadvantages.

Table 9.1. Summary of common types of mouse cages and shelving.

System and description	Advantages	Disadvantages
Conventional cages and shelving: • Boxes sit on, or fit into, open metal shelves. • Boxes have wire lids that can contain bins for food and water bottles; lids may or may not have covers. • Air circulation in cages is determined by room circulation and by air flow caused by the thermal heat load of the mice (Reeb *et al*., (1997).	• Is least expensive. • Allows easy access to mice. • Allows cage changing and any work with mice on open tables.	• Even with filter covers, provides lowest level of pathogen protection. • With filter covers, poor air circulation can affect intra-cage temperature, ammonia levels, humidity. • Requires more frequent cage changing than other systems.
Microisolator cages and conventional shelving: • Similar to conventional systems, except individual boxes have covers containing high efficiency particulate air (HEPA) filters	• Is a cost-effective, flexible way to provide higher level of pathogen protection than elsewhere in a mouse room.	• To maintain higher level of pathogen protection, cage changing and any work with mice must be done in HEPA-filtered hood. • Air circulation may be poor, usually worse than conventional cages and shelving. • May require more frequent cage changing.
Ventilated, HEPA-filtered caging systems, with heating, ventilation and cooling (HVAC) functionality: • Boxes fit snugly into closed, forced ventilated shelving system.	• Provides highest possible pathogen protection for the mice and allergen protection for the technicians. • Provides good air exchange. • Cages remain dry, which may reduce cage change frequency and labor expense.	• Requires capital expense. • Cage changing and any work with mice must be done in HEPA-filtered hoods. • Ventilation can result in drafts within the cages, which might stress some mice.
Portable ventilated caging systems: • Similar to stationary ventilated units, but - boxes fit into standalone units on wheels, and - incoming air is filtered room air; exhaust is filtered back into the room.	• Provides flexible solution for pathogen protection. • Works well for light mouse loads. • Cages remain dry, which may reduce cage change frequency and labor expense. • Cages can be pushed together for floor space economy without inhibiting air circulation for animals.	• Numerous portable racks can increase the heat load in the room. An option is to modify rack ventilation so it is exhausted outside the room.

Note: Caging made of polyphenyl-sulfone or other chemically resistant material is highly recommended if there is any possibility of exposure to quaternary ammonium cleaners or other high pH agents (Koehler *et al*., 2003).

9.A.1.b. Cage space requirements for mice

The NRC height requirement for laboratory mouse cages is 12.7 cm (5 inches). Table 9.2 provides the specifications for floor space requirements for mice of a specific weight. When caging mice of different sizes, the floor space requirements for the largest mouse is assigned to all mice in the cage.

Table 9.2. Requirements for floor area for laboratory mice; cage at least 5 inches high.

Weight (g) of the heaviest mouse in the cage	Floor area per mouse	
	(sq. cm)	(square in.)
< 10	38.7	6
10–15	51.6	8
15–25	77.4	12
> 25	> 96.75	> 15

National Research Council (1996), *Guide for the Care and Use of Laboratory Animals*, p. 27.

9.A.1.c. What we do at The Jackson Laboratory

Throughout The Jackson Laboratory, we use all four types of caging systems.

In almost all of our production rooms, we use ventilated caging systems that provide heating, ventilation, and cooling (HVAC) functionality, with 99.97% high efficiency particulate air (HEPA) filtering, directly to the cages. Air circulation within each room is provided by a separate, networked HVAC system. We conduct all cage changing and procedures within HEPA-filtered changing stations.

In some of our production rooms, and in a majority of our research colonies, we use conventional, "double shoebox" caging systems with polycarbonate or polyphthalate carbonate boxes with overall dimensions of 28 cm x 27 cm x 13 cm deep; individual units (pens) are 28 x 13 x 13 cm. Weaning cages, which are used to house segregated groups of male and female mice after weaning and before pairing, are 28 x 28 x 13 cm. Cage lids made of heavy wire contain bins for food and water bottles. Cages are topped with a snug fitting filter hood made of non-woven ramie fabric attached to a plastic frame. Air circulation within each room is provided by a networked HVAC system.

In some research colonies in which we use standard caging systems, we also use microisolater cages for mice that are immunologically compromised.

9.A.2. Water delivery

9.A.2.a. Types of water delivery systems

Two basic types of watering systems are commonly available for laboratory mouse cages: automatic and bottle-based. A third type, less commonly used, incorporates plastic bags. Some caging systems may require a specific type. Table 9.3 provides an overview of these systems.

Table 9.3. Summary of types of water delivery systems.

Type of system	Advantages	Disadvantages
Automatic	• Individual cages will never run low on water. • No sterilization of water bottles and lids is required.	• It requires a capital expense. • System must be maintained (per manufacturer's instructions) to prevent buildup of mold and bacteria. • Some mice may need "training" to learn how to use an automatic system. • It is difficult to determine water usage for specific cages. • Leakage in some systems may cause mice to drown if cages fill with water. • Cages must be checked daily for evidence of water leakage.
Glass or plastic bottles in following configurations: • Rubber stopper with sipper tube. • Solid stopper or solid lid with gasket; hole in bottle to provide water. • Metal lid with gasket; "sipper" hole in the middle of the cap.	• Water usage per cage can be easily monitored. • Sterilization of bottles and lids is very straightforward; pathogen protection is high. • Treatments can be easily provided in the water on a cage-specific basis.	• Individual bottles and lids must go through sterilization process. • Technicians must deal with individual bottles when changing cages; if sipper tubes are used, they must check for air bubbles. • If lids or gaskets are broken or not seated properly, water can leak. Even small leaks can dampen bedding enough to cause hypothermia. • Bottles must be checked daily for leaks and to ensure adequate water supply. • Cages must be checked daily for evidence of water leakage.
Plastic bags filled with water: • Plastic bag punctured with a sipper stem and encased in a mouse proof holder.	• Water can be stored long term, treated or untreated. • Disposable bags eliminate labor and expense to wash and process bottles. • Bags can be sterilized separately and then filled behind the barrier. • Viable option for emergency planning.	• It requires a capital expense for new equipment, new technology. • Governmental or institutional policies may require incineration of disposed bags.

9.A.2.b. What we do at The Jackson Laboratory

At The Jackson Laboratory, we provide water to almost all mice in plastic water bottles capped by metal lids with small "sipper" holes. We have found that leakage is less likely with these lids compared to lids with sipper tubes and bottles with "drip" holes.

9.A.3. Room environment

Criteria for the room environment—temperature, humidity, air flow, light and noise, for example—are specified by the National Research Council (NRC) and the American Association for Laboratory Animal Science (AALAS). Table 9.4 highlights these requirements.

Table 9.4. Environmental parameters for mouse rooms (NRA or AALAS requirements); what we do at The Jackson Laboratory.

Parameter	NRC or AALAS requirements		What we do at The Jackson Laboratory	
	Requirement	Comments	Requirement	Comments
Temperature	17.8–26.1 C (64–79 F)	AALAS recommends 21.1–24.4 C (70–76 F); optimal temperatures are higher for some mice (such as hypothyroid mice).	18.9–21.1 C (66–70 F)	• Measured in common air exhaust duct as air exits mouse room. • Monitored by alarm system.
Relative humidity	30–70%		45% ± 15% (Engineering notified when humidity exceeds 70%.)	• Measured in common air exhaust duct as air exits mouse room. • Monitored by alarm system.
Air flow	10–15 room air changes per hour	AALAS has less specific ventilation requirements that are performance based rather than quantitative (such as air change per hour). They consider air quality from the perspective of both the general room and cages.	• Conventional caging: 15–18 room air changes per hour • Fixed ventilated systems: up to 60 cage air changes per hour	• Air is 100% HEPA-filtered. • For fixed ventilated caging systems, because separate systems provide cage and room ventilation, sharing of air by mice and caretakers is minimized. • Rooms are positive pressured.
Light period	Normal:12:12 L:D Breeding: 14:10 L:D	AALAS specifications	Normal:14:10 L:D Alternate: 12:12 L:D	• A longer light cycle is used because it may promote breeding.
Light intensity	130–325 lux (cage level)	AALAS specification (Mice prefer low light levels.)	≥ 323 lux (30 foot candles), fluorescent	• 30 foot candles at 1 meter above the floor is standard for worker safety and productivity. Otherwise, AALAS requirements are followed.
Noise	25–40 decibels acoustic (dBA)	AALAS specification; recognized as “relatively quiet environment.”	65 dBA	• We strive to limit maximum facility-related noise to no more than 85 dBA. (Typical noise levels in active animal rooms are 65 dBA.) • Levels above 95 dBA may induce seizures in certain mice.

Our environmental monitoring systems are monitored 24 hours per day, 365 days per year by The Jackson Laboratory security staff, which contacts the Facilities Operations staff, also on call on the same schedule, whenever alarm limits are exceeded. All production and research animal room systems are backed up by diesel-fired emergency/standby generators that turn on automatically when they detect a power outage.

9.A.4. Cage bedding

9.A.4.a. Choices of bedding

Bedding has two obvious purposes: providing warmth and absorbing moisture. Bedding affects the bacterial microenvironment and thus affects the rate at which bacteria break down urea to produce ammonia in the cage. Bedding also provides environmental enrichment. Unfortunately, no bedding material is perfect, so your choice depends on the needs of your specific mice and colony. Table 9.6 provides advantages and disadvantages of the most used types of bedding.

Table 9.6. Advantages and disadvantages of common types of bedding for mice.

Type of bedding	Advantages	Disadvantages
Hardwood shavings	• Are cost effective. • Are physiologically inert; do not elevate cytochrome p450 (Cunliffe-Beamer *et al*., 1981; Weichbrod *et al*., 1988).	• May be dusty and irritating. • Is not as absorbent as other materials. • May be from an environment in which chemicals were used.
Softwood shavings (cedar, pine)	• Are cost effective.	Same as for hardwood shavings, plus… • Cedar and pine can be dusty and irritating. • Cedar, in particular, elevates expression of hepatic p450 enzyme systems (Wade *et al*., 1968).
Fractions or pellets of corn cob	• Are non-irritating. • Are physiologically inert; do not elevate cytochrome p450 (Pick & Little, 1965).	• Is moderately expensive. • Is not good for nesting; additional nesting material may be required. • May be prone to mold. • Is less absorbent than other bedding.
Recycled paper, cellulose chips (alpha cellulose)	• Provides superior absorbency and ammonia control.	• Is moderately expensive. • Can be dusty. • Fibers may be irritating to certain strains (e.g., nude or SKH-1) that have no eyelashes (White, 2007).
Cotton-based material	• Is highly absorbent. • Is good for ammonia control when shredded.	• Is expensive. • Is effective against odors only if shredded (some strains do not shred it.) • Fibers may entangle infant limbs and cut off circulation.

9.A.4.b. What we do at The Jackson Laboratory

At The Jackson Laboratory, we use a dispenser to add autoclaved bedding to sanitized cages at a depth of 16–26 mm before cages are delivered to mouse rooms. We provide additional bedding and non-woven cotton fiber (such as Nestlets®) when necessary—for breeding mice or when we must single-house mice, for example. We use various types of bedding for our mice, and we continually monitor research on bedding. For information about bedding used for specific strains of JAX® Mice, contact Customer Service at 1-800-422-6423 (North America) or 1-207-288-5845 (International).

9.B. Day-to-day care

Day-to-day care for your animals involves several major efforts: to protect the animals from pathogens, to keep them well fed and watered, and to maintain their genetic integrity.

Never forget that pathogen protection is only as effective as its weakest link. Thus, it is mandatory that *all* activities related to animal care adhere to the pathogen protection standards of your institution. (Details of setting up a pathogen protection plan are provided in Chapter 7, "Animal Health: Preventing, Identifying, and Eradicating Microbial Contamination.")

9.B.1. Mouse room entry and exit procedures; traffic patterns within and among mouse rooms

9.B.1.a. Considerations

It is remarkably easy to introduce pathogens into a mouse colony, even a colony that is fairly well protected. Although the most common source of pathogen introduction is mice from other colonies, pathogens also can enter a mouse room via the air or other vectors such as humans, clothing, or supplies. They can be easily spread by contamination of clean supplies with "dirty" food, water bottles, bedding or cages.

What about protection of humans from mouse allergens?

Procedures that protect animals from human-borne pathogens also protect technicians from animal-borne allergens. In addition to following normal SOPs in the mouse room, technicians susceptible to allergies should wash their hands frequently, keep their hands away from their faces and eyes, handle mice within ventilated tables, and vacuum floors rather than dry sweep. Showering after leaving the mouse room is an additional option for anyone suffering from allergy symptoms. For further details, see Chapter 15, "Human Health Concerns—Mouse Allergies, Bites, Zoonotic Disease."

9.B.1.b. What we do at The Jackson Laboratory

At The Jackson Laboratory, each production and research mouse room has a materials lock and a personnel lock for entry and exit of material and people. All sterilizable materials (such as food, cages, water, supplies) are sterilized in a central area and wrapped in plastic before delivery into the materials lock. Before entry into the mouse room, material is unwrapped. Any non-sterilizable material (such as electrical devices or laminated paper) is completely "wiped down" with a solution of 70% ethanol before entry into the mouse room.

Once in the mouse room, all clean material is covered and kept separate from used cages, bedding, food, and water. In all mouse rooms we have specific unidirectional traffic patterns for clean and dirty supplies, and in some mouse rooms, we have "clean" and "dirty" doors.

All personnel that enter a mouse room must pass through a personnel lock. We have multiple levels of barrier protection, depending on the mouse room. Our minimal level of pathogen protection for technicians includes hand washing, either dedicated in-room shoes (that have been autoclaved before being brought into the room) or disposable booties, and sterilized garments with snap closures and long sleeves with rib-knit cuffs. In most production facilities, we have 5-stage dressing locks, 2-piece scrub uniforms with knit cuffs at the ankle and wrists, socks, dedicated shoes, face masks, hair (and beard) covers, and eye protection. For rooms that house the most vulnerable and valuable mice, we have an additional level of protection: an air shower or a wet shower for use upon both entry and exit.

We discourage movement of animals or people between mouse rooms. However, we know that our employees must often visit more than one mouse room in a day, especially in our research facility. Thus, we have a written policy: If technicians or researchers must visit multiple mouse rooms in a single day, they must start in the room with the highest level of pathogen protection and work their way down to the rooms with lower levels. The policy also includes a way to handle emergency exceptions. An integral part of the policy is publication of a list of all mouse rooms and their health statuses, which are evaluated quarterly. This information is available to the public at www.jax.org/jaxmice/health.

9.B.2. Changing cages

9.B.2.a. Considerations

Procedures for changing cages involve distribution of clean cages, food and water, removal of used and dirty cages, cage contents, and equipment, all performed under requirements that meet or exceed the level of pathogen protection for any single room. This means, for example, that if you use special filter covers on your cages or ventilated system, technicians must change cages—and researchers must handle mice—in a HEPA-filtered hood. If requirements do not meet those standards, your pathogen barrier is not as strict as you might think, and pathogens have an entryway into your colony.

Cage changing: a great opportunity to monitor the health of your animals.

Animal caretakers see all the animals in all the mouse rooms. We recommend training them to watch for symptoms that might indicate illness in the mice. These symptoms include diarrhea, unusual fighting or marking, unusual levels of scratching, and non-responsiveness to routine cage disturbance.

9.B.2.b. What we do at The Jackson Laboratory

At The Jackson Laboratory, we have standard operating procedures (SOPs) that detail strict conditions and procedures for changing cages. Within a room, any cage changing or work done with the mice must be done in an environment that is at a protection level at least equal to that of the cages. All mouse room personnel are trained in these requirements and procedures.

When handling mice, technicians wear medical examination gloves. Because of the identification of latex allergies over the past several years, we now use non-latex gloves exclusively.

For cages in an open, conventional caging system, technicians transfer mice to clean cages weekly. For ventilated caging racks, technicians change the cages bi-weekly (more often if cages are excessively soiled). Cages with special requirements are changed more frequently. Filter covers are changed when they are soiled and discarded when they are damaged.

When transferring mice, technicians use one of two procedures:

- With a 10-inch dressing forceps, they grasp the mouse by the tail near its body, gently lift it into the clean cage, and release it when its feet touch the bedding of the clean cage.
- With gloved hands, they gently pick up the mouse and place it in the clean cage.

Forceps are generally used for mice older than 15 days of age. Gloved hands are used for frail, obese, or tailless mice. For mice between zero and 15 days of age, often part of the nest is scooped and moved to the clean cage in the same approximate location.

Cage changing techniques that can minimize escapes.

Escaped mice are costly. They disrupt the cage changing procedures, and because of the possibility of misidentification of an escapee, the mouse can rarely be used, even if it is caught quickly. To minimize escapes, whenever lifting a cage top, check for mice that might be clinging to it. Return those mice immediately to the cage. Also, never have two cages open at the same time. This is especially critical with duplex caging.

Technicians take several steps to prevent spread of pathogens from cage to cage. When they are finished changing a cage, they soak the forceps in an iodine disinfectant. (The usual procedure is to have two forceps, one that is being used and one that is being sanitized in the disinfectant.) They either change gloves or sanitize them using 70% ethanol. They wipe down changing tables and shelves per an SOP.

For immunocompromised mice and mice held in our highest level of pathogen protection, technicians change cages in laminar flow cage changing units. When these mice are not within a laminar flow system, technicians always protect them with a filter cover. All materials that come into contact with these mice are sterilized prior to use.

Based on the needs of specific mice, we do make exceptions to the above procedures. For example, when changing male mice, some technicians take a small lump of dirty shavings and place this, with the mice, into the new cage. This familiar scent often prevents “scuffling” as the males adjust to their new environment.

We segregate all dirty cages, cage covers, filter hoods, and water bottles in a dedicated spot in each mouse room until we return them to our processing plant for cleaning, sterilizing, and recycling. We never reuse dirty supplies.

Although it may seem obvious, we feel it is worth mentioning: Mice are master escape artists that can squeeze through very small openings. Our technicians are trained to check for any misalignment or misshaping of cage, cage top, food or water hopper wires. Sometimes, just a small bend enlarges an opening enough for a mouse to escape. When technicians notice a problem, either they reshape the wire or discard the piece of equipment.

9.B.3. Providing food and water

9.B.3.a. Considerations

Care must be taken to ensure that food and water are not entry points for pathogens into the mouse room. Food should be pasteurized, sterilized, or irradiated. If you have an automatic watering system, you should follow daily or weekly maintenance routines recommended by the manufacturer. If you use water bottles, water can be either prepared in the mouse room or brought into the mouse room following the procedure set up for that room. Once water has been sterilized, humans should not touch it before it is given to the mice.

9.B.3.b. What we do at The Jackson Laboratory

At The Jackson Laboratory, we either 1) sanitize empty water bottles, fill them with treated water, and cap them, or 2) fill them with treated water, cap them, and then sanitize the *filled* bottles. We wrap all filled, sanitized bottles in plastic in a central area before distribution to mouse rooms. We autoclave food bags received from the supplier and wrap them in plastic. We introduce the filled water bottles and autoclaved bags of food to mouse rooms via the materials lock. Exceptions are made for irradiated food, which is not autoclaved. Rather, we thoroughly disinfect the outside of the bag and wrap it in plastic before distributing it to the materials lock.

How much do "normal" mice eat and drink each day?

According to AALAS (2006), per 10 g of body weight, mice fed ad libitum consume approximately 1.5 g of dry pelleted food per day and drink 1.5 ml of water per day.

In the mouse room, technicians transfer the food to portable plastic containers for distribution. To minimize contamination, they use a disinfected plastic scoop to transfer food from the portable food container to the food bin in the cage. They never touch clean food with their bare or gloved hands. Technicians "top off" food in the cage covers on a weekly basis or whenever they notice that the food supply is low. They break up any clumped feed that may have resulted from the autoclaving process.

Our mice drink from plastic water bottles with "sipper" holes in the caps. When changing cages, technicians provide clean, full water bottles. We never refill empty or partially empty bottles.

To ensure that mice do not run out of water or suffer from wet cages due to a water bottle leak, we assign specific technicians to check cages for both conditions once per day, following an SOP. If the supply of water is low, technicians provide a clean, full bottle. If a cage is wet, technicians change the cage and provide fresh water. If a bottle leaks for any reason other than a mis-seated cap, it is sent to the Process Quality Control department for inspection.

In some of our research colonies, investigators use the water as a delivery system for treatments. It is the responsibility of the investigator to prepare the treated water, using the water given routinely to our mice as a base.

For immunocompromised mouse strains (such as mice with severe combined immunodeficiency) that are vulnerable to pneumocystis, we treat the water with Sulfatrim®, 2% solution. We administer this treatment on a one-week-on, one-week-off schedule.

Note: For a discussion of nutrition and health as well as details about the mouse food and water we use at The Jackson Laboratory, refer to Chapter 10, "Food and Water—Nutritional and Health Implications."

9.B.4. Keeping mouse rooms clean

9.B.4.a. Considerations

The main goal of room cleanliness is pathogen protection. All hard surfaces must be disinfected regularly. Requirements must reflect the level of pathogen protection chosen for a specific facility. A procedure with a lower level of protection lowers the level for the entire room.

9.B.4.b. What we do at The Jackson Laboratory

On a strict schedule and according to detailed SOPs, we clean and disinfect rooms and associated areas as follows:

- At least daily, we disinfect changing tables, all work surfaces, floors, animal husbandry tools, cleaning tools, floor drains, sinks.
- Every week, we disinfect all work tables (including any associated filters and grain hoppers), any plastic or glass surfaces, all vertical surfaces (including walls, doors, window frames, etc.), mouse traps, and miscellaneous items (such as storage and trash containers, telephones and electronic equipment, chairs, stepstools, and toilets), and wet and air shower areas.
- Every month, we disinfect caging racks, wall filters, overhead vents and lights, and ceilings in all mouse rooms, materials and personnel locks, and bathrooms.

9.B.5. Minimizing genetic contamination

9.B.5.a. Considerations

To prevent the disaster of genetic contamination due to unintended breeding, technicians—and anyone who works with the mice—must be watchful on a routine basis for any indication of a change in expected phenotypes. As an example, timely discovery of a mouse with a phenotype that differs from the normal strain—a different coat color, for example—may provide quick identification of a breeding or genotyping error, which can lead to a quick response.

9.B.5.b. What we do at The Jackson Laboratory

At The Jackson Laboratory, we have a stringent genetic quality control program to prevent genetic contamination. (Details of our program are provided in 8.B.2.a, “Our genetic quality control [GQC] program,” and at www.jax.org/jaxmice/geneticquality.) Highlights of our plan include the following:

- We do our best to house separate strains in separate rooms. If we do house multiple strains in the same room, we try to organize the colony so that the strains that share a room have different coat colors. If this isn’t possible, we at least try to avoid adjacent housing of strains with the same coat color.
- We keep meticulous records. (For details, see Chapter 11, “Recordkeeping and Identification of Mice.”)
- We educate our technicians to identify and report unusual physical characteristics or behaviors that might indicate a mutation or cross-breeding.
- Upon report of suspected genetic alteration, our colony managers immediately access breeding records and establish the pedigree. We stop setting up new matings—except for test matings—to determine if the altered phenotype is genetic.

9.C. Other issues related to animal husbandry

Following are some topics regarding husbandry and routine care that we are often asked about.

9.C.1. Providing environmental enrichment to alleviate stress

9.C.1.a. Implications of environmental deprivation

Environmental deprivation may be defined as a lack of species-appropriate environmental stimuli that is sufficient to cause stress. Stressed animals do not breed well, and their biochemistry (elevated glucocorticoid levels, for example) can confound research results. The relevant issues are these: Just how do we identify environmental deprivation in a mouse? And from the perspective of a mouse, exactly what "enrichment" reduces deprivation stress?

9.C.1.b. Identifying stress

Several behaviors may be indicators of stress, but none is definitive. Stressed mice may exhibit stereotypic (repeated) behavior patterns, but some repeated behaviors may simply reflect adaptation, to the cage environment, of a normal drive. For example, a mouse that repeatedly jumps up and down in the corner of a cage may simply be expending energy, similar to running on a wheel. Increased aggression may be a sign of stress, but normal murine social interaction, such as barbering or dominance behavior, may be incorrectly interpreted by human observers as signs of aggression. One of the more sensitive indicators of stress is reproductive performance. However, many types of stresses can impair reproductive performance, and deprivation stress is not considered a cause of diminished breeding. Thus, when mice exhibit signs of stress, deprivation should be considered as just one possible source.

9.C.1.c. Identifying appropriate environmental enrichment

If we assume that animals have "enriched" lives in the wild, we can look at their natural characteristics and behaviors for clues to the types of enrichment that may be most meaningful to them in captivity. Mice have a strong sense of smell. They are territorial and they like to dig and burrow. They generally prefer the edge of an area rather than the middle—even in their own cages. (Interestingly, many strains of laboratory mice are blind and/or deaf by the time they are eight months old, implying that visual and auditory stimuli are probably unnecessary.)

For strains that like to dig and build nests, one method of enrichment is to provide material for nest building—if the material is a type that mice of that strain like to use and if the material is not harmful when eaten.

Some recommendations are to include toys in cages. But the toy must be appropriate for a mouse. Dogs fetch and cats chase toy birds on strings because these activities mimic hunting behavior. This implies that an effective mouse toy would encourage burrowing, climbing, chewing, and running.

It seems that one of the most important features of successful enrichment is provision of the mouse with some control over his environment, for example, by providing nest building material and, in some ventilated caging systems, by providing a way to escape from drafts (Baumans *et al*., 2002). This behavior also is strain dependent.

A few words about exercise wheels: Many people consider the relatively small cage environment, as compared to mouse territories in the wild, as a source of stress due to spatial limitations. One suggested remedy is the provision of running wheels. In fact, laboratory mice *do* love to run. Flurkey (unpublished) has measured an average voluntary distance for a young C57BL/6J (000664) mouse with only three days of "training" at six miles per day, every day. However, chronic voluntary exercise has wide-ranging effects on the physiology of the mouse, and the outcome is not necessarily positive. For example, Harrison (2008) found that group housed mice given a running wheel from four months of age throughout their entire lives may have a longer or a shorter lifespan or no difference in lifespan. The chronic exercise interacted with sex and genotype to produce unpredictable effects.

Probably the greatest single source of enrichment to a mouse is…another mouse (National Research Council. 1996). Cagemates provide a constantly changing, species-relevant source of stimulation—as well as a source of warmth. In fact, some consider single housing to be a type of stress. Of course, concerns about aggression, especially with group-housed male mice of certain strains, must be considered.

9.C.1.d. What we do at The Jackson Laboratory

At The Jackson Laboratory our Animal Care and Use Committee has developed a policy about enrichment. In all colonies, our technicians are trained to watch for any behavior that is outside the range of "normal" for the mice they tend, and, if they notice anything that might indicate stress, they consider environmental deprivation as a possible cause.

We provide nesting material for all breeders. For group-housed mice that receive bedding that is not conducive to burrowing or nest building, we provide supplemental, commercial nesting material such as Nestlets®. We do not provide any cardboard items such as empty paper towel rolls because they may contain contaminants that could affect the health of our mice.

For mice housed singly for two weeks or less, we provide supplemental nesting material for both warmth and enrichment. For mice housed singly for more than two weeks, we provide the supplemental nesting material as well as a "toy" that promotes gnawing, climbing or hiding. Approved objects include a short length of sterilized PVC pipe (with polished edges), a cage card holder (without holes), a commercial plastic tunnel or igloo, a metal ring, or a Shepherd Shack® or "refuge." We transfer durable toys with the mouse from a dirty to a clean cage. We thoroughly sanitize any toy before giving it to a different mouse.

As with other issues related to animal care and health, we continually evaluate new research and comments from our technicians to ensure the best care for all of our animals.

9.C.2. Managing aggression in a colony

Signs of aggression in mice range from patchy hair loss to death. Aggression appears to be more common among males of specific strains (for example, SJL/J [000686] and most BALB/c strains) and much more likely to occur among unrelated males who are first caged together after they are sexually mature. However, even among male littermates, aggression may increase or appear for the first time during adult maturation, even as late as 15–18 months of age.

With male mice, it is important not to confuse signs of dominance with signs of aggression. Often, submissive male mice back down following a show of dominance from another male mouse. Fighting often ensues only when this balance is interfered with. Of course, sometimes true aggression does occur, in which case intervention is required.

Managing aggression may seem more art than science. Here are a few suggestions:

- Mice use scent, primarily from urine, to mark territories and establish dominance. Thus, when group-housing males who have not previously lived together, use a clean cage, and try not to transfer any scents from any of the used cages to the new one. On the other hand, when moving all the mice from a dirty cage to a clean one, it sometimes helps to transfer some of the old scent, by moving some of the dirty nesting material litter into the clean cage.
- Sometimes, male aggression is heightened if a female is present. Be sure that a female hasn't been placed in the cage inadvertently.
- Depending on the strain, the addition of nesting material may help divert the energies and activities of the animals.

Also, check references such as Ambrose & Morton, 2000; Emond *et al*., 2003; Hurst, 2005; Singleton & Hay, 1982; Smith *et al*., 2004, 2005; National Research Council (1996). Also, check current and back issues of JAX® NOTES (www.jax.org/jaxmice/jaxnotes).

9.C.3. Caring for wild-derived inbred mice

Wild-derived inbred mice, which were trapped in the wild and inbred without domestication, generally are more sensitive than common inbred strains to stresses associated with shipping, new surroundings, and new handlers. A few extra precautions can make these mice—and their caretakers—less stressed and more productive.

- When you receive a shipment of wild-derived mice, take special precautions when removing them from their shipping container. (For details, see 12.B.2, "Special handling for newly arrived, wild-derived inbred mice.")
- Minimize activity and noise around wild-derived mice. Try to house them in a quiet room, away from areas of heavy traffic and noise. Minimize rack and cage movement. It may help to put "wild mice" labels on the cages as a reminder of their presence.
- Handle wild-derived mice as little as possible, and always in a calm manner. Allow animal caretakers adequate time to work slowly and patiently when changing cages, weaning, or working with wild-derived mice. When being moved, some wild-derived mice might be less stressed if forceps are used; others might be less stressed if gloved hands are used. If mice are moved by gloved hand, either change or sanitize the disposable gloves between cages.
- Regarding breeding, allow 8–12 weeks for a pair of wild-derived mice to settle down and produce a litter. If a breeding pair does not produce pups after this time, place a different female in the male's cage. (If you swap males and females, it is preferable to keep the male in his "home" cage.) Adding Nestlets® or Kimwipes® to cages can encourage nesting and improve overall pup productivity.
- Be prepared for aggressive male behavior, especially fighting that typically begins at 6–8 weeks of age. Aggression is particularly prevalent among mice of the CZECHII/Ei (001144) strain and in progeny of any crosses involving *Mus musculus castaneus*. Try housing the males singly or pairing them with females.
- Keep a watchful eye on new mothers, who may abandon or cannibalize their litters. To minimize these behaviors, avoid changing a cage if it contains a litter less than three days old. If cages containing newborns must be changed, move the nest and pups as a unit using a clean, gloved hand. (Human scent on pups may induce abandonment or cannibalizing.) If the problem persists, consider fostering the pups to another female. (For details on pup fostering, see 13.E.1.c, "Fostering a litter.")
- Make sure that anyone who works with wild-derived mice knows the special precautions required for their care.

9.D. Sources of information regarding animal care

9.D.1. Organizations/agencies

Following is a list of organizations involved with animal care issues:

- The National Research Council, a component of the National Academies: advises the federal government on the broad community of science and technology. (www.nationalacademies.org/nrc)
- Institute for Laboratory Animal Research (ILAR), a component of the National Academies of Science: evaluates and disseminates information on issues related to the scientific, technological, and ethical use of animals and related biological resources in research, testing and education (http://dels.nas.edu/ilar_n/ilarhome)
- Office of Laboratory Animal Welfare (OLAW), a component of the National Institutes of Health (NIH): provides regulations for organizations applying for NIH funding. (http://grants.nih.gov/grants/olaw/olaw.htm)
- The American Association for Laboratory Animal Science (AALAS): a nonprofit association that promotes education and expertise in the care and use of laboratory animals (www.aalas.org). AALAS is the organization that requires the formation of an Institutional Animal Care and Use Committee (IACUC) at all research and instructional organizations (www.iacuc.org). AALAS also provides certification programs for managers of animal resources and animal care technicians.
- The Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International: a private, nonprofit organization that promotes the humane treatment of animals in science. Institutional membership and assessments are voluntary, but are an important indication of high quality animal care by institutions and technicians. (www.aaalac.org)

9.D.2. Publications

Following is a list of publications that provide detailed information about laboratory animal care and use:

- *Guide for the Care and Use of Laboratory Animals* (often referred to simply as *The Guide*. 1996. National Research Council. (The main resource used by AAALAC International.)
- *The Laboratory Mouse*. 2001. Suckow MA, Danneman, P, Brayton C. CRC Press.
- *Laboratory Mouse Handbook.* American Association for Laboratory Animal Science (AALAS). 2006.
- Journal of the American Association for Laboratory Animal Science (AALAS)
- *The Mouse in Biomedical Research*, Volume 3, Normative Biology, Husbandry, and Models. 2007.(Fox JG *et al*. (eds). Academic Press.

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Chapter 10: Food and Water—Nutritional and Health Implications

Kevin Flurkey, Joanne M. Currer

The National Research Council (NRC) has published guidelines for acceptable food and water for laboratory mice. Details are provided in the *Guide for the Care and Use of Laboratory Animals* (1996) and *Nutrient Requirements of Laboratory Animals* (1995). Within these guidelines, colony managers must make a number of decisions that can directly impact the health of their mice and the outcome of their research.

Reputable manufacturers of feed pride themselves in maintaining high—and consistent—nutritional standards for their feed. But due to the nature of some ingredients, the manufacturing and decontamination processes, and shipping and storage conditions, the feed that goes into the food hopper has the potential to vary considerably from batch to batch in nutrient composition and in levels of contamination—including both pathogens and toxins. Thus, if researchers or colony managers observe anything unexpected in their animals, they should consider diet as a possible etiologic factor.

In this chapter, we provide background information on manufacturing, decontamination, and storage of feed, and discuss how nutrition is affected. We also explain the choices we make at The Jackson Laboratory regarding mouse diet and water treatment.

Please note that this chapter provides information on the food and water itself. The ways in which we provide both to the animals are discussed in Chapter 9, "Animal Husbandry."

The chapter is organized as follows:

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10.A. Choosing a diet and arranging for decontamination and storage of feed

When selecting a diet for your animals, you must consider several interrelated factors: the various types and forms of the diets, the nutritional needs of the animals, decontamination methods, and storage requirements.

10.A.1. Types of diet

Mouse diets are classified according to type of

- ingredients (natural, purified, or chemically-defined), and
- formula (open or closed).

10.A.1.a. Ingredient types

10.A.1.a.1. Natural

Natural ingredient diets, the most common diet type, contain agricultural products and by-products such as whole grains, mill by-products, high-protein meals (animal and vegetable), yeast, and mined or processed mineral sources (NRC, 1995).

10.A.1.a.2. Purified (semi-synthetic) and chemically-defined

Purified diets (also known as semi-synthetic diets) contain ingredients that represent single nutrients: casein, starch, dextrins, sucrose, glucose, specific oils, cellulose, vitamins, and minerals. Purified diets are more expensive than natural ingredient diets, but are useful when the exact composition of a diet must be controlled—as in nutritional studies, toxicological studies in which results might be sensitive to low concentrations of contaminants, and immunological studies in which animals may be influenced by antigens in the diet. But purified diets are not recommended for long-term use. For example, survival rates in a lifelong treatment study were lower in rats fed AIN-93M (American Institute of Nutrition, 1993, maintenance diet) compared to a natural ingredient diet (Duffy *et al.*, 2002).

A *chemically defined diet* is a type of purified diet in which individual amino acids are used in place of a protein source, and specific fatty acids are used in place of oils. Chemically defined diets are very expensive and are used mainly for studies of amino and fatty acid metabolism.

10.A.1.b. Formula types

10.A.1.b.1. Open and fixed

Open formula diets, which can be made of natural or purified ingredients, are fixed formula diets, i.e., the macronutrient source is invariant. Manufacturers openly publish the exact proportions of ingredients.

10.A.1.b.2. Closed and variable

Closed formula diets, which are always made of natural ingredients, are proprietary. Manufacturers publish the ingredients, but not the specific formulations. Generally, the information provided by the manufacturer includes at least the food sources that may have been used and a proximate analysis of the concentrations of protein, fat, and ash.

In a closed formula diet, the concentrations of nutrients are specified, but the macronutrient source may vary according to considerations such as market price. The source of dietary protein, especially, is liable to vary in a closed formula diet. (For example, milk protein may be substituted for fish meal). Manufacturers use variable formulations because considerable variation in nutrient composition exists for the same raw foods from different locations and in different seasons. Using proximate analysis, they can adjust proportions of raw foods in variable formulations to achieve a highly consistent concentration of protein, fat, and ash in the diet.

Closed formula diets may vary in other nutrients that are not used for standardization, as the raw food source varies. Knapka (1997) has argued that the varying ingredient inclusion rates in

variable formula diets poses a considerable risk of experimental variability. Researchers that are concerned about the possible effects of varying dietary nutrients should use purified fixed formula diets or have the relevant nutrients assayed independently by commercial laboratories.

As noted above, in variable formula diets, protein sources will vary. This can affect the biology of the mouse. For example, simply varying the source of the protein, not the amount, could double the inducible tumor incidence (Guo *et al*., 2004).

10.A.2. Physical form of the feed

10.A.2.a. Pellets and extrusions

The most common physical form of feed are pellets and extrusions (collets). Both manufacturing processes start the same way: raw food material is ground, sifted, and mixed with vitamin and mineral supplements into a meal.

10.A.2.a.1. Pellets

For pelleting, the meal is mixed with steam, which raises the temperature to 65–80 C and gelatinizes the starches, thus binding the diet ingredients together. The heat also reduces the microbiological load by about two orders of magnitude. The meal is forced through a die and cut at a specific length. Then, the resulting pellets are dried so that moisture content typically is about 12%, a level at which free water content is too low to support growth of microorganisms. As a result, pelletized food, when protected from moisture, can remain on a shelf for about six months before loss of nutrient value becomes significant or growth of mold becomes a concern.

Purified diets require a manufacturing method slightly different than described above due to the inclusion of casein (typically used as the protein source in purified diets) and simple carbohydrates, both of which are particularly sensitive to heat. To minimize loss or alteration of nutrients, meal is mixed with water, pelletized, and then dried at a low temperature (less than 60° C) in a vacuum. Purified diets generally have a shelf-life of about four months when refrigerated or frozen.

10.A.2.a.2. Extrusions (collets)

For extrusion, meal is ground finer than for pelleting. Meal is mixed with steam and hot water, brought to 80–95 C, and extruded under pressure (about 35 atmospheres), allowing temperatures of 150 C. Because steam is trapped within the food during extrusion, the extruded pellets (collets) become honeycombed. The collets are dried by hot air to a moisture content of 8–12%.

Because of the higher temperatures used during extrusion, the loss of vitamins is greater during preparation; however, because the higher temperatures also denature enzymes that break down vitamins during storage and destroy mold and bacterial spores, extruded diets have longer shelf lives than pelleted diets.

10.A.2.b. Other forms of food

10.A.2.b.1. Ground diets

Occasionally, ground diets are required. For example, some mice cannot eat pellets or collets due to physical problems such as malocclusion or malformed, poorly developed, or broken teeth. And sometimes researchers administer treatments through feed. Either purchase ground feed or grind your own from pellets or collets. Note that grinding pellets or collets can reduce vitamin stability, which reduces shelf life (Tobin *et al.*, 2007).

Food cups can be used to serve ground food. It is perfectly acceptable, however, to simply place the meal directly on the bedding in a dry area of the cage. While this may seem unsanitary, mice are coprophagic, so placing feed on the cage bottom does not put the mice at risk as long as it does not become moist and thus become a growth medium for bacteria or mold.

A word of caution about using ground diets: Because mouse incisors grow continuously, it may be necessary to provide gnawing material when giving mice ground feed for more than several weeks.

10.A.2.b.2. Gel diets

Gel diets are a special type of diet that combines food and water into one product. Gel diets are useful for mice that either cannot access water from the standard water delivery system or cannot eat normal food or both. Gel diets are also used during shipping. But gel diets are expensive. They do not provide gnawing opportunities, and they require special precautions to prevent spoilage—both during storage and in the cage (NRC, 1995).

10.A.3. Decontamination of feed

The risk of contamination by insects and microbiological organisms is unavoidable in food manufacture, packaging, and shipping.

Historically, diets were sterilized or pasteurized primarily for use in specific pathogen free (SPF) mouse colonies. Today, however, because of the recognition of infectious agents as a source of uncontrolled variables as well as agents of overt disease, and because of the common use of genetically modified mice, many with compromised immune systems, the elimination of microorganisms from diets has become a standard practice.

Autoclaving and irradiation are processes used for sterilization and pasteurization. Sterilization is defined as destruction of every living organism, including spores as well as vegetative forms. Pasteurization eliminates vegetative bacteria and some spores. Pasteurization minimizes major chemical alteration of the diet; however, it does not destroy bacterial spores.

10.A.3.a. Autoclaving

Autoclaving is the process of sterilizing or pasteurizing with steam at a specific temperature and pressure for a specific length of time. Autoclaving is the most common method of sterilizing mouse food. It is generally performed on site, typically in a double-door autoclave, after which it is passed into a barrier animal facility. Because the process of autoclaving affects feed, manufacturers produce feed specifically to be autoclaved and mark the feedbags accordingly.

Nutrients that are susceptible to damage by heat, moisture, and oxygen are especially affected by autoclaving. “Browning” reactions among amino acids, especially methionine, cysteine, and lysine, and between amino acids and carbohydrates, alters the structure of the amino acids and can substantially diminish protein bioavailability. Milk proteins, such as casein, and simple sugars, which are commonly used in purified diets, are particularly susceptible. Normally, feed manufacturers counteract the diminished bioavailability of proteins due to autoclaving by increasing the protein content of the autoclavable diet above minimal recommended levels or by supplementing the diet with methionine, cysteine, and lysine.

Heat-labile vitamins (B_1, B_{12}, B_6 and pantothenate) are particularly sensitive to autoclaving; modest losses of vitamins A, D_3, and folate also occur. Thus, manufacturers generally add additional vitamins to diets that will be autoclaved. Because of this, toxicity can result if mice are fed autoclavable feed that has not been autoclaved. (Also see 10.A.5.e, “Essential amino acids and vitamins.”)

Autoclaving can affect the hardness of pellets. The degree of the effect varies with the specific ingredients and formula of feed. Hardness tests on autoclaved food can identify any that is too hard for mice to eat. Collets, which are made by extrusion and have a honeycombed structure, are less susceptible to hardness problems.

Another issue with autoclaving is the clumping of food during the sterilizing process. Coatings such as silica dioxide or calcium bentonite have been used in the past to minimize clumping. Although these coatings are considered to be inert because they do not alter the normal pathology of research animals, the potential exists for interference with specific end points in research, and Tobin *et al.* (2007) recommend that coated diets be avoided.

10.A.3.b. Irradiation

Irradiation is the process of exposing feed to radiation for the purpose of destroying microorganisms. The radioactivity damages the DNA and prevents replication. Although irradiation is produced from a radioactive source, no radioactivity is transferred to the irradiated feed. Irradiation is performed at specialized facilities rather than on-site.

Gamma irradiation is the most commonly used form of irradiation for diet decontamination. Irradiation at doses less than 10 kGy (radicidation or radurization) is equivalent to pasteurization. A minimum dose of 21 kGy is sufficient to kill most bacteria, molds and fungi, and is considered a sterilizing dose, although killing *Clostridium* and *Bacillus* spores may require doses above 30 kGy. Doses of at least 30 kGy may be necessary to inactivate some viruses (Baldelli, 1967). The sensitivity of many pathogens to irradiation is provided by the World Health Organization (WHO; 1999).

Because the radiation dose will vary throughout the product due to load pattern, density of the product, and thickness of the load, the dose is usually stated as the minimum received by the load (typically, at the center of the load). Often, a color indicator label is provided on each bag. Colors may change somewhat with exposure to light and also vary among label manufacturers, so labels and color keys should be checked carefully.

From the standpoint of nutrition, irradiation at doses typically used for rodent diets (20–25 kGy for barrier facilities) has no effect on protein bioavailability (Ford, 1979; Eggum, 1979) and losses of most vitamins are less than 20 percent (Ford, 1979; Isler and Brubacher, 1999). Because the effects of irradiation are transmitted by the production of free radicals, the main concern for damage to the diet is the free radical-induced oxidation of fats, producing peroxides. Ford (1979) reported a six- to eight-fold increase in peroxide values in a high fat diet irradiated at 25 kGy; the increase was reduced to three- to four-fold by irradiating under a vacuum (in the absence of oxygen). Peroxide levels continued to increase during storage.

Keep in mind that, although irradiated feed is free from pathogens, by the time the feed reaches its destination, outside packaging might not be. Thus, standard operating procedures (SOPs) for pathogen protection must be followed when bringing the food into mouse rooms (disinfecting the outer bag, for example).

10.A.3.c. Comparison of decontamination methods

Each decontamination method has advantages and disadvantages. For larger operations, where the expense of the autoclaving equipment and operating expertise is already assumed for other needs, autoclaving will be the most cost effective. Autoclaving also may be necessary where lipid peroxides in the diet may be a particular concern. And it has the additional advantage of on-site decontamination, so that contamination during transport is of minimal concern. Irradiated diets provide more consistent protein quality and levels of nutrients, and they are a uniform hardness, but they are more expensive. Irradiated diets are typically preferred for small barrier colonies or isolators where large autoclaves are unavailable. Pasteurization may be preferred over sterilization when a more consistent dietary protein quality or vitamin level is necessary, but neither pasteurization or sterilization by autoclaving provides the consistency of irradiation (Tobin *et al*., 2007).

10.A.4. On-site storage of feed

The two most important factors affecting food during storage are temperature and humidity. Natural-ingredient diets are typically stored at room temperature (22 C), and shelf lives are calculated on that basis. Because the rate of biological processes typically doubles with each temperature increase of 10 C, reducing the storage temperature to 12 C would reduce the rate of vitamin destruction and the oxidation of fats by half, thus doubling the shelf life.

Because, compared to yeast or bacteria, mold starts growing at a lower relative humidity (about 80%), it will be the first microorganism to appear in stored food that is exposed to moisture. Mold is a particular problem because of the production of mycotoxins, such as aflatoxins, vomitoxin (deoxynivalenol [DON]), zearalenone, and fumonisin. Aflatoxins and DON are especially dangerous for mice. For storage guidelines, the National Research Council (1996) recommends a maximum exposure of 23 C and 70% relative humidity with a continuous exposure of less than 21 C and 60% relative humidity.

It is important to heed storage conditions and the "use by" date recommended by the supplier. Deviation from these guidelines can affect the nutritional value of the food.

10.A.5. Nutritional composition of feed and requirements for healthy mice

To produce research results that are unambiguous regarding the health of the animals, any diet you consider should be designed specifically for laboratory rodents. The Guide (NRC, 1995) provides a comprehensive listing of nutritional requirements for mice, and Tobin *et al*. (2007) provide a thorough review of nutrition.

Nutrients can be divided into five broad categories: protein, fat, available carbohydrates, fiber, and essential nutrients. Essential nutrients, which include essential fatty acids, essential amino acids, vitamins and minerals, are listed in Table 10.1.

Table 10.1. Essential nutrients for mice.

Essential amino acids:	**Macroelements:**	**Vitamins:**
• Arginine (may not be essential for adult mice)	• Calcium	• A (retinol)
• Histidine	• Phosphorus	• Vitamin D_3 (cholecalciferol)
• Isoleucine	• Magnesium	• Vitamin E (tocopherols)
• Leucine	• Potassium	• Vitamin K_3 (menadione)
• Valine	• Sodium	• Vitamin B_1 (thiamine)
• Threonine	• Chloride	• Vitamin B_2 (riboflavin)
• Lysine	• Sulfur	• Available niacin
• Methionine		• Vitamin B_6 (pyridoxine)
• Phenylalanine	**Trace elements:**	• Panthothenate
• Tryptophan	• Iron	• Vitamin B_{12} (cobalamin)
	• Zinc	• Available biotin
Essential fatty acids:	• Manganese	• Folate
• Linoleic	• Copper	• Choline
• α linolenic	• Selenium	
	• Iodine	

Ultratrace chemicals, which are not yet established as essential but may be required, include arsenic, boron, chromium, cobalt, fluoride, lithium, molybdenum, nickel, silicon, tin, vanadium. Tabular information compiled from Tobin *et al*., 2007.

10.A.5.a. Protein

Nutritional requirements for protein differ for the three classical physiological states: reproduction (including lactation), growth, and maintenance.

10.A.5.a.1. Reproduction

The NRC (1995) states that natural ingredient diets containing 18 percent protein are appropriate for reproduction in mice. In fact, diets with higher protein percentages (24%, for example), may be sub-optimal (Knapka *et al*., 1977).

What percentage of protein in the diet is most common?

In most mouse colonies in the United States, commercial diets used for breeding, growth, and maintenance are about 18% protein. In Europe, maintenance diets are more typically 14–16% protein.

10.A.5.a.2. Growth

A maximal growth rate to mature body weight typically is achieved on diets containing at least 13–16% protein. Growth rate immediately after weaning may be higher on 18% protein diets, compared to 14%, which may reflect an interaction between the stress of weaning and dietary composition. Catch-up growth occurs on the diets with 14–16% protein, and subsequent growth to mature body weight continues at the same rate with diets that range in protein composition from 13–18% (reviewed in Tobin *et al*., 2007). Though the NRC (1995) recommends 18% protein (or 20% casein in purified diets because casein is about 88% protein), Tobin *et al*., (2007) state that optimal growth rates can be maintained on diets of 14–16% protein, assuming good-quality protein, i.e., protein with an amino acid pattern that matches the requirements for mice.

10.A.5.a.3. Maintenance

The few estimates of maintenance requirements for dietary protein in mice indicate that a 5% protein diet, with good quality protein, is sufficient to sustain adult body weight (Tobin *et al*., 2007). In fact, in adults, restriction of protein to 4% (casein), compared with 26%, typically increased lifespan for multiple strains of mice (Leto *et al*., 1976; Goodrick, 1978; Stolzner, 1977). In rats, high protein diets are associated with kidney damage; in mice, however, a comparable association of dietary protein and kidney damage has not been reported (Tobin *et al*., 2007).

10.A.5.b. Fat and essential fatty acids

For growth and maintenance of most strains, diets of 4–6% fat weight/weight (w/w) are generally appropriate (Knapka *et al*., 1977). For breeding, strain variation exists for the optimal dietary fat content, which ranges from 4–12%. For example, BALB/c mice may perform better with diets of 8% fat, compared to 4% or 12% (Knapka *et al*., 1977); however, diets with fat content above 6% could promote obesity in other strains, which may reduce fertility. General guidelines for dietary fat content do not exist specifically for poorly reproducing strains, and optimal amounts should be determined by each researcher.

Both linoleic and α-linolenic acids are considered essential fatty acids. Linoleic acid is a precursor to arachadonic acid and other omega-6 fatty acids, and arachadonic acid can substitute in the absence of dietary linoleic acid. Although α-linolenic acid is necessary for the synthesis of certain prostaglandins, the NRC has not yet determined a specific requirement for it.

Interestingly, animal fats are poor sources of the essential fatty acids. For example, about four times more lard, compared to soya or corn oil, is needed to satisfy the requirement for linoleic acid.

10.A.5.c. Carbohydrates and fiber

Complex carbohydrates provide the predominant energy source in natural diets. Crude fiber is the indigestible material remaining after a defatted feed sample is successively boiled in mild acid and then base, which digests proteins and starches and removes the amino acids and soluble carbohydrates. Total carbohydrate value overestimates the amount of carbohydrate available for metabolism because it includes insoluble (crude) fiber. Typically, the amount (in percent by weight) of crude protein, crude fat, and crude fiber will be provided as the proximate analysis with each bag of feed. The remaining weight is made up of carbohydrate, moisture, and ash.

10.A.5.d. Ash

Ash is the inorganic material that remains after burning a feed sample at a minimum of 525° C for 12–18 hours, depending on the temperature. Ash is composed of minerals; it is used in proximate analysis as a rough measure of the amount of minerals in a feed sample. Natural ingredient diets typically contain 4–8% ash.

10.A.5.e. Essential amino acids and vitamins

Amino acid requirements for maximal growth rates for mice are provided by the NRC (1995). Although the optimal amount of total protein differs for reproduction, growth, and maintenance, available information indicates that the relative amounts of each essential amino acid remain about the same for all three physiological states (Tobin *et al*., 2007). Essential amino acids for mice, as specified by the NRC (1995), are listed in Table 10.1, "Essential nutrients for mice."

Should you be concerned about phytoestrogens in the diet?

Today, with the increased use of soy products, there is some question about the biological effects of phytoestrogens in food. If this is a concern for you, we recommend conducting a bioassay to determine if the diet you are using contains a level of estrogenic activity high enough to interfere with your study. One of the most sensitive measures of physiologically relevant estrogen is uterine weight in ovariectomized females (Gordon *et al*., 1986; Mobbs *et al*., 1985).

The NRC (1995) also lists essential vitamins (Table 10.1) and their estimated levels required for growth, which probably are also suitable for reproduction. No estimates have been provided for maintenance. Vitamins are added to natural ingredient diets at levels usually well above minimal requirements to allow for processing losses, possible inefficiencies in absorption, and losses during storage. Because autoclaving diminishes certain vitamins, diets specified as "autoclavable" are fortified for autoclave-sensitive vitamins so that minimal requirements typically are still met following autoclaving. However, because autoclaves and autoclaving protocols vary from site to site, and because the temperature and steam penetration will vary even within a pallet of feed, actual amounts of vitamins in each batch of feed can vary considerably. Diets manufactured specifically for autoclaving should never be fed to mice without autoclaving due to the possibility of vitamin A or D toxicosis.

Conditions of storage and method of feed preparation contribute to the loss of vitamin potency. This loss differs considerably among various vitamins. Whereas loss of most vitamins over a 6-month period ranges from 5–25% in pelleted food, loss of menadione can range from 50–80%, depending on the specific form of the vitamin; loss of vitamin D3 can range from 20–65% (Tobin *et al*., 2007). Storage losses depend on the moisture content of the feed, the environmental temperature and humidity, and on the packaging (e.g., vacuum packing). (For information on storage, see 10.A.4, "On-site storage of feed.")

10.A.5.f. Minerals, including calcium and phosphorus

More than twenty minerals are considered essential (Table 10.1). They are categorized as macroelements (elements at concentrations greater than 80mg/kg in the body) and trace elements. Although minerals are derived from the various ingredients in the feed, a mineral premix will often be added to provide appropriate levels and balance among the various minerals. The level of each mineral in a feed sample is assayed from an ash sample using specific spectroscopy techniques.

Although requirements in mice for most minerals have not been determined in detailed studies, Reeves *et al*., (1993) report that the main standard purified diets, AIN-76A, AIN-93G (for growth), and AIN-93M (for maintenance), have not been linked to mineral deficiencies.

A particular concern for rodent diets is the ratio of calcium to phosphorus. In rats, deposition of calcium phosphate crystals in the kidney (nephrocalcinosis) is a relatively common condition (e.g., Cockell *et al*., 2002) that is related to both levels of dietary calcium and the ratio of calcium to phosphorus. A more generalized calcium mineralization, called dystrophic calcinosis, has been reported for some mouse strains. This condition involves deposition of calcium in soft tissues, particularly the heart and kidney, in susceptible strains (Van den Brock *et al*., 1997). Yuen and Draper (1983) reported that when B6D2F1 mice were fed a purified diet containing 0.6% calcium (normal) and 1.2% phosphorus (an amount present in some diets), kidney calcium levels were more than double the level in mice fed the 0.6% calcium with 0.3% phosphorus. The relationship of dystrophic calcinosis to nephrocalcinosis is unknown. Tobin *et al*. (2007) recommend that, for natural ingredient diets, where a considerable proportion of phosphorus is bound in an unavailable form (phytates), calcium and phosphorus weight ratios of at least 1 (molar ratios of 0.77) are acceptable, although a weight ratio of 1.2 may be safer. (Total amounts of each mineral should be less than 1%; NRC requirements, even for breeding, are only 0.5% for calcium and 0.4% for phosphorus.) For purified diets, where the phosphorus is more available, Reeves *et al*. (1993b) recommend a weight ratio of 1.7.

More than 40 minerals have been suggested to have some essential role in the health of the mouse. Besides the 13 minerals and trace elements that have been identified as essential in studies of mice or rats (Table 10.1), and that are typically added to commercial rodent diets in a mineral premix, numerous elements have been ascribed some essential function, but are required in such low amounts that the requirement is thought to be met by the concentrations found in the ingredients of natural-ingredient diets or in drinking water. In contrast, in purified diets, some of these elements may be missing (Reeves *et al*., 1993a). For example, long-term survival (beyond two years) of rats fed the purified diet AIN-93M (maintenance) was lower than on a natural-ingredient diet (Duffy *et al*., 2002), and the benefit of diet restriction was diminished for rats on AIN-93M.

10.A.6. Quality control

Typically, near infrared spectroscopy is used for proximate analysis to rapidly determine concentrations of protein, fat, fiber, and ash in feed. Chemical analysis is used for determination of caloric content and concentrations of essential nutrients.

Reputable manufacturers provide batch analysis certificates when they ship feed. These certificates provide information on proximate analysis, analysis of selected nutrients, and of selected contaminants. It is good practice, however, to use the services of an independent laboratory to confirm nutrient concentrations in your feed at least annually. This gives you precise values for food under storage conditions at your facility. This is especially important if you autoclave your food.

10.A.7. What we do at The Jackson Laboratory

At The Jackson Laboratory, we use several standard diets. For most production colonies, we use a closed formula, custom diet based upon the NIH-31, 4% fat (w/w) or 6% fat (w/w) diet (LabDiet® 5LG6/5K52, 4%; 5K52/5K67, 6%). In certain breeding colonies, we use a comparable formulation that is a minimum of 10% fat (LabDiet® 5K20). All of our mouse food is decontaminated before use. For most mice, we sterilize mouse food by autoclaving. For specialized needs in some colonies, we use pasteurized or irradiated food. The diets for our production mice are available on the strain datasheets (www.jax.org/jaxmice/query), under the "Health & husbandry" tab. For additional information, telephone us at 1-800-422-6423 (North America) or 1-207-288-5845 (International) or email us at micetech@jax.org.

We check autoclaved feed for hardness and clumping, which are common consequences of the autoclaving process. To ensure that the autoclaved pellets are not too hard for the mice, we regularly conduct hardness tests on our autoclaved feed. We follow strict SOPs and reject pellets that exceed our hardness limit. To break up any clumping, animal care technicians break up large chunks while the feed is in the bag, and break up smaller clumps as required when feeding the mice.

To precisely determine the nutrient levels in the diet and evaluate the performance of our autoclaves, we use an independent testing laboratory to analyze feed twice yearly. Normally we test protein, fat, fiber, ash, calcium, and phosphorus levels in January and July; we test vitamin A, vitamin B, and lysine in April and October. We follow strict SOPs for food collection and analysis.

We never store food for longer than six months after the mill date.

10.B. Treating water

10.B.1. Guidelines for safe water

Water for mice must be treated to minimize microbiological contamination. To date, although no organization has published specific guidelines for safe water, several water treatment methods—including autoclaving, acidification, hyperchlorination, reverse osmosis, and ultraviolet light exposure—are in common use. Both the NRC (1995, 1996) and AALAS (2006) provide details.

There is no standard for the type of water to provide mice: tap, bottled, or distilled. Tap water has widespread variations, such as in mineral content, general hardness, or presence of flouride, but the generally accepted convention is that if water is good enough for the community, it is good enough for the mice. Please note, however, that this axiom does not recognize the potential for the influence of water variation on research results. And, because there is no concerted effort to standardize water composition, the best general strategy for a researcher is to use a consistent source of water and consistent treatment method.

It also is important to ensure that your water delivery system is clean. If you have an automatic watering system, this means following appropriate maintenance per the manufacturer's recommendations. If you use water bottles, they should be sanitized—or even sterilized—between uses.

10.B.2. What we do at The Jackson Laboratory

At The Jackson Laboratory, to suppress the growth of bacteria and mold in the water we use in all mouse colonies, we treat water with hydrochloric acid (HCl) to a pH of 2.8–3.1. In our trials, this pH level suppressed growth of *Pseudomonas aeruginosa* for more than five weeks. To replace any vitamin K that is destroyed by autoclaving the mouse food, we also add menadione sodium bisulfate (MSB) to the water at a rate that results in an average concentration of 0.40 mcg MSB/ml of water. Water bottles are filled, capped, and wrapped in plastic in a central facility and shipped to individual mouse rooms.

10.C. References

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Chapter 11: Recordkeeping and Identification of Mice

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Accurate recordkeeping is critical to a colony manager. In a production colony, accurate records allow you to track—and maximize—production of your colony and monitor the health and genetic integrity of your mice. In a research colony, accurate recordkeeping is a mandatory requirement to avoid the corruption of data.

The objective of this chapter is to provide some guidelines for recordkeeping activities necessary in both production and research colonies. We'll also explain some of the recordkeeping strategies we use at The Jackson Laboratory.

The chapter is organized as follows:

11.A. Identifying individual mice

11.A.1. Identification methods

The five most common ways of identifying individual mice are ear punch, ear tag, tattoo, toe clip, and microchip. Table 11.1 summarizes the advantages and disadvantages of each method. Further details, including references, are provided at www.jax.org/jaxmice/faq/idsystem.

Table 11.1. Advantages and disadvantages of common mouse identification methods.

Identification method	Advantages	Disadvantages
Ear punch	• When done by experienced technician, procedure is quick and accurate.	• Cannot be administered until mouse is 3 weeks old. • Works best when numbers are limited from 1–99. • May be altered or lost through fighting or grooming. • Mistakes are difficult, perhaps impossible, to reconcile. • Immobilizing mice while retaining access to ear pinnae requires extensive practice. For less experienced technicians, it may be necessary to anesthetize the mouse to accommodate more legible punching.
Ear tag	• When done by experienced technician, procedure is quick. • Provides a wide range of ID numbers.	• More expensive than ear punching. • Ear tag is often lost over time through grooming or fighting. • Ear often becomes inflamed.
Tattoo	• On all but heavily pigmented tails, tattoo remains visible throughout the mouse's entire life. • Can be used on infant mice (tail or toe pad); it is not disabling (as is toe clipping).	• When used on infant mice, tattoos fade as animal grows. • Requires special equipment.
Toe clipping	• Unambiguous method for identifying pups after 4 days of age. • When done by experienced technician, procedure is quick and accurate.	• Not recommended for weanlings or older mice. • Anesthesia is recommended if pup is older than 7 days. • Permanently affects ability to grip; may affect grooming.
Microchip (subcutaneous transponder)	• Provides unambiguous, non-invasive identification. • Lasts throughout the life of the mouse. • Combined with computerized recording, mouse numbers can be transferred directly to a computer, eliminating transcription errors. • Chips can be recycled.	• Mice must be 4–5 weeks of age before being chipped. • Chips are more expensive than other methods. • Implantation requires anesthesia.

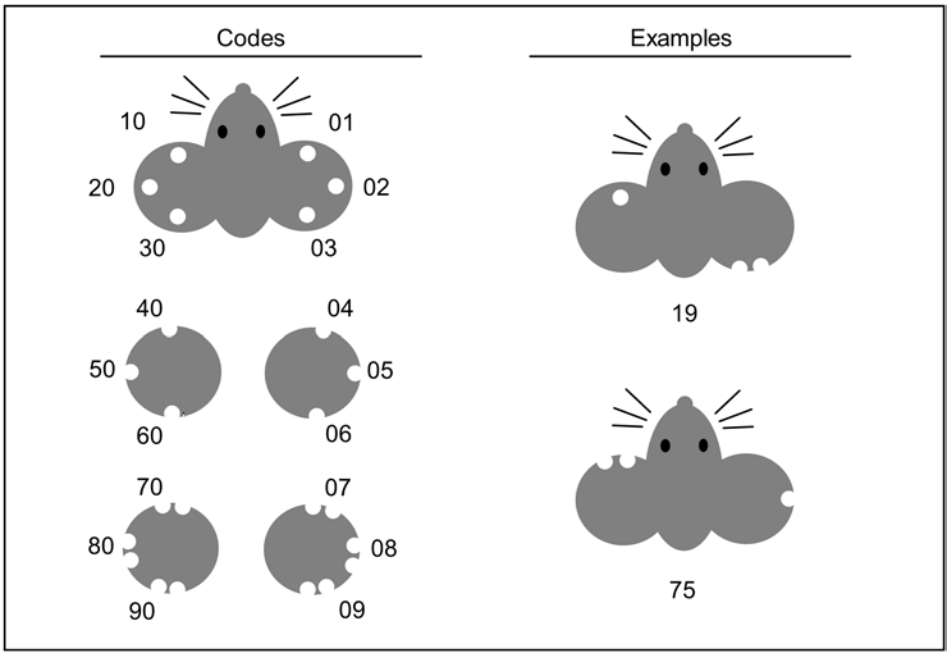
11.A.2. What we do at The Jackson Laboratory

We use multiple methods of mouse identification at The Jackson Laboratory. If we need to identify specific mice in our production colonies, we use ear punches (Figure 11.1). In our research colonies, we may use ear punches or ear tags. For some studies, such as aging studies, when it is essential that identifiers are readable for more than one year, we use microchips.

11.A.2.a. Our ear punch codes

Figure 11.1 illustrates the ear punch codes we use at The Jackson Laboratory. These codes allow us to distinguish 99 mice. When we need to unambiguously identify 15 or fewer mice, we sometimes use a subset of the codes—the punches for 01, 03, 10, and 30, which are on the rostral and caudal edges of the ear pinnae and are very easy to distinguish from each other. Using this method, codes would represent just the following numbers: 1, 3, 4, 10, 11, 13, 14, 30, 31, 33, 34, 40, 41, 43, 44. (*Note:* Sometimes, customers may receive JAX® Mice that have ear "clips." While not an official ID system, the clips provide a way to identify mice within a cage for genotyping purposes.)

Figure 11.1. Dorsal view of ear punch coding used at The Jackson Laboratory.



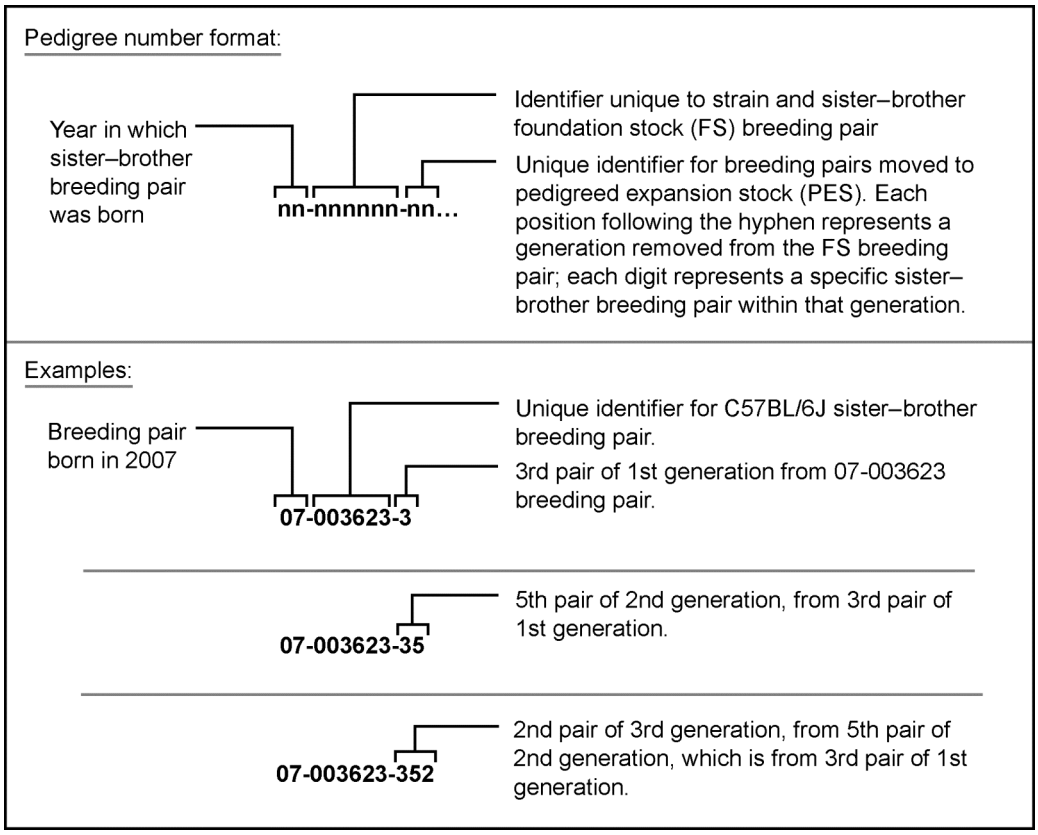
Adapted from Dickey (1975).

11.A.2.b. Our pedigree numbering system for JAX® Mice

At The Jackson Laboratory, our pedigree system for inbred strains was designed to track individual animals and their ancestral relationships in our production colonies. This system allows our colony managers to unambiguously identify and remove any mice that could compromise the genetic integrity of the inbred line. Each breeding unit (a sister–brother mated pair) within a foundation stock (FS) is given a unique pedigree number that is entered into the pedigree register.

When we transfer mice from an FS to a pedigreed expansion stock (PES) to begin colony expansion, we trace the full sibling breeding units by expanding the parental FS pedigree number to track the generations removed from the FS pair as well as the specific litter from that mating. Figure 11.2 illustrates our pedigreed numbering system and provides several examples of pedigree numbers for specific PES mice of our C57BL/6J (000664) strain. (For a description of our production colony structure and its advantages, see 8.B.1, "Our mouse colony structure: how it helps us maintain genetic quality control.")

Figure 11.2. Pedigree numbering system used for inbred mice at The Jackson Laboratory



We also have standard operating procedures (SOPs) that address issues related to lengthy pedigree numbers and numbering systems for non-standard pedigrees. If you have any questions about pedigrees of any JAX® Mice, please call Customer Service at 1-800-422-6423 (North America) or 1-207-288-5845 (International), or email us at micetech@jax.org.

11.B. Keeping day-to-day records

Keeping accurate records for an entire colony of mice is a challenge. For some mice, the most relevant data represent their ancestry and birthdates; for other mice, relevant data must cover the animal's entire life, including dates and effects of treatments and date of death. Data also must be stored securely. Over the years at The Jackson Laboratory, we have developed some recordkeeping strategies and techniques that work for our production colonies as well as our research colonies.

11.B.1. Recommendations and strategies

The birth record for a mouse pup should include a unique ID number that is linked to the unique ID numbers of its parents. Other information generally includes strain or genotype, generation, and dates of birth and weaning. Breeding records also might include pedigree number (if applicable); dates of mating setup, observation of vaginal plugs (if applicable), dates of parturition; quantities and sexes of pups born and weaned; date retired from breeding; other observations. In a research facility, daily recordkeeping can also include treatments, dates of treatments, date of death, and reason for death.

Technicians without access to a data entry program must handwrite information on cage cards, and perhaps in pedigree books. Cage cards of multiple colors can help distinguish groups of mice. To avoid problems with illegible handwriting, we recommend the use of either sharpened pencils with lead of a medium hardness (greater than 2.5 HB) or permanent marking pens with fine points. Softer lead may smear, standard ink may bleed, and markers with broad or flattened tips may create unclear text or numbers.

We recommend taking the time to set up SOPs or "shortcut" codes or both for certain studies. As examples, in a study that relies on necropsies, both the person who necropsies the mice and the pathologist who reads the reports must know exactly what distinguishes a "lump" from a "light pink mass" from a "tumor."

Store cage cards and other paper-based data in a safe place. Label storage boxes or file drawers clearly, including strain and date. Remember that, if and when you need records (for example, if you suspect genetic contamination), you probably will not have the luxury of time to search through thousands of records to find the ones you need. Back up computer data regularly. If necessary, store backup files in a separate building.

11.B.2. What we do at The Jackson Laboratory

11.B.2.a. Production and repository colonies

In our production and repository colonies we have recently implemented a state-of-the-art computer system that integrates our colony management, ordering, and invoicing systems. Because we know exactly how many of each strain of JAX® Mice are available at any given time, when our customer service representatives get an inquiry or an order, they can rapidly provide current, accurate information. Once an order is placed, shipping documents and invoices are automatically created, speeding delivery and simplifying billing.

The data analysis features of our system enable our colony managers to easily review breeding records and to manage anticipated requirements for mice so they can set up breeding accordingly. This integrated system will help us achieve our goals of providing the best customer service possible and of managing our colonies so that we have JAX® Mice available when researchers need them.

11.B.2.b. Research colonies

In our research colonies, it is the responsibility of investigators to maintain and organize their own data. Some investigators keep handwritten records. Some use combinations of handwritten records and Microsoft® Access™ or Microsoft® Excel®. Others use computer systems such as The Jackson Laboratory's Colony Management System (JAX-CMS), a multi-user relational database management system for managing animal colonies in a research environment (see 11.C.3). Any records on a computer connected to our network can be backed up automatically on a daily basis.

11.C. Choosing colony management software

11.C.1. Advantages of a colony management system

Colony management software provides several advantages:

- Accurate, complete records. A well-designed data entry form can "screen" data input and help prevent missing data or erroneous data entry. A hand-held data entry device makes data entry convenient.
- Laser-printed cage card data. Printed cage cards can present complete information—including full strain names—in identical formats and also prevent legibility problems related to faint or illegible handwriting or smeared ink. Laser printing is permanent and will not "bleed" when wet.
- Flexible data analysis. Computerized data can be filtered and sorted to facilitate reporting and analyses.
- Thorough, efficient recovery from breeding errors and genetic contamination. Should breeding errors occur, the pedigree can be tracked via the computer rather than by gathering and sorting cage cards.

11.C.2. Considerations when choosing a colony management system

When selecting a software package, considerations include the following:

- Ease of data entry. Are input forms designed so they are logical for technicians to use? Will you have enough terminals or data entry devices so that no one has to wait to enter data?
- Types of "tasks." Does the system accommodate any tasks you need to track? Is the system flexible enough to accommodate any new activities you might want to track in the future?
- Flexibility of reporting. Do the standard reporting and printing templates meet most of your needs? Does the system allow you to design your own reports?
- Compatibility with other software. Is the file structure compatible with, or can the files be exported into, a software package you routinely use? This is especially critical if you like the data analysis and reporting flexibility of packages such as Microsoft® Access™ or Microsoft® Excel® and want to continue to use them with data captured by the new software. Compatibility with Microsoft® Excel® is also important if people in your organization use multiple types of statistical software.

  This flexibility is especially relevant for future use of the data: Should the colony management system become obsolete and unsupported, if you cannot use the data in another system, they will be unusable unless you manually re-enter them or develop a way to convert them.
- Robustness. Is the system designed to accommodate the amount of data traffic you can expect on a routine basis? Will it allow for expansion?
- Barcode scanning. Can the system scan barcodes? This is an important feature if you use microchips for mouse identification.
- Product support. Is documentation technically correct and usable by your staff? Are quick reference cards available? Is online help available? Is technical support available?

11.C.3. The Jackson Laboratory's Colony Management System (JAX-CMS) for research mouse colonies

The Jackson Laboratory's Colony Management System (JAX-CMS) was written by researchers at The Jackson Laboratory in response to demand within The Jackson Laboratory for a system that would execute the core functionality of colony management from an intuitive, easy-to-use interface. Since its initial release in 1998, it has been widely used within The Jackson Laboratory and by a number of outside institutions.

JAX-CMS currently runs as a Microsoft® Access™ application. It can be executed remotely from UNIX® or Macintosh® environments using technologies such as a Citrix® server. Features include the following:

- Tracking of animal status, pedigree, mating and litter records.
- Logging of genotype.
- Management of animal pens.
- Printed cage cards.
- Tracking of experimental data.
- Advanced database queries.
- Report generation.
- Bulk data entry.

JAX-CMS is free!

JAX-CMS, tutorials and support are available free to the public at http://colonymanagement.jax.org. End user support is provided through our moderated listserv discussion group, which you can join after you download the software.

11.D. References

Dickey MM. 1975. "Keeping Records," in *Biology of the Laboratory Mouse*. Green EL (ed). Dover Publications, NY.

Chapter 12: Introduction of New Mice into a Colony

Joanne M. Currer, Dorcas Corrow, Kevin Flurkey

The challenges of introducing new mice into a colony include preventing the introduction of pathogens, managing the potential for genetic contamination, and acclimating the mice to their new environment so that the physiological effects of any shipping stress have lapsed before they are used for research.

The objective of this chapter is to provide guidelines for meeting these challenges. We'll also provide specific steps to take when you introduce JAX® Mice into your facility. And we'll highlight what we do at The Jackson Laboratory.

The chapter is organized as follows:

***Acknowledgments**: We would like to thank Peggy Danneman for her valuable input to this chapter.*

12.A. Precautions when introducing live mice

12.A.1. Protecting against pathogens

Each mouse you introduce into a colony has the potential to carry infectious agents from its previous colony. Once a new infectious agent becomes established in a colony, it is very difficult and expensive to eradicate. And as infestations from new mice accumulate, a poorly protected colony can become a menagerie of pathogens.

Even the shipping container can introduce pathogens…

Keep in mind that the outside of the shipping container may have been exposed to a variety of infectious agents. Before you unpack the mice, we recommend disinfecting the outside of the shipping container by wiping it thoroughly with a solution of a disinfectant such as 70% ethanol.

When introducing mice into conventional colonies, if mice are purchased from reputable suppliers or are transported from another mouse room on site with higher pathogen protection standards, precautions for pathogen protection are generally not taken. For colonies in which pathogen protection is a concern, however, quarantine of new mice is a necessity.

Quarantine procedures involve separation of new mice until you are satisfied that their health status is equal to or higher than that of other animals in the colony. Technicians must take special care to keep the mice separate and to disinfect the caging system and changing areas to prevent the spread of any pathogen that might be present. Another precaution is for technicians to handle quarantined mice only after all other mice are cared for. (For details on pathogen protection, please see Chapter 7, "Animal Health—Preventing, Identifying, and Eradicating Microbial Contamination.")

12.A.2. Protecting against genetic contamination

When you introduce mice that are a new genotype, you must take precautions to ensure that existing stock does not become genetically contaminated. If you cannot house the new mice in a separate room, try to house them next to mice that have a different coat color. Keep diligent records. If a mouse escapes, find it immediately. Do not run the risk that it might breed. Details on preventing genetic contamination are provided in Chapter 8, "Genetic Quality Control—Preventing Genetic Contamination and Minimizing Effects of Genetic Drift."

12.A.3. Identifying and recovering a loss of phenotypic expression

Because expression of a phenotype can depend on internal or external environments, when a strain is introduced into a new colony, conditions may be sufficiently different to alter phenotypic expression. For example, in most mouse models of inflammatory bowel disease (IBD), a complex enteric flora is required to produce gut pathology in young adults. In colonies where not all the required enteric flora are present, the phenotype is not expressed until middle age.

It also is not unusual for a phenotype to be lost when a mouse strain is rederived. For example, when the NONcNZO10/LtJ (004456) strain, a polygenic model for male type 2 diabetes, was re-derived into a production barrier colony at The Jackson Laboratory, diabetes was attenuated. A higher fat diet was required to reconstitute the phenotype. Although a genetic basis for any phenotypic loss must be considered, altering the environment may restore the phenotype.

12.B. Handling newly arrived mice

12.B.1. Recognizing and managing the physiological effects of stress related to transportation

When new mice arrive at your facility, they are not ready for research. Since they were packed for shipment, they have been under extreme stress and their physical condition is not normal. Elevated stress-related hormones have promoted catabolic states and inhibited anabolic states that will have physiological effects for at least a week after arrival. Eating behavior, aggressiveness, and numerous other biological systems related to stress response can be affected. This response is so universal that any research results obtained from mice within 72 hours of receipt must be considered unreliable.

Keep in mind that even moving your mice from one mouse room to another within your facility is stressful to them. Although the actual "trip" might be short, their new location might have different odors, light levels, sounds, air circulation, temperatures—even new technicians. This combined stress will affect some biological variables within minutes, especially those that are influenced by epinephrine. The stress might not affect other variables for 24 hours.

We recommend taking the following precautions when you receive new mice:

- Disinfect the shipping container and remove the mice as soon as possible. Provide fresh water and food. Even though the mice have been transported with a moisture source, they might be hungry or relatively dehydrated.
- Monitor the mice to ensure that they are drinking and eating. If they are not drinking water using the water delivery system in your facility, provide an alternate supply of water until they are familiar with your system. If they are not eating, provide an alternate food source or place some food on the cage floor.

How can you tell when mice are no longer stressed and are ready for research?

One way to monitor effects of stress on specific strains following shipping (or in specific environments) is to monitor body weight. Body weight recovery will stabilize when the mice have adjusted to new conditions. If possible, compare weight data on newly arrived mice against "normal" mice of the same age, sex, and strain in your own colony. Because an adult mouse at a stable weight consumes, and burns, about 10% of its body weight each day, body weight will rapidly reach a new steady-state level in response to a change in environment or nutrition. Typically one week is sufficient time for adjustment.

So, just how long will it take for the physiology of your mice to normalize in their new environment so they are ready for research? If the mice were moved to a new facility in a vehicle such as an airplane, truck, or van, wait for a week before conducting research. If the mice were moved from room to room within a facility, wait three days.

12.B.2. Special handling for newly arrived, wild-derived inbred mice

Wild-derived inbred mice present challenges additional to those of standard inbred mice. For example, in contrast to most inbred mice, which run *from* light, wild-derived mice may run *toward* light, presumably associating it with an escape route. And, they are generally hyperactive and exceptionally quick to bolt. Following are several hints for unpacking a shipment of wild-derived mice:

- Provide an environment that will keep escapees contained. Place the entire shipping container in a closed, hooded changing station or at the bottom of a deep container such as a sterilized garbage can.
- Before opening the shipping container, have a new cage ready.
- If you have the space to maneuver, place an extra cage lid over the opening of the shipping container. As you open the container, slide the lid into place over the opening and move it around as needed to create smaller openings from which to remove the mice. (This strategy also allows you to see the mice and perhaps anticipate escape moves.)
- Because some wild-derived inbred mice tend to burrow in bedding, always check the bedding thoroughly for any hidden mice. If the container has a shipping label, verify the quantity of mice in the container with the quantity noted on the label.

After you have transferred your new wild-derived mice into their new cages and you are certain that they are eating and drinking, try to disturb them as little as possible. For more information on housing and caring for wild-derived mice on a routine basis, please refer to 9.C.3, "Caring for wild-derived inbred mice."

12.B.3. What to do if you have an automatic watering system and your newly arrived JAX® Mice won't use it

Mice at The Jackson Laboratory drink from water bottles with "sipper holes" in the bottles or caps. Sometimes, upon arrival at an institution that uses an automatic watering system, JAX® Mice will not—or do not know how to—use it. This problem is more likely with very young or recently weaned mice (i.e., shipped within 24 hours of weaning) and appears to be strain dependent. (For example, anecdotal evidence suggests that some NZB/BlNJ [000684] mice have difficulty adapting to a new water source.)

If your facility is equipped with an automatic watering system, we recommend that you make sure your newly unpacked JAX® Mice are drinking water from it. If you notice any symptoms that might indicate a problem—weight loss, lack of appetite or dehydration, for example—here are a few steps you might take:

- Tap the water valve in the mouse cage until a small bead of water forms on the surface. Most mice will find this water and recognize it as their new water source.
- Place a water bottle on the cage or set a small container of water or semi-moist food or gel pack on the floor of the cage. After a few days, remove the alternate water source and try the automatic system again.

It is important to note that, if mice are not drinking adequate water, they are experiencing stress. Before you use mice for research, be sure they have been drinking water normally for at least 48 hours.

12.C. What we do at The Jackson Laboratory

When we move mice from our production facilities to our research facilities at The Jackson Laboratory, mice spend less than one hour in a shipping container. We follow strict entry procedures through materials locks at the receiving mouse room. We also allow the mice to acclimate to their new environment before using them for research.

When we move mice among our research colonies, we never introduce them into a room that has a health status higher than that of the room in which they originated. We wrap the cages in the materials lock of the room they are leaving and unwrap them in the materials lock of the room they are entering. We do not conduct research on the mice until we know they are acclimated to their new environment.

When we receive mice from outside The Jackson Laboratory or transfer mice from one of our own researchers to our production facility, the mice are quarantined and rederived. These mice are bred in a separate "dirty" facility and their offspring are introduced into the "clean" area of our campus, following testing for excluded microorganisms. For information about our importation program, please see Chapter 7, "Animal Health—Preventing, Identifying, and Eradicating Microbial Contamination."

Chapter 13: Breeding Strategies and Techniques

Joanne M. Currer, Dorcas Corrow, Marge Strobel, Kevin Flurkey

The obvious goal of a breeding colony is to provide healthy, genetically well-defined mice that are suitable for use in research. Colony managers have an additional challenge: to do this in the most efficient, cost-effective way possible.

The objective of this chapter is to provide guidelines for maximizing the breeding of your mice. We include biological breeding data, factors that can affect breeding, and several techniques that assist with breeding. At the end of the chapter, you'll find a table to help you troubleshoot breeding problems you might encounter.

It is worth noting that, although the logic of breeding strategies is quite straightforward, the behavior of mice often is not. Even littermates might behave differently under very similar circumstances. Thus, wise colony managers and animal caretakers customize strategies and techniques that work best for *their* mice in *their* environment.

Note: This chapter covers general breeding strategies and techniques. For information about breeding *schemes* for developing and maintaining specific categories of mice, see Chapter 3, "Categories of Laboratory Mice: Definitions, Uses, Nomenclature."

The chapter is organized as follows:

Acknowledgments: *We would like to thank James Yeadon for his valuable input to this chapter.*

13.A. Factors that affect the breeding of laboratory mice

Breeding performance of laboratory mice depends on both biological and environmental factors. Some factors apply to all strains; others are strain dependent. Some factors relate specifically to your own situation and requirements.

13.A.1. Biological breeding data

Table 13.1 provides a summary of general reproductive characteristics for mice. For many of our most popular strains of JAX® Mice, we provide more detailed information in Chapter 4, "Characteristics of Popular Strains of JAX® Mice, Including Reproductive Performance." Also see the JAX® Mice strain database (www.jax.org/jaxmice/query) and the Mouse Phenome Database (MPD; www.jax.org/phenome).

Table 13.1. Reproductive characteristics for most inbred strains of laboratory mice.

Characteristic	Normal value	Comments
Sexual maturity	4–8 weeks of age	Males usually reach sexual maturity by 6 weeks, females by 4–6 weeks.
Estrous cycle	4-day or 5-day cycle.	The normal cycle can be interrupted by mating, pheromones, vibration, noise, and other environmental stresses.
Post-partum estrus	6- to 8-hour period	This is the estrous period females enter within a few hours after giving birth. If a male is in the cage, mating and pregnancy are likely.
Ovulation rate	4–12 ova per estrus	Strain dependent.
Litter size	2–12+ pups (average 6–8 pups)	Strain dependent. Pattern is for litter size to increase, reach a peak at about 3–4 months of age, then start decreasing.
Fertility rate	50–100%	With some strains, all pairs of mice are fertile; with others, as few as 50% are.
Gestation length	18–21 days	Gestation length is typically 18–19 days for most strains; may be longer if litters are smaller (4 or fewer pups) or mothers are older.
Time between litters	22–50 days	Typically the shortest time between litters occurs between 3–4 months of age (between the first and second litter), then progressively increases.
Generation time	About 10 weeks	Strain dependent. Conception-to-conception time is determined as follows: 3 weeks gestation, 3–4 weeks suckling, 1–3 additional weeks until sexual maturity. But, generation time for the first litter is often longer.
Weaning age	21–28 days	In husbandry context, the age at which pups are removed from their mother, not when they start eating solid food. Varies among strains; depends on weanling size and maturity. Most strains are weaned at 21 days, some at 28 days. Do not remove pups from mother before 17 days unless they are transferred to a foster mother.
Total litters	2–8	Total litter number varies by strain. A total of 4+ litters is typical, but some strains produce as few as 1 or 2 litters.
Reproductive lifespan (female)	Terminates at 6–12 months of age	Strain dependent. For inbred strains, fecundity usually begins to decrease with the 3rd litter. By 6–8 months, some females become infertile, and litter size for most females of most strains has diminished to the point that it is more economical to set up a new breeding pair.
Reproductive lifespan (male)	Terminates at 12-–14 months of age	Strain dependent. Fertility in some strains decreases starting at 10 months.
Delayed implantation	n/a	Implantation normally occurs during the fifth day after conception. However, if a female is nursing, implantation may be delayed for more than a week, during which time embryonic development is held in suspension.
Seasonal breeding fluctuations	n/a	Some strains are susceptible to changes in breeding behavior based on season.

13.A.2. Environmental factors that can affect breeding performance

Whether you are maintaining a large production colony or simply breeding several pair of mice for a small experiment, one of the most important things you can do to foster successful breeding is to provide your mice with a quiet, comfortable, stable environment, free from noise and vibration, with a consistent light cycle. You cannot overestimate the adverse affect that environmental stress can have on breeding. Table 13.2 provides information that can help you alleviate stress and improve the breeding performance of your laboratory mice.

Table 13.2. Environmental factors that can affect breeding.

Factors	Action to promote successful breeding	Comments
Location of cages	• Avoid locating cages near a heavily trafficked or noisy area, such as near a door or sink or a loudspeaker used for paging. • Place cage racks several inches from the wall to prevent building vibrations from transferring to the cages.	• Changes in levels of noise and vibration can decrease breeding performance and may induce mothers to resorb litters, cannibalize their pups, or stop breeding. • Construction-related noise and vibrations may be especially problematic.
Lighting	• Maintain a single, consistent, lighting cycle. The most common cycles are 14:10 (on:off) and 12:12. • For wild-derived mice, use less intense lighting.	• Disruption of the light cycle may have an adverse effect on breeding.
Barometric pressure	• Maintain a stable barometric pressure.	• Falling barometric pressure can cause some strains to become hyperactive, which reduces breeding performance.
Temperature and humidity	• Keep the temperature at 16–26 C (64–79 F) • Keep the humidity at 40–60%.	• Thermoneutral temperature for mice is around 27–28 C (80–82 F). In contrast, for humans, it is about 22–23 C (72–74 F).
Air quality and odors	• Keep the room air fresh, free from strong odors. • Establish a "no perfume" policy for caretakers, technicians, or anyone who works with the mice. • Use clean forceps or clean (or new) gloves for each cage.	• Mice are extremely sensitive to strong or noxious odors, but they like their own scent to be strong in their environment. • Noxious fumes may reduce breeding performance.
Handling	• Work gently, slowly, and quietly when handling breeding mice. • Try to regularly assign caretakers to the same cages so that the mice receive consistent care with familiar handling and odors. • Avoid changing cages more often than once a week; if females are ready to give birth, skip cage changing until pups are 2 days old or you can see milk spots. • Minimize handling and checking on the mice, especially if mice are pregnant or giving birth, or have new litters. • Unless absolutely necessary, do not handle pups until they are 3 days old.	• Laboratory mice respond best to calm, consistent handling. • Often, mice do not like new technicians. • Wild-derived mice are especially sensitive to handling stress.
Bedding and nesting material	• Provide nesting materials in the cage, especially if bedding (such as pellets from corn cob) does not lend itself to nesting.	• The addition of material such as Kimwipes® or Nestlets® can promote nesting. • Nesting behavior is strain dependent.
Diet	• Provide food with a dietary fat content of 4–11% fat w/w. • If mice have bad teeth, broken teeth (e.g., from gnawing or malocclusion), no teeth, or other phenotypes that affect their ability to eat normal mouse food, provide an alternate food supply, perhaps either ground or dampened food. • If mice cannot reach the "normal" water or food supply, provide access to both on the cage floor.	• The optimal percentage of fat in the diet, with respect to fecundity, is strain dependent. Optimal fat content ranges from 4–12% w/w; a commercially available breeder diet with 9% fat w/w works well for most strains of mice.

13.A.3. Strategies for setting up and monitoring breeding to optimize colony production

Some breeding strategies involve timing and setup issues: when to set up mating, when to "swap" breeders, when to replace breeders, how to improve breeding behavior. Table 13.3 provides guidelines for some of these issues.

Table 13.3. Strategies for maximizing productivity of a breeding colony.

Factors	Action to promote successful breeding	Comments
When to set up breeding	• Mate mice when they are 6–8 weeks old.	• Note that it is not unusual for the first litter to be smaller than the second or third, which are typically the largest.
When to foster pups	• Foster pups - if the mother does not "nest" the pups right away but leaves them scattered around the cage , or - if milk spots do not appear in the pups by the time they are 24-hours old.	• If you need offspring from a female who is a poor mother, plan ahead to have foster mothers ready when she gives birth. • Housing 2 females together will often allow them to work together and successfully raise 2 litters.
When to replace breeding pairs for optimal performance	• Replace breeding pairs as their reproductive performance declines (typically 6–8 months of age; see Table 13.1.) • For a large colony, maintain a stable supply of breeding pairs at various ages by replacing a specific percentage on a weekly or monthly basis.	• A colony of mixed-age breeders produces a more consistent quantity of pups than does a colony with mice of the same age.
When to replace individual female breeders	• Replace female breeders when they - produce no litter within 60 days of pairing (unless this delay is normal for the strain), - produce no litter within 60 days of their last litter, or - produce litters but wean no pups for 2–3 litters.	
When to replace individual male breeders	• Replace male breeders - when they reach 1 year of age, or - if they are infertile with a young, fertile female.	
When to cull a litter	• Reduce the number of pups in a litter if the mother is having problems feeding the pups.	• Some mothers may be unable to provide milk for more than a few pups. • For segregating strains, remove unwanted pups as soon as they can be phenotyped or genotyped.
How to improve breeding behavior	• With young females, use experienced males. • Isolate males for 2 weeks before pairing. • Rotate males within a strain among cages.	• Males mature later than females; therefore age at first litter for same-aged breeding pairs is often determined by the male.

13.A.4. General guidelines for successful breeding

1. Breed healthy animals.

- Specific acute illnesses that affect breeding include parvovirus (losses at 7–10 days) and MHV (losses at weaning).
- Chronic infectious illnesses that affect breeding include *Pneumocystis* (pneumonia) for immunodeficient mice and *Helicobacter* spp. (if associated with colitis).
- Infestation by ectoparasites, such as mites, can impair breeding.
- Obese males often lose interest in breeding, and obese females are less likely to get pregnant.

If you must breed animals that are unhealthy, consider assisted reproductive techniques, pup fostering, or rederivation.

2. Know your animals.

- Make allowances for strains with characteristics that require special housing or care. For example, some males, such as those of the SJL/J (000686) strain, attack their mates and offspring. With very aggressive males, you may need to remove the male as soon as you observe a vaginal plug (13.E.1.a). Also, do not place a new male in a cage with a female and her litter; he may kill the pups. (This instinct is suppressed if the male is present when the female gives birth.)
- In cases where a constitutive disease such as type 1 diabetes will seriously impact breeding, it may be possible to use a disease prevention strategy to keep the breeders disease free through the reproductive period. For example, with NOD/LtJ (001976) breeders, the injection of a single dose of complete Freund's adjuvant (CFA) at five weeks of delays the onset of diabetes.
- Be aware that interaction of a mutation with the genetic background may affect breeding performance. If you transfer a mutation to a new background by backcrossing, maintain earlier backcross generations until you are certain that the new background does not affect any phenotypes of interest, especially those related to reproductive performance and survival.

3. Keep meticulous and accurate breeding records; review them regularly.

- Analyze breeding performance on a regular basis, especially the time between litters, litter size, born:wean ratio. Adopt a detective-like demeanor and cultivate habits such as the following:
  - Monitor genotype ratios for breeder units so you know what to expect.
  - Investigate deviations in breeding performance and phenotype immediately.
  - Consider strain characteristics when analyzing productivity. For example, females of some strains routinely lose their first litters. Others, such as some 129 substrains, are poor mothers.
- Compare the breeding performance of the strain in your colony with data provided by your supplier. If breeding data are not available for mutant strains, use data for the inbred strain background. But recognize that all mouse colonies, even within a facility, are different and that strains that breed well in one colony may not do as well in another. Thus, "standard values" should not be considered absolute performance standards. Rather, they should be used as guidelines to indicate the breeding potential of the strain and to help identify breeding problems.

4. Choose a female:male breeding ratio based on your need for pedigree vs. productivity

Choices of female:male ratios include

- pair mating,
- trio mating (two females and one male that remain in the cage together), and
- harem mating (generally two females, with a male that might be rotated to other cages).

Most strains produce more progeny per cage with trio mating because inbred mice can tolerate the relatively high density and all adult cage mates generally help care for the pups. Thus, for strains that have small litters or are poor parents, harem mating may be a viable strategy. Keep in mind, however, that if you use trio or harem mating and want to maintain a pedigree lineage, you must house females separately when they become pregnant.

Another issue is whether to leave the male in the cage with a pregnant female or females or to remove him from the cage. Removing the male can lengthen the time interval between litters in two ways: First, if he is not in the cage during post partum estrus, which occurs shortly after a female gives birth, a mating opportunity is lost. Second, once he is removed, we recommend delaying his return until all pups have been weaned and removed. Otherwise, he may kill the pups, even if they are his. In contrast, if the male remains in the cage, he can impregnate the female as soon she gives birth or at the first estrus after her pups are weaned. Furthermore, the male's instinct to kill pups is suppressed if he is present during the pregnancy.

13.B. Breeding schemes

Choice of breeding scheme depends on the strain you are breeding. In this handbook, we discuss breeding and maintenance schemes for specific categories of mice in Chapter 3, "Categories of Laboratory Mice: Definitions, Uses, Nomenclature."

13.C. Sizing a breeding colony for a research program

The exact quantity of mice for a study is determined by the experimental design, the variance of the phenotype, and the desired statistical power. (For an illustration of using power analysis to calculate quantities of mice required for an experiment, see Appendix L, "Simplifying Power Analysis to Determine Sample Size.") Especially in cases where specific genotypes are required, it may be surprising to learn how many mice must be produced to get the required quantity. For example, assume that on a weekly basis you need 20 males that express a recessive, infertile phenotype. Only 1/4 of the mice you produce will express the recessive phenotype (with heterozygous-by-heterozygous mating), and only 1/2 of those will be male. Thus, to get 20 males with the recessive phenotype, you will need to produce 160 mice per week.

Once you know the quantity of mice you need to produce and the production schedule, the next decision is whether to breed the mice or purchase them on a regular basis from a supplier. If you choose to breed the mice, you must determine the quantity of breeders to purchase. This decision is based on values for strain productivity—average pups per litter, wean:born ratio, average number of litters per breeding female, and rotation period of the females. For many strains of the most popular JAX® Mice, this information is provided in 4.B, "Reproductive performance," and in the JAX® Mice strain database (www.jax.org/jaxmice/query). Additional information on some strains of JAX® Mice, as well as inbred strains from other suppliers, is also provided in the Mouse Phenome Database (MPD; www.jax.org/phenome). It is important to keep in mind that these breeding data represent *guidelines* for a strain, and are not a guarantee of a given productivity. In fact, a more relevant source for this information would be the experience you or a colleague at your institution has had for your strain of interest or a similar strain.

You also must decide whether to breed your own replacement breeders or purchase them from your supplier. Consider that purchasing replacements from your original supplier can help minimize genetic drift in your colony. If you do choose to breed your own replacements, set up a schedule as stringent as the one for producing the experimental mice so that they will be available when you need them.

A few words about inbreeding a line of heterogeneous mice: Typically, a line will become less fertile within about three generations and may eventually become infertile. Make plans to refresh your breeding stock or set up extra lines at the beginning of your program.

13.D. Strategies for maintaining a line or strain without expansion

Often, researchers may wish to maintain a newly-generated strain without expanding it. A common reason to do this is to retain valuable mice while awaiting program funding. One challenge is to maintain the strain as efficiently as possible while protecting against its loss. Another challenge is to minimize the development of a substrain background. Following are several strategies to consider:

- Maintain a minimum of two generations of mice, with each generation consisting of two or three sister–brother breeding pairs (total of four to six pairs). Do not eliminate any older generation until a younger generation is proven fertile. Unless you have a concern about breeding performance, such as with inbreeding depression, set up each new generation from only one breeding pair of the parental generation.
- Closely monitor breeding performance. As performance declines with age, promptly set up the next generation of breeders.
- Try to keep the age range of your breeding pairs between two and eight months of age. Breeding pairs older than eight months may become infertile and "suddenly" stop breeding, without exhibiting a progressive decline in litter size.
- When maintaining a mutation on a standard inbred background, consider backcrossing the strain every 10 generations to prevent substrain divergence.
- Consider cryopreservation of the strain, so that the unique genetic characteristic (for example, mutation, congenic region, new strain) can be recovered in the event of breeding failure or a catastrophe such as fire or flood.

13.E. Using reproductive techniques

For the purposes of this handbook, we have divided reproductive techniques into two groups:

- *Standard reproductive techniques* that require no special equipment and that are straightforward to learn.
- *Assisted reproductive techniques (ARTs)* that require special equipment and very specific training.

13.E.1. Standard reproductive techniques

Standard reproductive techniques are used to determine and time pregnancies and to foster pups. These techniques may seem simple and old fashioned, but when used with "artful skill" by experienced technicians, they are some of the most valuable colony management tools available.

13.E.1.a. Determining pregnancy: vaginal plugs and palpation

Although there is no accurate early pregnancy test for mice, there is an easy and accurate way to tell if breeding has occurred recently: the presence of a copulatory (or vaginal) plug in the female's vagina. The copulatory plug is a waxy, solid mass of cream-colored, coagulated ejaculate that is produced by the male's seminal vesicle and coagulating gland. (One of the plug's purposes is to prevent other males from mating with the female.)

Generally, vaginal plugs remain intact for 10–12 hours post mating. Because mating usually occurs over a period of 15–60 minutes about 4–6 hours into the dark cycle, the best time to look for vaginal plugs is as early into the light cycle as possible, before they dissolve or become dislodged. The nature and depth of the vaginal plug can be a strain and age characteristic: it is superficially evident in some strains or in young females, but deep in the vagina in other strains and in older females. If the plug is not visible when looking at the vaginal opening, technicians can use a blunt, flat, autoclaved toothpick or blunt disinfected metal probe or forceps to open the vagina slightly and expose the plug.

Although a vaginal plug does not confirm conception, it does prove that mating has occurred. Even mating with a vasectomized male—one of the steps in creating pseudopregnant females—produces a vaginal plug. The likelihood of pregnancy ranges from about 30–100%, but it is stress and strain dependent (see sidebar). We confirm pregnancy with palpation after the 10th day of the pregnancy. (The day the vaginal plug is found is day zero.)

The likelihood of pregnancy following mating is strain dependent. For most strains, the rate of pregnancy among estrus-suppressed (group-housed) females that were induced to ovulate by housing with a male is highest for females with vaginal plugs found the third day after being set up with a male. C3H/HeJ (000659) and BALB/cJ (000651) strains are examples (table below). C57BL/6J (000664) females are an exception: 39% of females with vaginal plugs on the 3rd day were pregnant; 69% of females with plugs on the 4th day were pregnant.

Day plug is found	Rate of pregnancy for these strains	
	C3H/HeJ (000659)	BALB/cJ (000651)
3rd	100%	44%
5th	62%	31%

Source: Frequently asked questions (husbandry) at www.jax.org/jaxmice/faq.

We do ship female mice in which we have observed a vaginal plug, but we guarantee pregnancy only after we have confirmed with palpation. We prefer to ship pregnant females between the 11th and 15th day of pregnancy, because they seem to withstand the rigors of shipping better at this stage of gestation. Up to day 11, hormonal maintenance of pregnancy is mediated through the hypothalamus and pituitary; during this period, a given stress is more likely to cause complete termination of the pregnancy (through fetal resorption) than after day 11, when the corpora lutea maintain the endocrine support of pregnancy independently of the hypothalamus and pituitary. For information on purchasing "plugged" or pregnant JAX® Mice, contact Customer Service at 1-800-422-6423 (North America) or 1-207-288-5845 (International).

13.E.1.b. Timing pregnancies using the Lee-Boot and Whitten Effects

The Lee-Boot Effect (Van Der Lee and Boot, 1955) describes the phenomenon of estrus suppression in a group of densely-housed female mice that is removed from male mouse urine for 28 days. The Whitten Effect (Whitten, 1956) describes the process of a female in anestrus being induced into estrus by exposure to male mouse urine.

You can take advantage of both of these effects to time and coordinate pregnancies:

1. To suppress estrus, house at least five females as densely as possible for 28 days. Keep all male mice at least four feet away in all directions, including front and back.

   Note: To adhere to your ACUC guidelines for housing density, you may need to use large cages such as weaning cages.

2. To induce the females to resume their estrous cycles simultaneously, expose them to male mouse urine by placing dirty bedding in the cage for at least three days. On the third day, set up your breeding. Typically females will go into estrus and breed on the third night.

   Note: Only the first estrus will be synchronized.

3. Starting on day 4, check for vaginal plugs.

13.E.1.c. Fostering a litter

Litter fostering is used when mothers are unavailable or unable to care for their pups. Two common fostering situations are following hysterectomy derivation and when a genetic defect prevents the mother from lactating. In research colonies, foster mothers typically come from the among the breeders. For hysterectomy derivation, foster mothers are specifically prepared in SPF colonies.

The fostering procedure is relatively straightforward: Replace some or all pups from a foster mother's natural litter with pups from the litter you are trying to save.

13.E.1.c.1. Considerations

- When fostering a litter, the younger the pups are, the better.
- Choose a foster mother that has successfully weaned a litter in the past and that currently has a healthy, well-fed litter of her own that is as close in age as possible to the age of the foster pups.
- For identification purposes, choose a foster mother whose natural offspring are a different color than the foster pups. This is an obvious precaution when mixing natural and foster pups. But even when replacing the entire litter, there is always a chance that a "natural" pup can be buried under the cage bedding and not removed with its littermates.
- Generally, try to limit the size of the foster litter to six pups or fewer. Of course, this number is strain dependent and should be based on experience. But the foster litter—all foster pups or the combination of foster pups and natural offspring—should contain no more pups than the natural litter. (Changing the litter size can affect the foster mother's milk supply.) If necessary, split the litter between two foster mothers.

13.E.1.c.2. How to foster a litter of pups

1. Transfer the foster mother to a holding pen. If you are mixing natural offspring with the foster pups, leave the appropriate number of the mother's pups in the pen; remove the others.
2. Place the foster pups into the foster mother's home pen and cover them with nest bedding so they acquire the scents of the foster mother and her natural pups. (One strategy is to rub some feces from the foster mother on the fosterlings.)

   Note: If any of the pups are cool to the touch, warm them with a heat lamp or heat pad.
3. Place the foster mother back in her home pen with the foster pups as soon as possible. The mother will be stressed when she is separated from her litter, so do your best to minimize the separation time.
4. Leave the foster mother and foster litter alone for about 30 minutes. Then, carefully check to be sure the mother has accepted them—whether the pups are in the nest or have been "kicked out." If the foster pups are between about 6 and 36 hours old, check for milk spots.
5. For the next several days, check on the foster litter periodically to be sure that the foster mother is feeding and caring for the foster pups, but try to disturb them minimally.

If, at any time, you notice that the foster mother is rejecting the foster pups, try spreading the scent of the nest or any natural pups on the foster pups. Or, try fostering to another mother.

13.E.2. Assisted reproductive techniques (ARTs)

Assisted reproductive techniques (ARTs) represent technical procedures that require special equipment and sometimes, dedicated facilities. Technicians must be trained in specific techniques. ARTs provide ways to expand a colony, rederive a strain, and maintain or rescue a strain that cannot or will not breed reliably. ARTs also are used to "remove mice from the shelf" without losing the strain.

Table 13.4 provides an overview of several ARTs, their application, and considerations. Note that ARTS are often used in combination. For additional information about ARTs and related training, see 14.G. "Resources."

13.F. Maintaining the genetic integrity of your colonies

13.F.1. Preventing genetic contamination and minimizing genetic drift

If you breed laboratory mice, it is imperative that you follow breeding and colony management practices that prevent genetic contamination and minimize the effects of genetic drift. Prevent genetic contamination by following stringent breeding and recordkeeping protocols. Minimize genetic drift by replenishing your breeding stocks on a regular basis with mice of the same strain from your original supplier. For details, see Chapter 8, "Genetic Quality Control—Preventing Genetic Contamination and Minimizing Effects of Genetic Drift."

13.F.2. Confirming phenotypes and genotypes

Sometimes, phenotypes can be lost through breeding. This can occur as a result of a breeding error, but it also is a predictable outcome when you breed certain types of mice. For example, when you breed transgenic mice, transgenes may be unstable in early generations.

If the phenotype of interest is physically observable, or if the phenotype is linked to an observable marker (such as a specific coat color), it is easy to determine its presence or absence. However, often phenotypes of interest are not physically observable or do not appear when an animal is young. In these cases, genotyping with a biological assay may be necessary to ensure that the mutant allele is present in the mouse.

If you need to genotype your JAX® Mice or have questions about genotyping…

The Jackson Laboratory website provides several ways to access information related to the genotyping of JAX® Mice:

- Display the datasheet for a strain of JAX® Mice and link to genotyping protocols: www.jax.org/jaxmice/query
- Browse a list of protocols (also search by gene, stock number or primer): www.jax.org/jaxmice/pub-cgi/protocols/protocols.sh
- Link to supplemental genotyping protocol information (e.g., genomic DNA extraction methods): www.jax.org/imr/supp_proto

For further assistance, email us at micetech@jax.org or call Tech Support at 1-800-422-6423 (North America) or 1-207-288-5845 (International).

Information about genotyping methods is provided in 2.E, "Genotyping: what it is and how it is used."

Table 13.4. Assisted reproduction techniques (ARTs): definitions, applications, considerations.

Technique and definition	When to use	Considerations
Hysterectomy derivation: Pups are taken via caesarean section from the birth mother and fostered with a "clean" foster mother.	• Importation of a new mouse strain into facility with higher level of pathogen protection. • Rederivation (due to health status or strain restoration).	• Females are sacrificed; males are not. • If done because of pathogen contamination, pups must be monitored for pathogens. • Good strategy if rederivation is necessary, IVF is not proven for the strain, or few mice are available.
Ovarian transplantation: Fresh or thawed ovary (or 1/2 or 1/4 of ovary) is surgically implanted in the ovarian bursa of 4- to 5-week-old female of histocompatible strain.	• When females cannot successfully support fetuses, give birth, or care for pups. Example: Homozygous B6C3Fe *a/a-Csf1*^{op}/J (000231) females fail to lactate, and homozygotes of both genders are extremely fragile. We transplant ovaries from a homozygous (*op/op*) female into a recipient female of a histocompatible strain. • When yield is not important as long as you get "something."	• Because you only need part of an ovary, one female can provide tissue for up to 8 recipients. • Allow 1–2 weeks of healing before breeding. First litter might be delayed. • If the recipient's ovaries were not completely removed, she may bear some of her own pups. Therefore, a recipient of a different coat color enables identification of any offspring from her residual ovaries.
In vitro fertilization (IVF): Eggs are collected from superovulated females, fertilized *in vitro* with fresh or thawed sperm.	• When females are of questionable health status. • When you need embryos for cryopreservation. • When you have only 1 male and multiple females.	• Can accommodate ratio of many females to 1 male.
Embryo transfer: Embryos—created via IVF, thawed, or removed from fertilized females of unacceptable health status and flushed—are implanted into pseudopregnant females of high SPF health status.	• Rederivation. • Strain rescue. • Rapid expansion of colony.	• Up to 15 embryos can be implanted per female. • If transplanting fewer than 4 embryos, add "carrier" embryos during transfer. • Consider using recipients that are readily available, stay trim, have large infundibulum, have a coat color that minimizes confusion with natural pups, and have "good mother" phenotype.
Intracytoplasmic sperm injection (ICSI): Insertion of frozen or thawed sperm head into egg cytoplasm *in vitro*.	• IVF with "dead" or thawed cryopreserved sperm.	• Sperm do not need to be live. • Sperm can be collected from dead male if sperm is collected and cryopreserved as soon as possible.
Embryo flushing (following *in vivo* fertilization): Superovulated females are mated; oviducts are collected and flushed.	• When cryopreserving embryos. • When IVF technology is unavailable.	
Superovulation: Females are induced into ovulating a greater number of eggs than normal via injection with gonadotrophins.	• Often used in combination with *in vitro* fertilization or *in vivo* fertilization and embryo flushing. • To create embryos for cryopreservation.	• Superovulated females at 21–35 days of age, weighing 12–14 g, produce the greatest number of fertilized eggs. • Response to gonadotrophin injection is strain dependent; preliminary dose injections may be necessary.
Pseudopregnancy: Females are induced into the neuroendocrine status of the first half of pregnancy by mating with a vasectomized male.	• As first step to prepare recipient for embryo transfer.	• Mating is confirmed with a vaginal plug.

13.G. Troubleshooting breeding problems

Table 13.5 will help you identify and resolve common breeding problems. For breeding information specific to popular strains of JAX® Mice, see Chapter 4, "Characteristics of Popular Strains of JAX® Mice, Including Reproductive Performance." Within the strain information in that chapter, "technician notes" often provide anecdotal breeding observations and hints from our animal care technicians. Also see the Mouse Phenome Database (MPD; www.jax.org/phenome). For breeding information on each strain of JAX® Mice, refer to individual strain datasheets, available at www.jax.org/jaxmice/query.

Table 13.5. Breeding problems and possible resolutions.

Problem	Possible resolution
• Mice are not breeding. • Females are not getting pregnant.	A: Make sure you have male and female breeders. B: Add nesting material specifically designed for that purpose. C: Try a different diet, for example, one with more or less fat. D: Minimize stress—including human contact with the mice and activity and noise in the room. Construction-related vibrations, even outside the building, can disrupt breeding. If possible, move mice to a quieter area. E: Try a 14:10 light cycle. F: Make sure mice are healthy and able to breed (for example, not too obese or too old). Try healthier, younger animals. G: If you never see vaginal plugs, try a new male. H: Determine whether the problem is the male or female: pair a proven breeder female with the male and a proven breeder male with the female. I: Surgically evaluate the reproductive tract. If the female appears normal, try low dose gonadotrophins, ovarian transplant, IVF; if the male appears normal, try IVF. J: Research the strain reproductive characteristics. For a transgenic, check the effect of the transgene on breeding. For a strain carrying a mutation, check the effect of the mutation. Adjust the breeding strategy accordingly.
• Mice get pregnant but you never see pups (females are resorbing fetuses).	D (above): The most common cause of fetal resorption is stress.
• Individual breeders are not producing an expected number of pups.	B, C, D, E, F, H, J (above)
• Females give birth, but don't raise their pups. • Pups disappear or do not survive.	D, J (above) K: If you introduced a male to a cage with pups, he may have killed them. Wait to add a male until all pups have been weaned and removed.
• Colony productivity has dropped.	L: Check for environmental factors that might have changed. Consider room conditions (temperature, vibrations, building construction, odors, etc.), caretaker, and diet. If more than one cage, area, or strain is affected, expand the search. If possible, restore environment to previous conditions.

13.H. Resources

In addition to the chapter references, following are some resources that provide information about breeding and ARTS:

Web-based resources

- The Jackson Laboratory website: www.jax.org
  Access to details on ARTs techniques and cryopreservation; information about workshops on shipping and reconstituting frozen mouse embryos, cryopreservation of mouse germoplasm, surgical techniques for the laboratory mouse; individual strain data sheets.
- Technical support and literature webpage: www.jax.org/jaxmice/support
  Links to online literature and requests for literature, including resource manuals, newsletters, and JAX® Notes; online requests for technical support.
- Mouse Genome Informatics (MGI) listserve webpage:
  www.informatics.jax.org/mgihome/lists/lists.shtml
  Subscription form to join our MGI email list service.

Print-based resources

- Appendix J, "Cryopreservation," in this handbook.
- Silver LM. 1995. *Mouse Genetics, Concepts and Applications*. Oxford University Press. (Also available online at www.informatics.jax.org/silver.)
- Staff of The Jackson Laboratory. 1968. *Biology of the Laboratory Mouse*, Second Edition. Dover Publications, Inc., NY.

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Chapter 14: Emergency Planning

Joanne M. Currer, Dorcas Corrow

Colony managers often think of emergencies in terms of major disasters such as fires, floods and earthquakes. But chemical spills and short power outages can result in an emergency event, too, as can suspicious letters or packages. Today, many colony managers also recognize the need to plan for the possibility of a pandemic and the resulting loss of employees.

All emergency planning is based on risk assessment: How much loss are you willing to accept? How much are you willing to spend to prevent a loss that may never happen? Or to recover from one that did?

The objective of this chapter is to provide some basic information about emergency planning as well as an overview of what we do at The Jackson Laboratory. The focus of this chapter is on the effects of an emergency on an animal facility, not an entire organization.

The chapter is organized as follows:

***Acknowledgments**: We would like to thank John Fitzpatrick and Janice Von Brook for their valuable assistance with this chapter.*

14.A. Developing your plan

Emergency plans have five major objectives: to minimize the initial effect of an emergency, to keep animals safe, to minimize loss of data, to return to normal operations as quickly as possible, and to manage a loss of employees.

14.A.1. Minimizing the initial effect of an emergency

To minimize the initial effect of an emergency, you must determine the scope of the event and initiate first response steps as quickly as possible. An emergency plan should include ways to quickly assess the emergency, procedures for immediate containment of the emergency, and if necessary, evacuation of people and animals from your facility. Considerations include, but are not limited to, a published list of responsible parties and responsibilities, a warning system (e.g., fire alarms), evacuation drills, a trained security force, trained floor monitors, and pre-arrangements with local authorities.

Procedures for keeping people safe should be appropriate for your institution and must be your highest priority. Perhaps you'll need trained medical personnel on site. Besides basic medical supplies, you might need protective equipment for first responders.

What about insurance?

Consider the real value of lost research animals, research data, and equipment when arranging for insurance coverage. Consult with experts to determine whether options such as business interruption or extra expense coverage or both make sense for your organization. Appropriate coverage can provide critical assistance to a recovery effort. And the process of an insurance audit might expose "risky behavior" that can be corrected.

Minimizing the effect of an emergency also includes planning to avoid it. For example, in an area susceptible to flooding, a wise strategy would be to house animals above ground. A wiser strategy might be to house them above possible water levels during a flood. As always, the cost to avoid a disaster must be balanced against the cost of the lost assets.

14.A.2. Keeping your animals safe

To keep your animals safe throughout an emergency, it is important to provide them fresh air, water and food in a suitable environment. You must be concerned about microbial contamination of any animals you move. Only your institution can determine the cost–benefit ratio of saving vs. destroying animals that may have been contaminated by any microbial agent.

It is always wise to have a backup plan for recovering from a loss of animals. Strategies include maintaining animals at another location (while avoiding the inadvertent development of a substrain) and cryopreserving embryos, ovaries or sperm off site.

How The Jackson Laboratory can help with your animal backup plan.

The Jackson Laboratory offers several JAX® Services related to cryopreservation and recovery of mouse strains. To learn about how these programs can help you plan for and recover from emergencies, visit our disaster planning webpage at www.jax.org/jaxservices/disasterplanning.

Keep in mind that once a pathogen barrier in a facility is broken, you must wait to reopen the facility until its condition is restored to an acceptable health standard. Thus, you might need to house animals in a temporary facility for a period much longer than the initial emergency.

14.A.3. Minimizing the loss of data

For any computer data, strategies include regular backups of institutional and research data to a server off site or over the Internet. You may need to set up special backup procedures for laptop computers, which may not be connected to your network on a regular basis.

Copy and store any critical paper-based data at a remote location or in containers or file cabinets resistant to fire or water or both.

14.A.4. Returning to normal operations

Plans for redeploying mouse rooms should be no less stringent than those for biosecurity breaks. (For details, see Chapter 7, "Animal Health—Preventing, Identifying, and Eradicating Microbial Contamination.") Of course, following an emergency, recovery might be on a much larger scale.

If the emergency involves loss of housing for your employees, arrangements for temporary housing can benefit both employees and your institution.

Having backup equipment at another site or planning ahead for replacement of equipment is also wise.

In times of crises, scientific institutions often do whatever they can to help. Assistance can include housing of animals and research space. Arranging ahead for this type of help can minimize confusion and uncertainty when you are in the middle of an emergency.

Specific issues related to pandemics

Pandemics present 2 specific challenges: First, pandemics affect the work force, not the animals. Therefore, you will have just as many animals to care for, but not as many personnel with which to do so. Second, pandemics can be of a lengthy duration. They are expected to occur in waves, which decline in severity as the disease runs its course.

Considerations include the following:

- Transportation in and out of an affected geographical area could be suspended during a pandemic. The implication is that enough food and water (for mice and employees), fuel for emergency generators, etc. must be planned for and in place before the pandemic strikes.
- Ways of doing business will be altered as "social distancing" is practiced. Face-to-face contact will be reduced in favor of telephone conversations, email, and teleconferences. Having protocols in place can prevent confusion, especially when the pandemic first appears.
- Staff preparedness involves planning and prevention at home as well as at work, both to protect against the disease and do whatever possible to minimize its effects.
- Focusing on critical functions rather than departments or titles can concentrate necessary personnel and energy on tasks that must be accomplished even under the most trying of circumstances.

14.A.5. Managing a loss of employees

In the event of a pandemic or any event that results in loss of employees, you must cope with a reduced work force. An obvious strategy to plan for this issue is cross-training of employees. But remember that effective cross training involves more than just animal care. You also might need to make arrangements for employee shortages in animal care support, including the wash area, janitorial services, shipping, and another other critical areas of support.

14.B. What we do at The Jackson Laboratory

At The Jackson Laboratory, we have standard operating procedures (SOPs) to manage emergencies at our facilities in Bar Harbor, Maine, and West Sacramento, California. We have specific SOPs for such events as fire, flood, earthquakes, bomb threats, contaminated letters or packages, chemical spills, and pandemics. Our security force and a designated group of employees are fully trained on these procedures and are responsible for managing any necessary implementation. We regularly remind employees to be watchful for any suspicious activities on or around our facilities.

To ensure the welfare of our employees and animals during a power outage, we have emergency generators in our facilities in Maine and California. And, we stock emergency supplies of food and water for mice. All supplies are substantial enough for a lengthy situation.

We train our employees in good health practices, including prevention of and response to a pandemic. Managers and employees are cross-trained in multiple areas of responsibility—with a focus on critical functions—to ensure that animals will be well tended even if we experience a reduction in workforce.

Our cryopreservation facilities, which store both our own and customers' biological material, are located in a secure area. We also store duplicate cryopreserved biologicals off-site.

Our institutional computers are backed up daily. We have redundant backups stored at multiple sites. And, we have a disaster plan in place to restore data as necessary.

We know a little about dealing with fires…

The Jackson Laboratory has twice experienced serious fires—in 1947 and 1989.

October 17,1947, a fire that started in a bog about 6 miles from our facility spread throughout Mount Desert Island, the 108-square-mile island on which The Jackson Laboratory is located. By the time the fire was declared "out" on November 14, (National Park Service, 2008) more than 17,000 acres of Mount Desert Island were destroyed, as was much of The Jackson Laboratory and its stock of mice. Fortunately, Dr. Little and the staff of The Jackson Laboratory had shared their research mice freely, and because the scientific community recognized the value of The Jackson Laboratory and our mice, they partnered with us to help restore our facilities and our mouse stocks.

May 10, 1989, a 5-hour fire that started in a construction area in a mouse room destroyed about 40% of our production facility and more than 400,000 mice. But many mice in affected buildings were saved, and many more throughout the campus were unaffected. We lost no strains of mice.

By May 23 we were shipping mice. We also assisted researchers dependent on JAX® Mice to maintain the momentum of their research by helping them set up their own mouse colonies. Within days of the fire we were rebuilding our facilities, and by spring 1990, we were operating at 80% of pre-fire capacity (The Jackson Laboratory Staff, 1989). Reconstruction employed a more modular building design, which makes our facilities less susceptible to the spread of fire or contamination.

14.C. References

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Chapter 15: Human Health Concerns—Mouse Allergies, Bites, Zoonotic Disease

Peggy Danneman, Kathleen Pritchett-Corning, Joanne M. Currer, Kevin Flurkey

Although working with mice is a relatively low-risk pursuit, three areas of potential risk exist: exposure to mouse allergens, injury from animal bites, and exposure to zoonotic disease.

Our objective for this chapter is to provide an overview of these issues and some strategies for addressing them. We will also highlight what we do at The Jackson Laboratory and provide resources where you can find further information.

The chapter is organized as follows:

15.A. Mouse allergens

In the United States, among the population of people who work with mice, approximately 10% routinely develop a laboratory animal allergy (LAA) to mice (JAX® Notes, 1999). Although an LAA can be a debilitating condition, sometimes resulting in severe physical reactions such as anaphylactic shock, reactions usually are much less severe—more commonly associated with nasal symptoms (sneezing, discharge), respiratory symptoms (wheezing, shortness of breath), cutaneous symptoms (rash, hives) or ocular symptoms (watering, redness). Workers with a history of atopic disease are at higher risk of becoming sensitized to laboratory animals.

15.A.1. The most common offending allergen: Mus m1

The main murine protein that causes allergic reactions in humans is Mus m1 (mouse urinary protein [MUP] in older literature), one of the many proteins in mouse urine. Mus m1 is a lipocalin protein that serves as a pheromone carrier. It is produced in the liver, and although it is excreted primarily in the urine, it also may be found in the hair follicles and dander of mice. Males produce approximately four times as much Mus m1 as females.

15.A.2. Protection from laboratory animal allergies (LAAs)

An obvious solution to an LAA is to limit total exposure to the animals. But often, this option is, at best, only partially workable. Following are several strategies for minimizing the effects of allergic reaction to mouse antigens:

- Caging and environment. The most effective caging system for minimizing LAA exposure is ventilated caging operated under negative pressure (Reeb-Whitaker and Harrison, 1999). Filter tops on non-ventilated cages will confine allergens, but their use must be balanced with their potentially detrimental effect on the cage micro-environment. Innovative room ventilation designs also can help improve the dilution and removal of airborne contaminants in a mouseroom. Harrison (2001) discusses options.
- Institution-wide husbandry practices. Specify workplace conduct rules and standard operating procedures (SOPs), such as changing cages in ventilated hoods (Schweitzer *et al.*, 2003) and prohibiting dry sweeping in animal facilities (Reeb-Whitaker and Harrison, 1999). Minimizing or, preferably, prohibiting animal traffic in personnel areas will help to protect sensitive individuals from unnecessary exposure to animal allergens.
- Personal protection. Consider LAAs when developing SOPs for entry to and exit from mouse rooms. Any procedure that prevents transmission of pathogens from humans to mice, such as those used in SPF colonies, also helps to limit exposure of humans to animal allergens. Components of these SOPs include frequent hand washing and use of booties, gowns with tight, rib-knit cuffs, gloves, and dust masks. Use of facility uniforms for animal caretakers and other individuals who spend a considerable amount of time in animal rooms will not only protect the individual worker but minimize the potential for carrying animal allergens to other parts of the facility or outside the facility (e.g., home). Facility uniforms should be laundered in the facility or by a professional laundry service.

  For workers who have symptoms of LAA or a history of asthma and are concerned about severe reactions to mouse allergens, personal protective equipment such as custom-fitted respirators or powered air-purifying respirators (PAPRs) provide high-efficiency particulate air (HEPA)-filtered air directly to the worker.

Most minor allergic symptoms can be treated with over-the-counter medications such as antihistamines or topical creams. More serious symptoms, especially respiratory ailments, should be treated by a physician.

15.B. Animal bites

If irritated or handled incorrectly, laboratory mice may bite. Although the use of gloves helps prevent injury to the skin, sometimes teeth do break through the gloves and the skin, creating small puncture wounds. Animal bites are always of concern, but laboratory mouse bites are of less concern than bites from most other animals. Nonetheless, even SPF mice, which are free of organisms that tend to cause disease in mice, still carry common bacteria in their mouths that can cause wound infections. Thus, any bite should be cleaned thoroughly with soap and water. (*Note*: Although people may associate the risk of tetanus with animal bites, the tetanus agent *Clostridium tetani* is not commonly found in laboratory mice. Nonetheless, vaccination against tetanus is commonly recommended when a human is bitten by any animal.)

Perhaps the greatest concern with bites from laboratory mice is allergic reaction. In particular, workers with a minor allergy to mice may have an exaggerated allergic reaction to a mouse bite if it breaks the skin. If swelling appears around the bite area, an occupational health care worker should be contacted to evaluate the need for treating and monitoring the wound. Anaphylaxis, a serious medical condition, can occur in highly sensitized individuals and requires immediate medical attention.

If the bite is from a wild-caught mouse, or a mouse exposed to wild-caught mice, there is greater concern (see 15.C, "Zoonotic disease"). A report should be made through appropriate institutional channels. Information about the health status of the mouse, if known, should be reported to an occupational health care worker for further evaluation.

15.C. Zoonotic disease

Most modern mouse colonies are kept in facilities designed to exclude wild mice and are regularly tested for various pathogenic organisms, including those transmissible to humans (zoonotic). Reputable suppliers of laboratory mice are especially vigilant.

However, wild mice—and even domestic laboratory mice—do have a potential for infection with and transmission of zoonotic diseases. Especially if investigators are using wild-caught mice in the laboratory, screening of representative animals for zoonotic organisms is highly recommended. If long-term use of wild-derived animals is anticipated, rederivation should be considered.

Zoonotic agents that are most likely to be carried by laboratory mice include lymphocytic choriomeningitis virus (LCMV), *Salmonella* spp., and *Streptobacillus moniliformis* (rat bite fever). In addition, wild mice may also carry other zoonotic organisms, including Hantaan virus, Seoul virus, Sin Nombre virus, *Leptospira* spp., or *Rodentolepsis* (formerly *Hymenolepsis*) *nana*.

Any animal infected with a zoonotic organism should be euthanized or housed in a containment facility, because many zoonotic bacteria, viruses, and fungi can persist in an animal even after treatment, resulting in a continued threat to the colony and the potential for further human infection.

The potential for mouse-derived biological products (e.g., serum or cell lines) to carry pathogenic organisms should be kept in mind. To protect both mouse and human health, such products should be screened for pathogenic and zoonotic organisms prior to use in the laboratory.

15.D. What we do at The Jackson Laboratory

15.D.1. Allergies

At The Jackson Laboratory, we include information about mouse allergies in our orientation for all new employees. We provide additional information to any employee who works with mice. We have a formal procedure for reporting possible allergic reactions, and, if warranted, we can screen for allergic reactions in our on-site health office. We recommend treatment based on severity of reaction.

We take animal allergies seriously at The Jackson Laboratory…

Researchers at The Jackson Laboratory have been studying mouse allergies since 1980—the biological perspective as well as the effects on our employees and institutional strategies for lessening those effects. Several published studies are referenced throughout this chapter.

Currently, we are partnering with Johns Hopkins University to study occupational mouse allergies and asthma. We also conduct annual medical surveillance for our employees who are at risk.

Our strict SOPs for mouse room setup and husbandry, which were originally designed to protect mice against pathogens, also help control allergens. We monitor mouse rooms and public areas to determine levels of airborne mouse allergens, and we take corrective action when necessary. We also provide personal protective equipment for any employee who requires it.

Our experience is that a majority of LAAs are relatively mild. Most affected employees manage their allergy by adhering to our SOPs for entry to and exit from mouse rooms, which include use of gloves and gowns in all mouse rooms at The Jackson Laboratory. We also have a formal respiratory protection program, which is required for all personnel who work in animal areas and have a history of animal allergies or asthma. This program is voluntary for employees interested in preventive protection. (It is worth noting that employees rarely need such extreme protection.) If allergies cannot be managed using respirators or PAPRs, an employee will be transferred to another position that involves less or no exposure to mice.

15.D.2. Bites

At The Jackson Laboratory, to minimize injury from animal bites, we train technicians in safe animal handling practices. We require the use of protective gloves when handling mice. Any bite wounds are reported to our Health Office and treated and monitored accordingly.

15.D.3. Zoonotic disease

In our mouse colonies, we regularly screen for zoonotic pathogens. Although it has been decades since we last found any of these organisms in any of our established colonies—the only zoonotic agent ever found in any of our established colonies was *Salmonella*, which was eradicated in the 1960s—our emergency response policy dictates euthanasia of any infected mice along with complete depopulation of any remaining mice in the room.

Mice imported from other institutions are rederived, primarily to eliminate the potential of introducing unwanted mouse pathogens into our colonies. Prior to rederivation, all newly imported mice are housed in a strict quarantine facility and handled with great care based on their potential to harbor zoonotic organisms. Similarly, we screen all biological products of rodent origin for organisms, including zoonotic organisms, that might threaten mouse or human health. (For information about our screening and importation procedures, see Chapter 7, “Animal Health—Preventing, Identifying, and Eradicating Microbial Contamination.”)

15.E. Resources

Selected Publications:

Bush RK, Stave GM. 2003. Laboratory animal allergy: an update. *ILAR J*. 44:28–51.

Curtin-Brosnan JM, Eggleston PA, Paigen BJ, O'Neil EA, Hagberg KA, Matsui EC. 2007. Respiratory protection and incident skin test sensitivity among laboratory mouse workers. *J Allergy Clin Immunol*. 119(Supplement 1):S65.

Division of Occupational Health and Safety. 2003. The National Institutes of Health Laboratory Animal Allergy Prevention Program (LAAPP). Web access: http://dohs.ors.od.nih.gov/publications.htm

Institute for Laboratory Animal Research. 2001. *ILAR Journal*. Entire Volume 42, Issue 1.

Matsui EC, Diette GB, Krop EJM, Aalberse RC, Smith AL, Eggleston PA. 2006. Mouse allergen-specific immunoglobulin G4 and risk of mouse skin test sensitivity. *Clin Exp Allergy*. 36:1097–1103.

Newcomer CE and Fox JG. 2007. "Zoonoses and Other Human Health Hazards," in *The Mouse in Biomedical Research. Vol. II Diseases,* 2nd Edition. Academic Press, New York, pp. 719–746.

Reeb-Whitaker CK, Harrison DJ, Jones RB, Kacergis JB, Myers DD, Paigen B. 1999. Control strategies for aeroallergens in an animal facility. *J Allergy Immunol*. 103:139–146.

Web resources:

Centers for Disease Control and Prevention: www.cdc.gov

National Institute for Occupational Safety and Health (NIOSH):
- NIOSH Safety and Health Topic—Asthma and Allergies: www.cdc.gov/niosh/topics/asthma
- "Preventing asthma in animal handlers": www.cdc.gov/niosh/animalrt.html

15.F. References

Harrison DJ. 2001. Controlling exposure to laboratory animal allergens. *ILAR J*. 42:17–36.

JAX® NOTES. 1999. Frequently asked questions about JAX® Mice. *JAX® NOTES*. 479:6–8.

Reeb-Whitaker CK, Harrison DJ. 1999. Practical management strategies for laboratory animal allergy. *Lab Animal*. 28:25–30.

Schweitzer IB, Smith E, Harrison DJ, Myers DD, Eggleston PA, Stockwell JD, Paigen B, Smith AL. 2003. Reducing exposure to laboratory animal allergens. *Comp Med*. 53:437–492.

Chapter 16: Vivarium Staff Development and Contribution

Joanne M. Currer, Kevin Flurkey

Researchers may know the genetics of mice down to the nucleotide, but often, technicians are more familiar with "normal" strain-specific characteristics and behaviors. In some ways, they know the mice best. Day after day, conscientious technicians keep animal colonies running smoothly, by caring for the animals, defending the pathogen barrier, and helping assure the genetic integrity and health of the animals. Should a problem arise, a well-trained, experienced technician will discover it at an early stage and—with the appropriate authority—take timely action that can minimize damage.

The reputation of a staff that unfailingly maintains high quality mice can also directly affect an organization's finances. In a production environment, an exemplary reputation affects revenue by warranting the confidence of customers. In a research environment, an exemplary reputation affects grant funding by inspiring the confidence of peer reviewers. Thus, a well-trained, involved animal care staff is a valuable resource for an entire organization—not just for colony managers.

The objective of this chapter is to highlight some issues related to training, communicating with, and maximizing the involvement of your staff. We also provide details about our training programs and several other strategies we use at The Jackson Laboratory.

The chapter is organized as follows:

***Acknowledgments**: We would like to thank Marcia Picard and Dorcas Corrow for their valuable input to this chapter.*

16.A. Training and career development for vivarium personnel

16.A.1. Considerations

16.A.1.a. Training

Simply put, if you are managing a production or research mouse colony, you need a well-trained staff. Protecting your mice from pathogenic and genetic contamination is time-consuming, meticulous work. If it is performed casually, your colonies—and research conducted at your institution—will suffer. One of the best ways to ensure that animal care is done well is to ensure that technicians understand the importance of even mundane tasks that are designed to assure health and genetic quality. Training, both on-the-job and classroom, is essential. It is important to note that on-the-job training does not imply informality. Although the training environment may be less formal than that of a classroom, objectives must be operationally defined, and evaluation and demonstration of proficiency must be specific.

Also, it is important to provide ongoing training. As technology changes, your staff must be well equipped to handle any advancements in husbandry and relevant research practices.

16.A.1.b. Career development

Some employees are eager to learn and advance—by increasing their skills, by learning more about the science and technology behind the work they do, by improving animal welfare, and by seeking new responsibilities. It is important to provide opportunities for these employees to continue their growth and advance their careers. Other employees are very happy doing one job well. Remember to recognize them for their consistent contribution.

And, remember the team that supports the technicians. Workers who wash, autoclave, and deliver supplies also must adhere to exacting standards. Ensure that they get recognition and advancement opportunities.

16.A.2. What we do at The Jackson Laboratory

It is an understatement to say that we are proud of our animal care technicians at The Jackson Laboratory, which, at the time this book was published, numbered at approximately 250. We know that our global reputation as one of the most reliable sources of genetically defined laboratory mice depends on the work our caretakers and technicians do day after day, year after year. We employ men and women who understand the seriousness of their work. We provide training and incentives to build their skill sets, and we recognize and reward hard work and dedication.

16.A.2.a. Training specific to The Jackson Laboratory

Following is overview information related to the training we offer our animal technicians. Detail is provided in Table 16.1.

16.A.2.a.1. New hire, on-the-job training

- *Phase I*. Upon hiring, animal care trainees attend orientation and receive extensive on-the-job training. Throughout this probationary period, employees receive ongoing coaching as well as three formal evaluations, which require that they show understanding and proficiency of standard operating procedures (SOPs) related to their job requirements.

  Upon completion of Phase I training, supervisors meet with trainees, evaluate their performance, skills, and preferences, and assign them to one of three areas—Production, Research Animal Facilities (RAF), or RAF Repository (where we maintain our special mutant strains).
- *Phase II*. Phase II training involves on-the-job instruction and evaluation tailored to Production, RAF, or RAF Repository.

- *Phase III.* Phase III training involves on-the-job instruction and evaluation tailored to the highest level barrier areas in Production. These areas house Foundation Stocks, Pedigree Expansion Stocks, and Pedigree colonies that distribute mice to other Production areas.

16.A.2.a.2. Required classroom training

We require that our technicians attend a formal course in addition to on-the-job training:

- *Laboratory Animal Sciences*. The Laboratory Animal Sciences course supplements on-the-job training. Technicians take this course after completion of primary training, generally six–eight months after they are hired. Much course material is drawn from the Assistant Laboratory Animal Technician (ALAT) training material provided by The American Association for Laboratory Animal Science (AALAS). Evaluation includes multiple quizzes and final written and practical exams.

16.A.2.a.3. Elective classroom training

Elective classroom training includes the following, all of which adhere to Animal Care and Use Committee (ACUC) and Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) guidelines:

- *Foundation of Basic Genetics*
- *Beyond Basic Genetics*
- *Biomethods*
- *Surgery*

A majority of our technicians attend both genetics courses, and approximately 75% of our technicians have completed biomethods training successfully.

16.A.2.b. Other training

16.A.2.b.1. Technician training

Training curriculum and materials from AALAS are incorporated into our required Laboratory Animal Sciences course. Employees also can use the AALAS materials as a self-study guide. We offer the Assistant Laboratory Animal Technician (ALAT) exam on site. Once certified, employees receive a monetary bonus. Although ALAT certification is not required for promotion, typically about 75% of the animal care technicians at The Jackson Laboratory are ALAT certified.

Higher levels of AALAS certification include the following, both of which offer a monetary bonus: Additional training in animal husbandry and certification as a Laboratory Animal Technician (LAT), and training in colony management and biotechnical skills (such as surgery) and certification as Laboratory Animal Technologist (LATg). LATg certification is especially important for jobs in our research and services groups. Although AALAS courses are self-study, exams are held at The Jackson Laboratory.

16.A.2.b.2. Management training

We recognize the importance of an effective supervisory and management team. Many of our supervisors and managers are trained and certified by the Institute for Laboratory Animal Management (ILAM), an AALAS educational program targeted to managers in the laboratory animal science industry. The 2-week course is taught 1 week per year over a 2-year period. Topics include management concepts, enhanced communication, team building, and networking.

On an ad hoc basis, The Jackson Laboratory provides off-site leadership training that comprises team building and coaching for supervisors and mangers who qualify.

Table 16.1. Training opportunities available to animal care personnel at The Jackson Laboratory

Training/ Class	Required/ Elective	Length	Content	Comments
On-the-Job, Phase I	Required	12 weeks	• Overview of The Jackson Laboratory, employee policies, and benefits • Basic husbandry, including animal handling and cage changing • Breeding • Recordkeeping • Materials handling • Pathogen monitoring • Deviant identification • Basic genotyping • Safety	Initiated upon hire.
On-the Job, Phase II	Required	4 weeks	Based on requirements specific to Production, RAF, or RAF Repository.	Follows Phase I training.
On the Job, Phase III	Elective	12 weeks	• High level barrier area • Mutant stock colony maintenance • Basic genotyping • Our pedigree numbering system	Follows Phase II training.
Laboratory Animal Science	Required	14 weeks, 1 afternoon per week	• Genetics • Chemical safety • Nomenclature • Recordkeeping • Diagnostics • Structure of organs, tissues, and cells • Genetic quality control • Breeding • Mouse room environment • Animal health • Drug therapy • Research techniques • Biological and genetic terms and concepts • Career development	Sometimes taken concurrently with on-the-job training.
Foundation of Basic Genetics	Elective	15 weeks, 1 afternoon per week	• Cell cycle • Genes and chromosomes • Rules of heritability • Concepts of gene therapy • Mitochondrial genetics	
Beyond Basic Genetics	Elective	15 weeks, 1 afternoon per week	• Mouse karyotype • Mitosis and meiosis • Oogenesis and spermatogenesis • *In vitro* fertilization • Preimplantation development • Relationship of mouse genetics to human health	
Biomethods	Elective	Multiple afternoons; length depends on topics and student skill levels	Required methods: • Animal handling and restraint • Sexing newborn mice • Injections • Euthanasia • Tail tipping • Ear notching • Blood sampling Optional methods: • Cardiac puncture • Other identification methods (ear tagging, microchip implantation, toe clipping, tattoo)	Taught on an "as needed" basis; often customized to a particular group of students.
Surgery	Elective	Multiple afternoons; length depends on topics and student skill levels	More than 30 various surgical techniques, including • Catheterizations • Implantations • Denervations • Cannulations • Organectomies	Taught on an "as needed" basis; often customized to a particular group of students.

16.A.2.c. Career advancement

Within our production and operations organization, we have multiple levels of trainee and technician positions. We reward initiative and hard work and regularly promote from within. Many former technicians have advanced to such positions as room supervisors, managers, and senior managers. Former technicians who moved into our research facilities now hold such positions as research assistants and technical support personnel.

Under our Shared Services program, vivarium technicians may choose to work part time with researchers. For a set amount of time per week, technicians perform research oriented tasks under the supervision of a member of the scientific staff. This is a way for vivarium technicians to broaden their knowledge base and gain critical skills for career advancement. Our Shared Services program has greatly facilitated introduction of animal care technicians to full-time jobs in research.

Tuition reimbursement for post-secondary education

At The Jackson Laboratory, we encourage all of our employees to take an active role in their education and career advancement. All technicians are eligible to participate in our lab-wide tuition reimbursement program, which applies to successful completion (grade C or above) of courses given by an accredited post-secondary institution. We also offer short term, interest-free loans through a local credit union for “up front” payment of tuition.

16.B. Effective communications

16.B.1. Considerations

One way to ensure that a mouse colony runs smoothly—and that technicians know exactly what is expected from them—is to develop and document any procedures or protocols for tasks that must be repeated in a consistent manner. This is especially critical when it comes to any institutional policy or standard operating procedures (SOPs) directly related to colony management (including animal care, protection from pathogens, safety, etc.). The more detailed this documentation is, the more consistently the tasks will be performed. Goals are to make it easy for technicians to learn and review procedures, and to remove subjectivity from performance evaluation.

Of course, communication works best when it is 2-way. This includes regular meetings, and it requires an environment where technicians are recognized as valuable team members. Encourage comments and problem solving. Critically evaluate comments you receive.

16.B.2. What we do at The Jackson Laboratory

At The Jackson Laboratory, we have detailed SOPs, formalized across the organization, for all tasks our technicians must perform. These include procedures for cleaning and sanitation of the facilities, animal care, and safety, as well as for compensation and benefits. These procedures are available on our internal website; some are posted throughout our campus. Whenever an animal care technician learns or undertakes a new responsibility, he or she must sign off on any relevant SOPs.

In our production facility we hold bi-weekly room supervisor meetings to disseminate information and discuss issues. We also hold quarterly open forums for our entire animal care staff, where our management team discusses what's happening in other parts of The Jackson Laboratory.

We encourage communications *from* our technicians, especially regarding changes to improve efficiency or ease stress for the mice or themselves. An example: One of our technicians, concerned about the unavoidable stress on mice when their cages are changed, redesigned a "mouse-holding" forceps to provide maximum grasping power with minimum pinching. Management listened and, in fact, The Jackson Laboratory pursued a patent for this design.

In our production facilities, we have a "know your mice" program that encourages technicians to look for unique characteristics or behaviors of their mice. It is through this program that we formally collect comments on physical appearance, behavior, and breeding that are the basis of the technician notes that we include on strain datasheets for JAX® Mice and in Chapter 4, "Characteristics of Popular Strains of JAX® Mice, Including Reproductive Performance."

In our research colonies, we have a "sick and injured animal program" that provides a formal way for technicians to make decisions about when to involve researchers and veterinarians in the care of individual mice. Because the characteristics of a research animal may be normal for that specific animal, it is important that the technicians use good judgment to ensure both the welfare of the animal and the validity of the research. Details of this program are provided in the sidebar in Section 7.B.3, "Our monitoring procedures."

The Senior Management Team at The Jackson Laboratory has initiated a program of quarterly forums, open to all employees. One goal of the open forums is to address any issue raised by any employee. Comments and questions can be submitted through a variety of channels. Additional goals of the program are to foster positive and constructive working relationships at all levels, and to facilitate understanding of the roles of The Jackson Laboratory in the field of biomedical research.

Chapter 17: Ordering JAX® Mice and JAX® Services—Contact Information for Customer Service and Technical Support; Frequently Asked Questions

To place an order or for Customer Service:	
Telephone:	United States: 1-800-422-6423 International: 1-207-288-5845 *Monday through Friday* *8:00 AM to 6:00 PM Eastern Time*
Fax:	1-207-288-6150
Email:	orderquest@jax.org
Web form:	www.jax.org/jaxmice/orders
Mail:	The Jackson Laboratory Customer Service 610 Main Street Bar Harbor, Maine 04609-1526 U.S.A.

For Technical Support:	
Telephone:	United States: 1-800-422-6423 International: 1-207-288-5845 *Monday through Friday* *8:00 AM to 6:00 PM Eastern Time*
Web form:	www.jax.org/jaxmice/micetech
Email:	micetech@jax.org

For JAX® Services:	
Telephone:	1-800-422-6423 1-207-288-6294
Website:	www.jax.org/jaxservices
Email:	jaxservices@jax.org

For general information about The Jackson Laboratory:	
Telephone:	1-207-288-6000
Web:	www.jax.org
Mail:	The Jackson Laboratory 600 Main Street Bar Harbor, Maine 04609-1526 U.S.A.

To expedite the order process:

Please provide the following information…

About your institution:

- Complete billing and shipping addresses.
- Names, telephone numbers, fax numbers, and email addresses of buyer and primary researcher.

About your order:

- Purchase order number (and release number, if applicable).
- Order type: one-time shipment or standing order.
- Requested delivery date(s).

About the mice:

- Stock number and full strain name.
- Genotype.
- Quantity, age, and gender required.

If you are a new customer:

To establish an account to order JAX® Mice and JAX® Services, please complete our new account form, available on our website: www.jax.org/jaxmice/orders/newcustomer

If I am in Europe or Asia, how do I order JAX® Mice?

Customers in Europe and Asia can purchase JAX® Mice one of two ways:

- Directly from Customer Service at The Jackson Laboratory.
- From one of our authorized distributors or breeders in Europe or Asia. For an up-to-date list and contact information, visit our website (www.jax.org/jaxmice/orders/international).

If you have any questions, please contact Customer Service. One of our international agents will answer your questions and help you with your order.

Can I set up a delivery schedule for my order of JAX® Mice?

We can schedule shipment of your JAX® Mice per your requirements. Contact Customer Service for assistance.

Are there ways to reduce the lead time for my order of JAX® Mice?

To expedite delivery of an order, consider the following:

- Partial shipments of smaller quantities of mice.
- Increased range of acceptable ages.
- Males and females vs. males or females only.

How are JAX® Mice shipped?

We ship JAX® Mice in protective polypropylene containers with air filters designed to provide maximum air flow and pathogen protection. (Air filter specs: media, 100% #5 polypropylene; basis weight, 2.25 ounces per square yard; nominal thickness [1 ply], 17 mils; air permeability, 125 cubic feet per minute per square foot.) We supply each container with bedding, a water source, and the normal feed for the mice being shipped. Supplies are adequate to accommodate mice for up to 10 days. We conduct the entire packing process in the facility in which the mice were raised.

Generally, we ship JAX® Mice to the United States and Canada via our dedicated ground transportation network. All trucks are specially engineered with environmental control systems that facilitate sanitation and air flow and ensure as safe and stress-free a ride as possible. Once we put the JAX® Mice on the truck at our facility, they remain on the truck in this tightly controlled environment until they are delivered to your site.

Sometimes it is imperative that we ship JAX® Mice via air. Because air travel requires loading and unloading of the animals in a variety of environments, we only use carriers that have proven to be reliable partners and who are sensitive to the needs of animal transport. If your order requires air shipment, we will work with you to ensure safe, reliable shipment of your JAX® Mice.

We schedule all shipments to minimize the time the mice are in transit.

For questions about shipping, please contact Customer Service.

Can I order pregnant JAX® Mice?

We distribute pregnant mice of some popular strains of JAX® Mice to most locations. We guarantee pregnancy for mice shipped after the 10th day of pregnancy, following confirmation of pregnancy using palpation. We prefer to ship mice between gestation days 11 and 15.

We also distribute female mice in which we have observed a vaginal plug, which indicates mating, not pregnancy. Because of the uncertainty of whether these mice are pregnant, we do not guarantee their pregnancy.

For more information, for a current list of strains for which we ship pregnant mice, or to schedule a shipment of pregnant mice, please contact Customer Service.

What should I do when my JAX® Mice arrive?

Before your shipment of JAX® Mice arrives, we suggest alerting your receiving department that you are expecting them. This notification can help avoid the problem of live animals sitting on a receiving dock for an extended period of time.

When you receive your shipment of JAX® Mice, we recommend that you do five things as soon as possible.

On the shipping dock:

1. Before you accept delivery of the mice, carefully inspect the shipping container for any visible damage. If you notice anything wrong,
   a. Describe it on the delivery manifest *before* you sign and return it to the driver.
   b. Immediately contact Customer Service at The Jackson Laboratory.
2. Check the shipping label to be sure that the JAX® Mice listed are the strain, genotype and age you ordered. If you have any questions or concerns, immediately contact Customer Service.
3. To ensure the health and welfare of your JAX® Mice, place them—in the sealed shipping container—in a safe place until they are delivered to a mouse room. A well-ventilated area, away from direct sunlight is best. Avoid temperatures below 8 C (46 F) or above 29 C (84 F).

When you are ready to unpack the mice in a mouse room:

4. Before you unpack your JAX® Mice, disinfect the outside of the shipping container by wiping it thoroughly with a disinfectant such as 70% ethanol. Although the mice were packed under extremely strict health regulations at The Jackson Laboratory, the outside of the shipping container may have been exposed to microbes since it left our shipping dock (especially if the mice were shipped via air). Taking the time to clean the container thoroughly before unpacking the mice can help ensure that the health status of your JAX® Mice—and your facility—remains high.
5. As soon as you open the container, check on the mice. They may need food or water.

Note: At The Jackson Laboratory, our mice drink water from bottles. If your facility uses an automatic watering system, be aware that the mice may need help learning how to use it. For strategies, refer to 12.B.3, "What to do if you have an automatic watering system and your newly arrived JAX® Mice won't use it."

What if there is a problem with my JAX® Mice when I receive them?

If you have any problem with your newly arrived JAX® Mice, immediately contact Customer Service. If the mice appear sick, you also may want to contact your staff veterinarian.

Note: Due to animal health issues, mice *cannot* be returned to The Jackson Laboratory. A customer service representative will advise what you should do.

Why do the coat colors of F2 hybrid JAX® Mice sometimes differ from order to order?

At the time this handbook was printed, we offered two strains of F2 hybrid JAX® mice—B6129PF2/J (100903) and B6129SF2/J (101045). These hybrids segregate on coat color, and we generally group mice of the same color when we ship them. This means that if you reorder the same F2 hybrid, you might get mice of a different color. Check the shipping label. If you suspect a problem, contact Customer Service.

Should I genotype my JAX® Mice when they arrive?

We guarantee the genotype of all JAX® Mice that we distribute. It is always a good idea, however, for you to confirm the genotype before undertaking studies.

When are newly arrived JAX® Mice ready for research?

Due to the lingering effects of shipping stress on their physiology, mice will *not* be ready for research for at least one week. During this time, however, mating pairs can be placed together. For details on introducing JAX® Mice into your colony and readying them for research, please refer to 12.B, "Handling newly arrived mice."

Should I try to duplicate the same diet used by The Jackson Laboratory to maintain my JAX® Mice?

The requirements for a specific diet depends on the individual strain or mutation. Sometimes it is important to duplicate the diet we use at The Jackson Laboratory to assure expression of a phenotype of interest. For example, we maintain the diabesity strain NONcNZO10/LtJ (004456) on a 10% fat diet (LabDiet® 5K20) to express the diabetes phenotype. For dietary requirements for any strain of JAX® Mice, please check the strain datasheet (www.jax.org/jaxmice/query).

Should I expect phenotypic differences from published descriptions of JAX® Mice that have been maintained in standard SPF vivaria if my vivarium is at full barrier status?

Certain phenotypes require the exposure to microorganisms that may be considered unacceptable in some vivaria. For example, the development of atherosclerotic plaques in mice may be diminished in vivaria that are full barrier status. For additional discussion, refer to 7.A, "Developing an animal health plan," in chapter 7. For information about specific strains of JAX® Mice, refer to the strain datasheet (www.jax.org/jaxmice/query) or contact Tech Support.

Is there a difference between JAX® Mice from Bar Harbor and Sacramento?

We offer a selection of JAX® Mice—including inbred and genetically-engineered strains and hybrids—from Sacramento based on customer requirements. We adhere to the same exacting standards for research, genetic and health monitoring, animal husbandry, and customer service in California as we do in Maine.

Because our breeding colonies in Sacramento are refreshed monthly using foundation stocks from Bar Harbor, JAX® Mice from Sacramento and Bar Harbor are genetically identical.

What does it mean to "register interest" in a new strain?

Each year, nearly 300 new mouse strains become available from The Jackson Laboratory. Some of these mice are from our own investigators, but many are strains that are donated by investigators from other institutions. The process of readying the strain for distribution includes importation, rederivation, colony building, and quality testing, which can take from 6–12 months. During this period, researchers can "register interest" in the strain, which places them on a waiting list for the mice.

Researchers receive confirmation of the registration and get advance notice (typically about three weeks) of the pending availability of the strain. They then have the option to place an order for the strain. (When the mice are available, these orders are filled on a "first come–first served" basis according to the date on which interest was registered.) Note that registering for a strain in no way obligates a researcher to purchase a strain.

For information on a specific strain of JAX® Mice for which we are registering interest, refer to the strain datasheet at www.jax.org/jaxmice/query. For information on registering for a strain, including a list of strains currently being readied for distribution, visit www.jax.org/jaxmice/interestlist or contact Customer Service.

Do I have to pay licensing fees for JAX® Mice?

At The Jackson Laboratory, one of our goals is to provide researchers with mouse models unencumbered by license restriction. However, some of the mouse strains we distribute were genetically engineered at other institutions and may require licensing for commercial use. Generally, academic and non-profit organizations are exempt from license restrictions of these types, but it is the responsibility of each institution to abide by all licensing and use requirements that might apply.

For any strain of JAX® Mice that requires a licensing agreement or that has use restrictions, we include relevant information in the strain datasheet (www.jax.org/jaxmice/query). The note "Use Restrictions Apply" will be displayed in the Availability area of the datasheet. For details of the use restriction, select the "Terms of Use" tab.

If you have questions about license restrictions, please review the information about conditions of use on our website at www.jax.org/jaxmice/cou. Or contact the Tech Transfer Office at tjlca@jax.org. Please include the stock number with your inquiry.

For more information…

To order technical literature, including a JAX® Mice Catalog, visit www.jax.org/jaxmice/support.

For frequently asked questions, visit www.jax.org/jaxmice/faq.

For any other assistance, please contact Customer Service.

Chapter 18: JAX® Services

One of the missions of The Jackson Laboratory is to enable research throughout the world. A significant way we do this is by providing JAX® Services to the global research community.

JAX® Services include a wide range of technologies. Each service was originally developed as a way for our own researchers at The Jackson Laboratory to share resources—equipment *and* expertise. Today we offer many of these same benefits to researchers throughout the world.

We generally group individual JAX® Services into five basic categories:

- Breeding and colony management
- Cryopreservation and recovery
- Phenotyping and efficacy testing
- Genetic analysis and research services
- Study-ready induced models

JAX® Services are designed to be flexible, either used individually or bundled, and tailored to meet a wide variety of research needs for both large and small organizations.

Where to get information about JAX® Services…

Phone:
1-800-422 6423 (North America)
1-207-288-6294 (International)
8:00 a.m. to 6:00 p.m., EST (or EDT), Monday through Friday

Email:
jaxservices@jax.org

Online:
www.jax.org/jaxservices

JAX® Services catalog:
www.jax.org/jaxmice/literature

This chapter provides just a brief overview of JAX® Services. For more detail, please visit our JAX® Services website (www.jax.org/jaxservices) or request a copy of our JAX® Services Catalog (www.jax.org/jaxmice/literature). To consult with us about how JAX® Services can augment your research program, please contact us at the telephone number or email address shown above.

Breeding and colony management

Aging service

Breeding and housing mice until they reach an age when you require them.

Applicable strains: Your research mice or any strain of JAX® Mice. Note that we also maintain colonies of several strains of JAX® Mice that we routinely have available at specific ages. For a list of these strains, visit www.jax.org/jaxservices/aging.

Breeding services

Breeding and maintenance of your mice at our facility, per your specifications; shipment to you per your schedule. This service also can be used to create F1 or F2 hybrids, to backcross progeny, or to maintain special stocks carrying multiple gene mutations or transgenes.

Dedicated supply services

Shipment of a specific quantity of JAX® Mice according to your schedule. Use when you need one shipment of a large quantity of JAX® Mice or a regular supply of JAX® Mice.

Applicable strains: Any strain of JAX® Mice, but generally used for strains that are in one of our repositories, strains not bred in large quantities, or strains that are difficult to breed.

Rederivation services

Speed rederivation (via IVF) with sperm cryopreservation (for transgenic, knockout, and single gene mutation mice)

Cryopreservation of sperm (at least 16 straws) from at least two males from your colony. On your schedule, use of *in vitro* fertilization (IVF) to create live mice (10 minimum) that are shipped back to you. The service is typically completed within 10–12 weeks from the time we receive your mice. Applicable to transgenic, knockout, and single gene mutation mice on the most commonly used genetic inbred lines, including BALB/cByJ (001026), BALB/cJ (000651), C3H/HeJ (000659), C57BL/6J (000664), DBA/1J (000670), DBA/2J (000671), FVB/NJ (001800), NOD/ShiLtJ (001976), hybrid combinations of these background strains, B6;129 hybrids.

Standard rederivation (for inbred and homozygous mice)

Importation and rederivation of your mice—four to five males and six to ten females from your colony; shipment to you of all the specific pathogen free (SPF) mice (minimum of two pair guaranteed) that we produce.

Applicable strains: Any strain, but the service is particularly appropriate for inbred and homozygous mice.

Speed expansion service

Use of *in vitro* fertilization (fresh or frozen sperm) and implantation of embryos into host females to provide accelerated expansion of a line of mice. Used for rapid expansion of a colony (for example, a small number of unique mutant mice), especially when you need a quantity of mice at the same age, if you have just a few viable males.

Strain rescue service

Importation of mice that have stopped breeding; use of assisted reproductive techniques to rescue the strain.

Sponsored strain distribution

Rederivation and distribution of your research mice. For an overview, see Appendix K, "Donating or Submitting a Strain of Mice to The Jackson Laboratory." For full details, visit www.jax.org/jaxservices/sponsoreddistribution.

Cryopreservation and recovery

Following are summaries of our JAX® Services related to cryopreservation. For general descriptions of cryopreservation techniques, see Appendix J, "Cryopreservation."

Our cryopreservation team can come to you…

We can deploy teams of reproductive specialists to your facility to cryopreserve large numbers of strains. This service is ideally suited for institutions or investigators with large rederivation projects or substantial backlogs of strains that must be cryopreserved. Our teams can routinely cryopreserve 120 strains or more on site in a week.

Cryostorage and recovery services

Cryopreservation of your mouse strain and storage of the frozen material in our secure facilities. Upon your request, either shipment of frozen material or recovery of the frozen material and shipment of liveborn mice.

IVF embryo cryopreservation service

Use of sperm from two male donors for *in vitro* fertilization of ova from superovulated donor females; cryopreservation of embryos and rapid recovery upon request.

Applicable strains: Your males and our females of these strains of JAX® Mice: BALB/cByJ (001026), C57BL/6J (000664), DBA/2J (000671), FVB/NJ (001800), NOD/ShiLtJ (001976).

Custom (homozygous embryo) cryopreservation service

Cryopreservation of inbred, mutant, and genetically modified lines of mice that cannot be cryopreserved under the conditions of our standard cryopreservation services. Requires male and female mice from your colony.

Sperm cryopreservation & recovery service

Cryopreservation of sperm from two male mice from your colony. Upon your request, either shipment of frozen material or recovery of the frozen material and shipment of liveborn mice.

Applicable strains: Any mouse strain.

Sperm cryopreservation is now a viable alternative at The Jackson Laboratory.

Historically, the use of sperm cryopreservation was limited by poor recovery rates. Researchers at The Jackson Laboratory are funded to study ways to improve cryopreservation methods. In the spring of 2006, we developed patent pending techniques that greatly improve the recovery of frozen sperm for strains for which recovery was poor (Ostermeier *et al*. 2008). This advancement enhances the application of sperm cryopreservation as a colony management tool.

Phenotyping and efficacy testing

In Vivo services

Implementation of treatment studies using mice housed and treated at The Jackson Laboratory. Areas of research include obesity and type 2 diabetes, type 1 diabetes, inflammation and autoimmunity, neurodegenerative and neuromuscular disorders, and xenografts.

Applicable strains: Your mice or JAX® Mice.

Compound evaluation services

Maintenance and treatment of your mice at our site. Optional services include evaluation of blood, serum, plasma, urine, and feces; and, necropsies and collection of tissue for histopathology, IHC, and gene expression.

Applicable strains: Any stock of mice or strain of JAX® Mice.

Phenotyping services

Model characterization, drug target validation, and efficacy testing in mouse models of disease; based on a broad selection of noninvasive physiological tests.

Genetic analysis & research services

Gene expression service

Nucleic acid preparation, chip/array hybridization, and detailed statistical analysis, using unique statistical tools developed at The Jackson Laboratory.

Gene mapping service

Mapping of a spontaneous genetic mutation or non-targeted transgenic mutation using SNP markers; based on tissue from a genetic cross between your mice and an appropriate strain of JAX® Mice.

Genome scanning service

Genotyping of your mice using single nucleotide polymorphism (SNP) markers; based on tissue sent to us. This service is especially useful when creating speed congenics, confirming strain identity, monitoring genetic purity.

Speed congenic development service

Creation of a congenic strain of mice using genotyping, reducing development time from 2.5–3 years to about 1.5 years.

QTL mapping service

Advanced identification of quantitative trait loci (QTLs) using selected markers from our validated panel of more than 2,000 SNP markers. Includes genotyping of DNA samples from a cross and the application of specialized statistical methods for QTL discovery.

Study-ready induced models

Aging service

Breeding and housing mice until they reach an age when they are useful as models of late onset disease or normal aging.

Applicable strains: Your research mice or any strain of JAX® Mice. Note that we also maintain colonies of several strains of JAX® Mice that we routinely have available at specific ages. For a list of these strains, visit www.jax.org/jaxservices/aging.

Alopecia areata surgical model

Development of mice surgically induced to develop alopecia areata.

Applicable strains: Strains on the C3H/HeJ (000659) background.

Diet-induced obesity (DIO) service

Treatment of mice to produce diet-induced obesity. Options include our shipping pre-treated mice to you or performing further procedures in our facilities.

Applicable strains: C57BL/6J (000664); other strains upon request.

STZ-induced diabetes service

Treatment of mice with a multiple, low dose, streptozotocin (STZ) protocol that induces pancreatic islet damage and subsequent diabetes.

Applicable strains: A/J (000646), BALB/cJ (000651) and FVB/NJ (001800) males are resistant; C57BL/6J (000664) males are moderately susceptible; males of strains CBA/J (000000) and NOD/ShiLtJ (001976) are highly susceptible.

VCD-induced model of menopause

Development of research models of perimenopause that can be used to study the interaction of menopause with age-related, chronic, cumulative diseases such as heart disease, diabetes, cancer, osteoporosis, and stroke.

Applicable strains: five genetic backgrounds—B6.129S7-*Ldlr*^{tm1Her}/J (002207), a strain with normally high serum cholesterol levels and extremely high levels when fed an atherogenic diet; B6.129P2-*Apoe*^{tm1Unc}/J (002052), another strain with high serum cholesterol levels; C3H/HeJ (000659), an atherosclerosis-resistant strain; C57BL/6J (000664), an atherosclerosis-susceptible strain; and NZBWF1/J (100008), a model of systemic lupus erythematosus—with an option to adapt the model on other backgrounds.

Surgical and histological services

Histology service

Tissue collection, tissue fixation, embedding, sectioning, straining; shipment of blocks and/or slides.

Applicable strains: Your mice or any strain of JAX® Mice.

Surgical and tissue collection services

A wide variety of standard surgical procedures and bio-specimen, organ and tissue collection, with the option of custom surgery.

Other research services

Mouse DNA Resource

Genomic DNA suitable for Southern blotting and amplification by PCR. Most DNA is extracted from spleen or brain and spleen of pedigreed male mice. Some DNA, however, is extracted from other tissues, and some is extracted from female mice. For details, call 1-207-288-6414, fax 1-207-288-6074, or email dnares@jax.org.

Applicable strains: Most strains of JAX® Mice. For a current list, visit www.jax.org/dnares.

References

Ostermeier GC, Wiles MV, Farley JS, Taft RA. 2008. Conserving, distributing and managing genetically modified mouse lines by sperm cryopreservation. *PLoS ONE*. 3(7):e2792. doi:10.1371/journal.pone.0002792.

Chapter 19: The Jackson Laboratory—West

In 1999 The Jackson Laboratory opened a facility in West Sacramento, California. Our objective was to provide a source for mice and related services in demand by academic, non-profit, pharmaceutical and biotechnology researchers in California and western North America. Since then, our staff and offerings have expanded as customer needs have warranted. We now provide a number of JAX® Mice and JAX® Services from The Jackson Laboratory—West.

Overview of The Jackson Laboratory—West facilities

We view The Jackson Laboratory—West as an extension of our Bar Harbor facilities. We adhere to the same exacting standards for research, genetic and health monitoring, animal husbandry, and customer service in California as we do in Maine.

Mouse rooms

Mouse rooms in Sacramento are maintained in a manner consistent with rooms of our highest health status in Bar Harbor.

Breeders are received monthly from Bar Harbor foundation stocks. Genetic monitoring is conducted in Bar Harbor.

Where to get more information…

About The Jackson Laboratory—West:
www.jax.org/jaxmice/jaxwest

About JAX® Services:
Telephone:
1-800-422-6423 (North America)
1-207-288-6294 (International)

Email:
jaxservices@jax.org

Web:
www.jax.org/jaxservices

Veterinary care and husbandry

The Comparative Pathology Laboratory at The University of California at Davis provides health monitoring and veterinary care for all mice in our Sacramento facility. Standards are consistent with those for our Bar Harbor facility. For details on husbandry at The Jackson Laboratory—West, visit www.jax.org/jaxmice/jaxwest/husbandry.

Overview of JAX® Mice and JAX® Services available from The Jackson Laboratory—West

Strains of JAX® Mice

We offer a selection of JAX® Mice—including inbred and genetically-engineered strains and hybrids—from Sacramento based on customer requirements. For a list of strains currently being bred and shipped from The Jackson Laboratory—West, visit www.jax.org/jaxmice/jaxwest/strains.

JAX® Services

Several of our JAX® Services are available at The Jackson Laboratory—West. Our ongoing goal is to provide the exact, customized services that best meet the needs of the research programs of our customers on the West Coast. One of our most popular offerings is JAX® *In Vivo* Services, which includes compound evaluation services and phenotyping services. Areas of research include obesity and type 2 diabetes, type 1 diabetes, inflammation and autoimmunity, neurodegenerative and neuromuscular disorders, and xenografts. We also offer breeding services based in "high health status" barrier rooms or flexible film isolators.

For details on these and other JAX® Services, please contact us as indicated above.

Appendix A: Strain Nomenclature Quick Reference

This appendix includes three tables related to nomenclature:

- Table A.1. Mouse strain nomenclature quick reference: examples, definitions, and comments.
- Table A.2. Abbreviations of inbred mouse strain and substrain names used in hybrid names.
- Table A.3. Nomenclature symbols and abbreviations used within strain names.

For more information about mouse nomenclature, including full guidelines set by the International Committee on Standardized Genetic Nomenclature for Mice, visit the Mouse Nomenclature Home page at www.informatics.jax.org/mgihome/nomen.

Table A.1. Mouse strain nomenclature quick reference: examples, definitions, and comments.

Name	Category and definition	Convention, comments, explanations
129 C57BL C3H DBA	Inbred strain: developed by intercross sibling mating (one lineage) for at least 20 consecutive generations.	Uppercase alphabetic and alphanumeric characters, starting with an alphabetic character. ***Exception***: Names assigned before adoption of current nomenclature rules may start with number or include lowercase alphabetic characters.
C57BL/6J DBA/1LacJ CBA/CaGnLeJ	Inbred substrain: branch of an inbred strain separated by 20 or more generations.	Inbred strain name, forward slash (/), ILAR code(s)*.
CAST/EiJ MOLD/RkJ	Wild-derived inbred strain: developed by importation of wild mice followed by intercross sibling mating for at least 20 generations.	Wild strain name, forward slash (/), ILAR code(s)*.
B6D2F1/J	F1 hybrid: first outcrossed generation of 2 inbred strains.	Abbreviation of strain of mother, abbreviation of strain of father, "F1," forward slash (/), ILAR code(s)*. ***Note***: Female strain abbreviation is always given first. (See Table A.2 for strain abbreviations.)
B6129SF2/J	F2 hybrid: first intercrossed generation of F1 hybrid siblings.	Name of F1 parental hybrid, "F2," forward slash (/), ILAR code(s)*.
C57BL/6J-*A*$^{w\text{-}J}$/J C3H/HeJ-*Mgrn1*^{md}/J	Inbred strain carrying a mutation. ***Note***: For details about the mutation, check with the supplier. For JAX® Mice, refer to the strain datasheet, at www.jax.org/jaxmice/query.	Background strain name, hyphen (-), gene symbol/allele (italicized), forward slash (/), ILAR code(s)*. ***Note***: Nomenclature does not reflect whether the strain is heterozygous (segregating inbred strain) or homozygous for the allele.
B6129PF1/J-*A*$^{w\text{-}J}$/*A*^{w}	F1 hybrid carrying a mutation.	Same as in previous row, with name of F1 hybrid as background strain.
C57BL/6-*Prf1*^{tm1Sdz}/J	Inbred strain carrying a targeted mutation.	Background strain name; hyphen (-); gene symbol/allele (italicized), targeted mutation number (*tm#*) and allele identifier (italicized superscript); forward slash (/); ILAR codes*.

*For Institute for Laboratory Animal Research (ILAR) codes, visit http://dels.nas.edu/ilar_n/ilarhome/search_lc.php.

Table continued on next page.

Table A.1. Mouse strain nomenclature quick reference: examples, definitions, and comments. (continued)

Name	Category and definition	Convention, comments, explanations
B6;129S4-*Nos1*^{tm1Plh}/J	Targeted mutation on a stock derived from 129S4 ES cells and C57BL/6J mice.	Same as in previous row, except abbreviations of the parental genotypes are separated by a semi-colon (;). If this stock is being backcrossed, this nomenclature indicates less than 5 backcross generations.
C57BL/6-Tg(CAG-EGFP)131Osb/LeySopJ	Transgenic strain: includes genetic material from a different strain or species.	Recipient strain, "-Tg," insert designation (in parentheses), line number and originator, forward slash (/), ILAR code(s)*.
STOCK-Tg(B19-RNAi:Il3)241Ckn/J	Transgenic strain on a mixed background.	Same as in previous row, with "STOCK" designation to represent a mixed background of 3 or more founder strains, founders of unknown genetic background, or outbred founders.
B6.129P1-*Lama2*^{dy}/J	Congenic or incipient congenic strain: in which a genetic locus is transferred, via outcrossing, from a donor to a recipient, followed by backcrossing for at least 5 generations. An incipient congenic has been backcrossed for 5–9 generations; a full congenic requires 10 backcross generations. ***Note***: For details about the number of backcross generations for a particular strain, check with the supplier. For JAX® Mice, refer to the strain datasheet at www.jax.org/jaxmice/query.	Abbreviation of recipient strain, period (.), abbreviation of donor strain, hyphen (-), genetic locus and allele of interest (in italics), forward slash (/), ILAR code(s)*.
B6.Cg-*A*^{y}/J	Congenic strain, with locus of interest from a mixed donor stock.	Same as in previous row, with "Cg" replacing name of donor strain to indicate mixed donor stock.
BKS.Cg-*m* +/+ *Lepr*^{db}/J	Congenic strain, with multiple loci from different sources.	Same as in previous row, with multiple loci of interest.
BXD1/TyJ BXD2/TyJ BXD5/TyJ	Recombinant inbred (RI) strain: generated by outcrossing 2 inbred strains (to create F1s), intercrossing of F1s (to create F2s), inbreeding single lineages of siblings into multiple inbred strains.	Abbreviation of strain of mother, "X," abbreviation of strain of father, numeral indicating line number within the panel, forward slash (/), ILAR code(s)*.
NONcNZO5/LtJ NONcNZO10/LtJ	Recombinant congenic (RC) strain: generated by outcrossing 2 inbred strains (to create F1s), backcrossing of F1s to one of the parental strains (recipient strain) for 1 or 2 generations (N2, N3), inbreeding single lineages of N3 siblings for 14 generations (F1–F14) into multiple inbred strains.	Uppercase abbreviation of female founder, "c," uppercase abbreviation of male founder, line number in the panel, forward slash (/), ILAR code(s)*.

*For Institute for Laboratory Animal Research (ILAR) codes, visit http://dels.nas.edu/ilar_n/ilarhome/search_lc.php.

Table continued on next page.

Table A.1. Mouse strain nomenclature quick reference: examples, definitions, and comments. (continued)

Name	Category and definition	Convention, comments, explanations
C57BL/6J-Chr $1^{A/J}$/NaJ C57BL/6J-Chr $X^{A/J}$/NaJ	Chromosome substitution strain (CSS; sometimes called a consomic strain): generated by outcrossing 2 inbred strains (to create F1s), backcrossing of F1s with marker-assisted selection for 9 generations (N1–N9), inbreeding single lineages of N9 siblings until all markers for the target chromosome are donor-type.	Full recipient strain name, hyphen (-), chromosome number, donor strain (superscripted), forward slash (/), ILAR code(s)*.
C57BL/6J-$mt^{PWD/Ph}$/ForeJ	Conplastic strain: type of congenic in which the mitochondrial genome of one strain is transferred to another strain. ***Note***: When a conplastic strain is part of a CSS (consomic) panel, it is also considered a CSS strain.	Full name of recipient strain, "-mt," full name of mitochondrial donor strain (superscripted), forward slash (/), ILAR code(s)*.
CBA/CaH-T(14;15)6Ca/J	Chromosomal aberration strain: includes rearrangements of the normal chromosomal structure.	Full background strain name, hyphen (-), abbreviation of aberration, affected chromosome(s) (in parentheses), series number of the aberration, ILAR code(s)* of discoverer or developer, forward slash (/), ILAR code(s)*. ***Note***: For translation of commonly used aberration abbreviations, see Table A.3 in this appendix. For translation of all abbreviations, visit www.informatics.jax.org/mgihome/nomen/anomalies.shtml.
Tac:ICR	Outbred stock: a genetically undefined population.	ILAR code* for the institution holding the stock, colon (:), the common strain root.

*For Institute for Laboratory Animal Research (ILAR) codes, visit http://dels.nas.edu/ilar_n/ilarhome/search_lc.php.

Table A.2. Abbreviations of inbred mouse strain and substrain names used in hybrid names.

Abbreviations and strains	
129P	129P substrains
129P1	129P1/ReJ (001137)
129P2	129P2/OlaHsd
129P3	129P3/J (000690)
129S	129S substrains
129S1	129S1/Sv-*Oca2*$^{+}$*Tyr*$^{+}$*Kitl*$^{Sl\text{-}J}$/J (000090)
	129S1/SvImJ (002448)
129S2	129S2/SvPas
129S4	129S4/SvJae
129S5	129S5/SvEvBrd
129S6	129S6/SvEvTac
129S7	129S7/SvEvBrd-*Hprt*$^{b\text{-}m2}$
129S8	129S8/SvEv-*Gpi1*^{c} *Hprt*$^{b\text{-}m2}$/J (002027)

Abbreviations and strains	
129X1	129X1/SvJ (000691)
A	A strains
AHe	A/HeJ (000645)
AK	AKR strains
B	C57BL
B6	C57BL/6 strains
B6Ei	C57BL/6JEi (000924)
B10	C57BL/10 strains
BR	C57BR/cdJ (000667)
C	BALB/c strains
C3	C3H strains
C3Fe	C3HeB/FeJ (000658)
C3Sn	C3H/HeSnJ (000661)
CB	CBA strains
CBACa	CBA/CaGnLeJ (001143)

Abbreviations and strains	
CBy	BALB/cByJ (001026)
D1	DBA/1 strains
D2	DBA/2 strains
HR	HRS/J (000673)
L	C57L/J (000668)
NZB	NZB strains
NZW	NZW strains
R3	RIIIS/J (000683)
SJ or J	SJL/J (000686)
SM	SM/J (000687)
SW	SWR strains
W	NZW strains

Numbers in parentheses are JAX® Mice stock numbers.

Table A.3. Nomenclature symbols and abbreviations used within strain names.

Symbol or abbreviation	Use, definition
STOCK	Indicates a mixed background of 3 or more founder strains, founders of unknown genetic background, or outbred founders. (Usage is unique to The Jackson Laboratory.)
/	Separates strain name from information such as genetic data, ILAR code(s)* for originator of the strain and institution that maintains the strain.
F#	Filial generations (sister–brother matings) ***Example***: F120. The inbred strain has been sibling mated for 120 generations. When followed by a question mark (F?), the number of filial generations is unknown. "?" is most often used when mice are imported into a repository and inbreeding history is unknown or unsubstantiated. ***Example***: F?+19. Unknown number of filial generations, followed by sibling mating for 19 generations.
N#	Backcross generations. ***Example***: N6. The stock has been backcrossed to the same recipient strain for 6 generations.
NE#	N equivalents. Used when creating a congenic with complex mating systems, to indicate equivalence to "normal" backcrossed generations. ***Example***: NE10. The mating system has produced a level of homozygosity equivalent to 10 backcross generations.
N#F#	Number of backcross generations (N) and number of filial generations (F). ***Example***: N8F20. The strain was backcrossed for 8 generations, and then intercrossed by sibling mating for twenty generations.
p	For cryopreserved strains, the generation when the material was frozen. ***Example***: F26p27. The strain was cryopreserved after 26 filial generations; since recovery, the strain has been mated for 27 additional filial generations.
G#	Generations that cannot be defined using "F" or "N" notation. For example, with advanced intercrossed lines, typically the first 2 generations are produced by sibling matings to produce an F2 population; the next generation, produced by a non-sibling intercross, is designated as the G3 generation. Subsequent generations of non-sibling intercrosses are designated as G4, etc.
. (period)	In congenic strain names, designates at least 5 generations of backcrossing. ***Note***: Congenic nomenclature is assigned after backcross generation 5 (N5). Congenics from N5 to N9 are called incipient congenics. A full congenic requires a minimum of 10 backcross generations.
; (semi-colon)	In mixed stocks, separates names of recipient and donor strains or, for non-donor/recipient situations, separates strain names of female and male founders. Indicates less than 5 filial generations or unknown number of filial generations.
: (colon)	In names of outbred stocks, separates the ILAR code* for the name of the institution holding the stock from the name of the common strain root. In names of advanced intercross lines, separates the ILAR code* of the developer of the line from abbreviations of the strains of the founders and generations of breeding.
c	In recombinant congenic strains, separates abbreviations of parental strain names.

*For Institute for Laboratory Animal Research (ILAR) codes, visit http://dels.nas.edu/ilar_n/ilarhome/search_lc.ph

Table continued on next page.

Table A.3. Nomenclature symbols and abbreviations used within strain names. (continued)

Symbol or abbreviation	Use, definition
Cg	In names of congenic strains, indicates that a single locus from an undefined (mixed) donor has been transferred or that multiple loci from different sources have been transferred.
Chr	In names of chromosome substitution (consomic) strains, precedes the chromosome number and the name of the donating strain.
mt	In conplastic strain names, precedes the strain name of the mitochondrial donor.
Tg	Indicates a transgenic strain; precedes the insert designation (in parentheses).
tm	Indicates a targeted mutation.
X	In recombinant inbred (RI) strain names, separates abbreviations of parental strain names.
In Is Rb T Ts Y	In chromosomal aberration strains, abbreviations for the aberrations. In: inversion T: translocation Is: insertion Ts: trisomy Rb: Robertsonian Y: Chr Y abnormality (For additional abbreviations and information, visit www.jax.org/jaxmice/type/chromosomal_abberati.)

Appendix B: 129 Strains—Nomenclature and Related ES Cell Lines

This appendix provides information about the 129 strain family: nomenclature and the embryonic cell lines.

129 strain nomenclature

Extensive outcrossing, residual heterozygosity, and documented contamination in 129 substrains resulted in a complex history and confusing genetic relationships (Simpson *et al*., 1997; Threadgill *et al*., 1997). Because of the extensive use of 129 substrains in targeted mutagenesis, it became important to establish a consistent nomenclature. In 1999, Festing *et al*. introduced new nomenclature for the 129 strains, which was approved by The International Committee on Standardized Genetic Nomenclature. Tables B.1 and B.2 provide information about the new nomenclature. For full nomenclature details, visit www.informatics.jax.org/mgihome/nomen/strain_129.shtml.

Table B.1. New parental categories for 129 strains.

This strain designation…	…represents strains derived from…
P	the original parental strain
S	a congenic strain made by outcrossing to introduce the steel locus
T	a congenic strain that originally carried the teratoma mutation
X	a strain where genetic contamination is documented*

*The 129X lines from The Jackson Laboratory have been fully inbred since the contamination event that occurred early in the history of the line.

Table B.2. Revised nomenclature for 129 substrains.

Revised nomenclature		Old nomenclature	Phenotype and genotype
Abbreviation	**Full designation (JAX® Mice stock number)**		
129P1-*Lama2*^{dy}	129P1/ReJ-*Lama2*^{dy}/J (000641)	129/ReJ-*Lama2*^{dy}	White-bellied, pink-eyed chinchilla, dystrophic: *A*^{w}/*A*^{w} *Oca2*^{p} *Tyr*$^{c\text{-}ch}$/*Oca2*^{p} *Tyr*$^{c\text{-}ch}$ *Lama2*^{dy}/*Lama2*^{dy} White-bellied, pink-eyed chinchilla, (non-dystrophic; unaffected): *Lama2*^{dy}/+ or +/+ or +/?
129P1	129P1/ReJ (001137)	129/ReJ	White-bellied, pink-eyed chinchilla *A*^{w}/*A*^{w} *Oca2*^{p} *Tyr*$^{c\text{-}ch}$/*Oca2*^{p} *Tyr*$^{c\text{-}ch}$
129P2	129P2/OlaHsd	129/OlaHsd	
129P3	129P3/J (000690)	129/J	White-bellied, pink-eyed, light chinchilla *A*^{w}/*A*^{w} *Oca2*^{p} *Tyr*$^{c\text{-}ch}$ *Oca2*^{p} *Tyr*^{c} Albino *A*^{w}/*A*^{w} *Oca2*^{p} *Tyr*^{c}/*Oca2*^{p} *Tyr*^{c}
129X1	129X1/SvJ (000691)	129/SvJ	
129S1	129S1/Sv-*Oca2*$^{+}$ *Tyr*$^{+}$ *Kitl*$^{Sl\text{-}J}$/J (000090)	129/SV-*p*$^{+}$ *Tyr*$^{+}$*Kitl*$^{Sl\text{-}J}$/+	White-bellied agouti *A*^{w}/*A*^{w}
	129S1/SvImJ (002448)	129/SV-*p*$^{+}$ *Tyr*$^{+}$*Kitl*$^{+}$/J	
129S2	129S2/SvPas	129/SvPas	
129S4	129S4/SvJae	129/SvJae	
129S5	129S5/SvEvBrd	129/SvEvBrd	
129S6	129S6/SvEvTac	129/SvEvTac	
129S7	129S7/SvEvBrd-*Hprt*$^{b\text{-}m2}$	129/SvEvBrd-*Hprt*$^{b\text{-}m2}$	
129S8	129S8/SvEv-*Gpi1*^{c} *Hprt*$^{b\text{-}m2}$/J (002027)	129/SvEv-*Gpi1*^{c} *Hprt*$^{b\text{-}m2}$@J	
129T1	129T1/Sv-*Oca2*$^{+}$ *Tyr*$^{c\text{-}ch}$ *Dnd1*^{Ter}/*J* (000091)	129/ Sv-*p*$^{+}$ *Tyr*$^{c\text{-}ch}$ *Ter*/+@Na	White-bellied, chinchilla *A*^{w}/*A*^{w} *Tyr*$^{c\text{-}ch}$/*Tyr*$^{c\text{-}ch}$
129T2	129T2/SvEms (002064)	129/SvEms-*Ter*$^{+}$?	
	129T2/SvEmsJ (002065)	129/SvEms-*Ter*$^{+}$?/J	

Table source: www.informatics.jax.org/mgihome/nomen/strain_129.shtml

ES cell lines and related 129 strains

To minimize background genetic variation when creating recombinant mice using embryonic stem (ES) cells, it is important to match the ES cells and the specific 129 substrain used as a recipient. Table B.3 lists the ES cell lines and their 129 strains.

Table B.3. ES cell lines and their 129 strains.

ES cell line	129 strains and (JAX® Mice stock number)	Coat color
AB1 (+*Hprt1-bm2*)	129S7/SvEvBrd-*Hprt1*$^{b\text{-}m2}$	White-bellied agouti
AB2.1 (+*Hprt1-bm2*)	129S7/SvEvBrd-*Hprt1*$^{b\text{-}m2}$	White-bellied agouti
AK7	129S4/SvJaeSor	White-bellied agouti
CJ7 (+*Kitl-SlJ*)	129S1/Sv-*Oca2*$^{+}$ *Tyr*$^{+}$ *Kitl*$^{Sl\text{-}J}$/J* (000090)	White-bellied agouti
CP1	129S6/SvEv	White-bellied agouti
D3	129S2/SvPas	White-bellied agouti
E14TG2a	129 P2/OlaHsd	Pink-eyed chinchilla
EK.CCE	129S6/SvEv	White-bellied agouti
HM-1 (*Hprt1*$^{b\text{-}m1}$)	129 P2/OlaHsd	Pink-eyed chinchilla
J1	129S4/SvJae	White-bellied agouti
mEMS32	129P3/JEms	Pink-eyed chinchilla
PJ1-5	129X1/SvJ (000691)	Light chinchilla or albino
R1 (+*Kitl-SlJ*)	129X1/SvJ x 129S1/Sv-*Oca2*$^{+}$ *Tyr*$^{+}$ *Kitl*$^{Sl\text{-}J}$/J* (000691 x 000090)	White-bellied agouti
RW-4	129X1/SvJ (000691)	Light chinchilla or albino
TC1	129S6/SvEvTac	White-bellied agouti
W9.5 (+*Kitl-SlJ*)	129S1/Sv-*Oca2*$^{+}$ *Tyr*$^{+}$ *Kitl*$^{Sl\text{-}J}$/J* (000090)	White-bellied agouti

*Origin of 129S1/SvImJ (002448). (Adapted from Simpson *et al*., 1997.)

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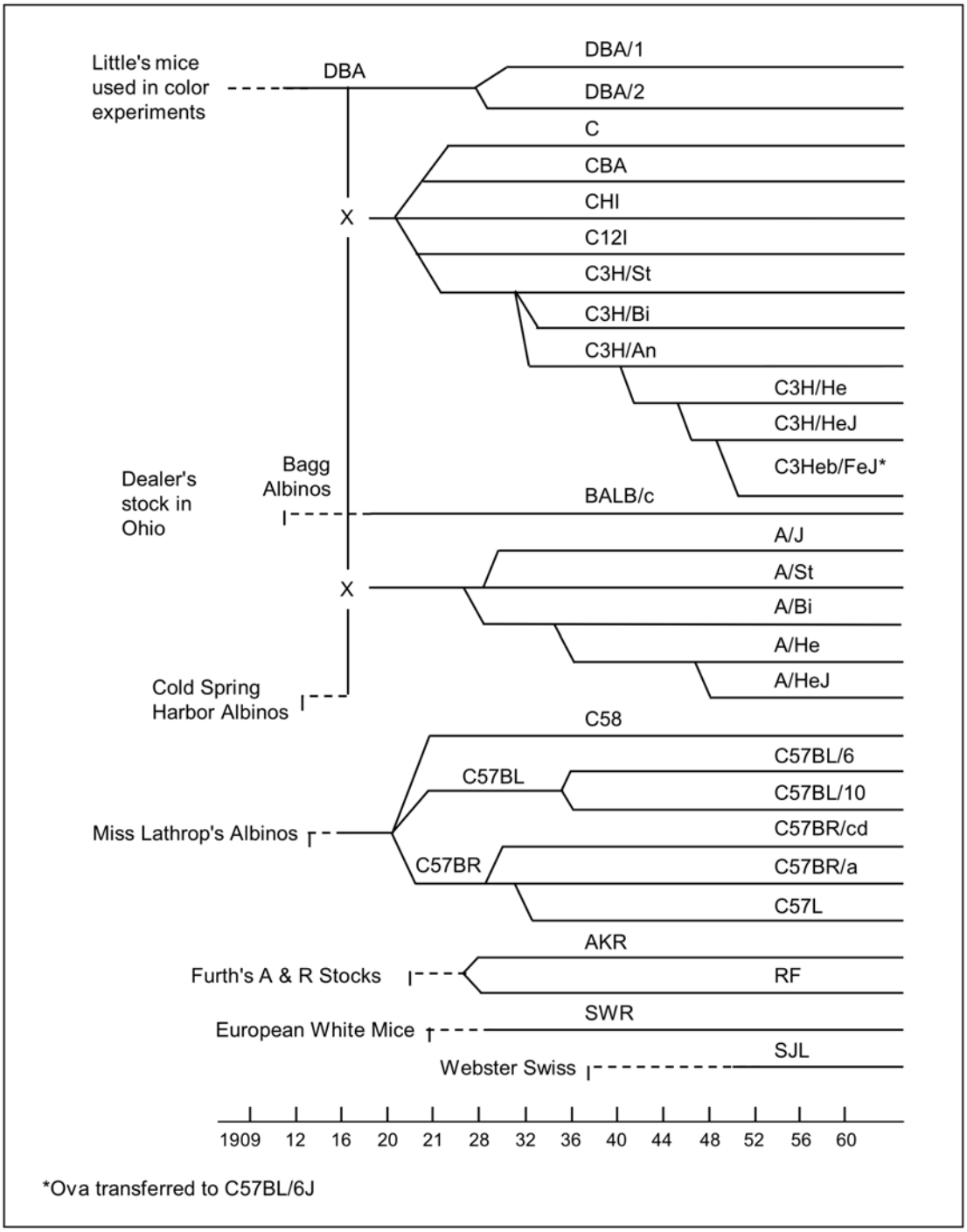
Appendix C: Origins and Relationships among Common Strains and Substrains of Laboratory Mice

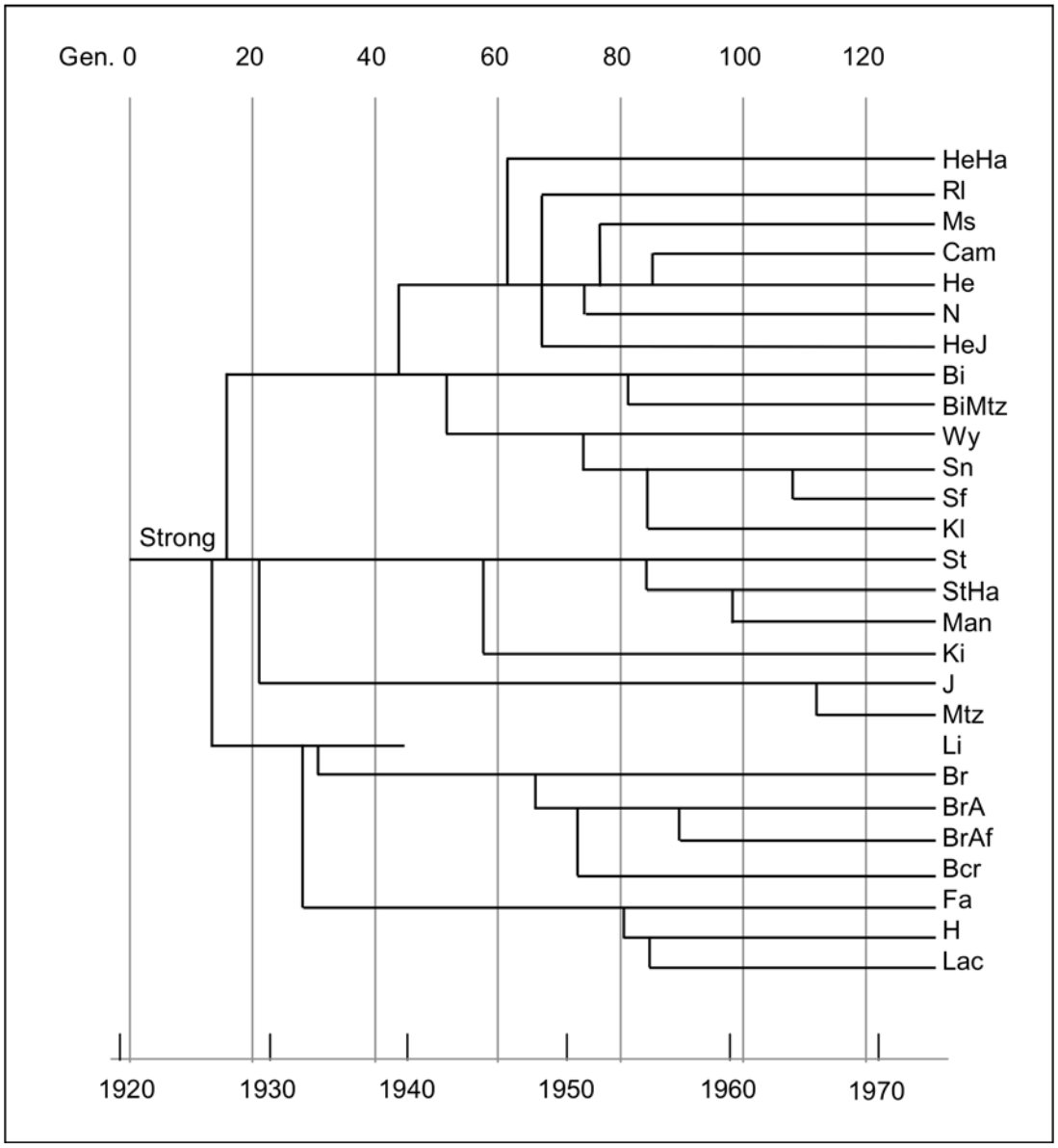
The relationships of the major inbred strains of laboratory mice are shown in Figure C.1. The derivations of many substrains of six of the major strains are shown in Figures C.2–C.7. These figures show the progress of inbreeding in years and estimates of the numbers of generations. For a comprehensive geneology chart of inbred strains, visit www.informatics.jax.org/mgihome/genealogy.

Figure C.1. The origins and relationships of some of the inbred strains of mice.



(Staats, 1980)

Figure C.2. Substrains of the A strain.



(Bailey, 1978)

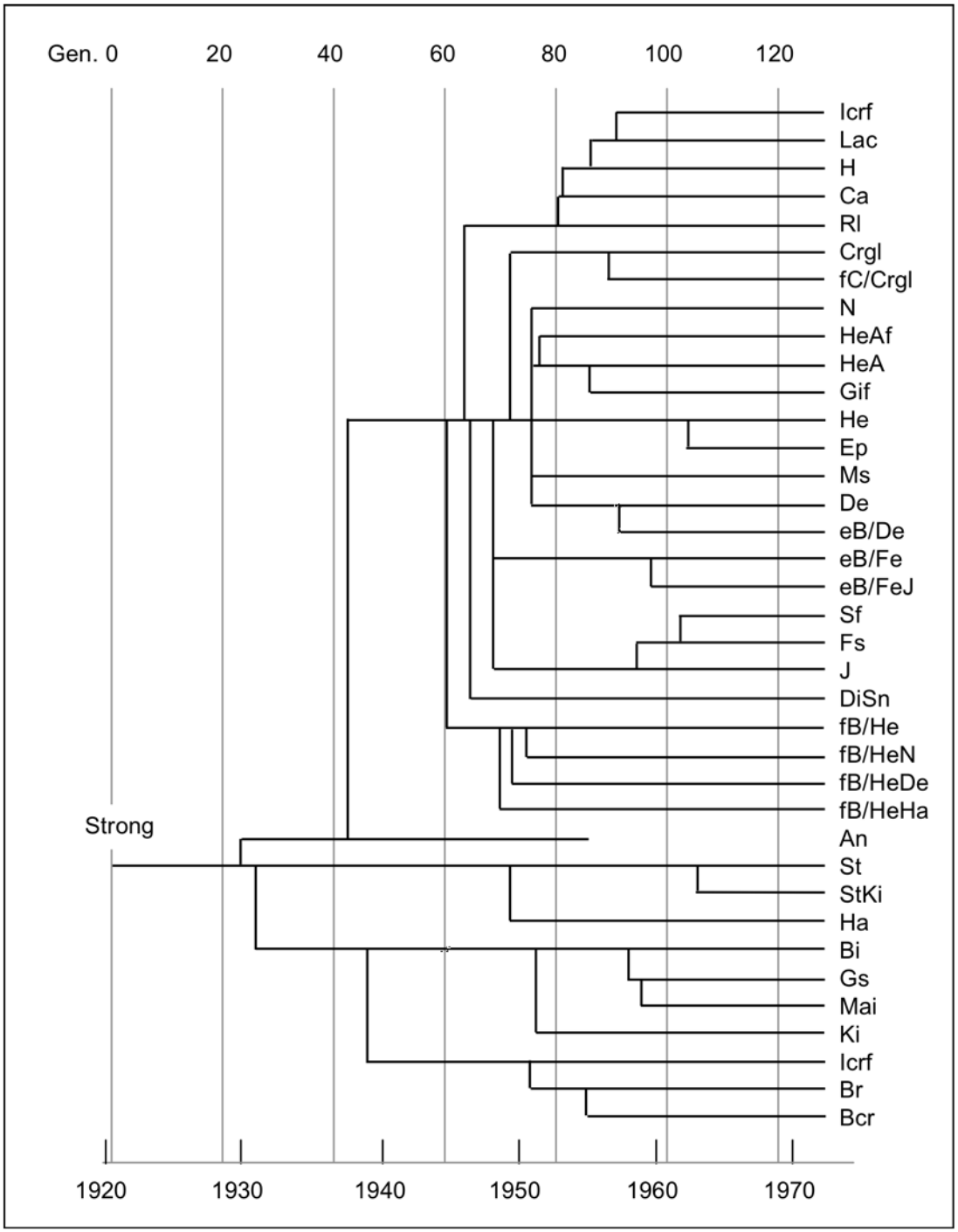
Figure C.3. Substrains of the BALB/c strain.

(Bailey, 1978)

Figure C.4. Substrains of the CBA strain.

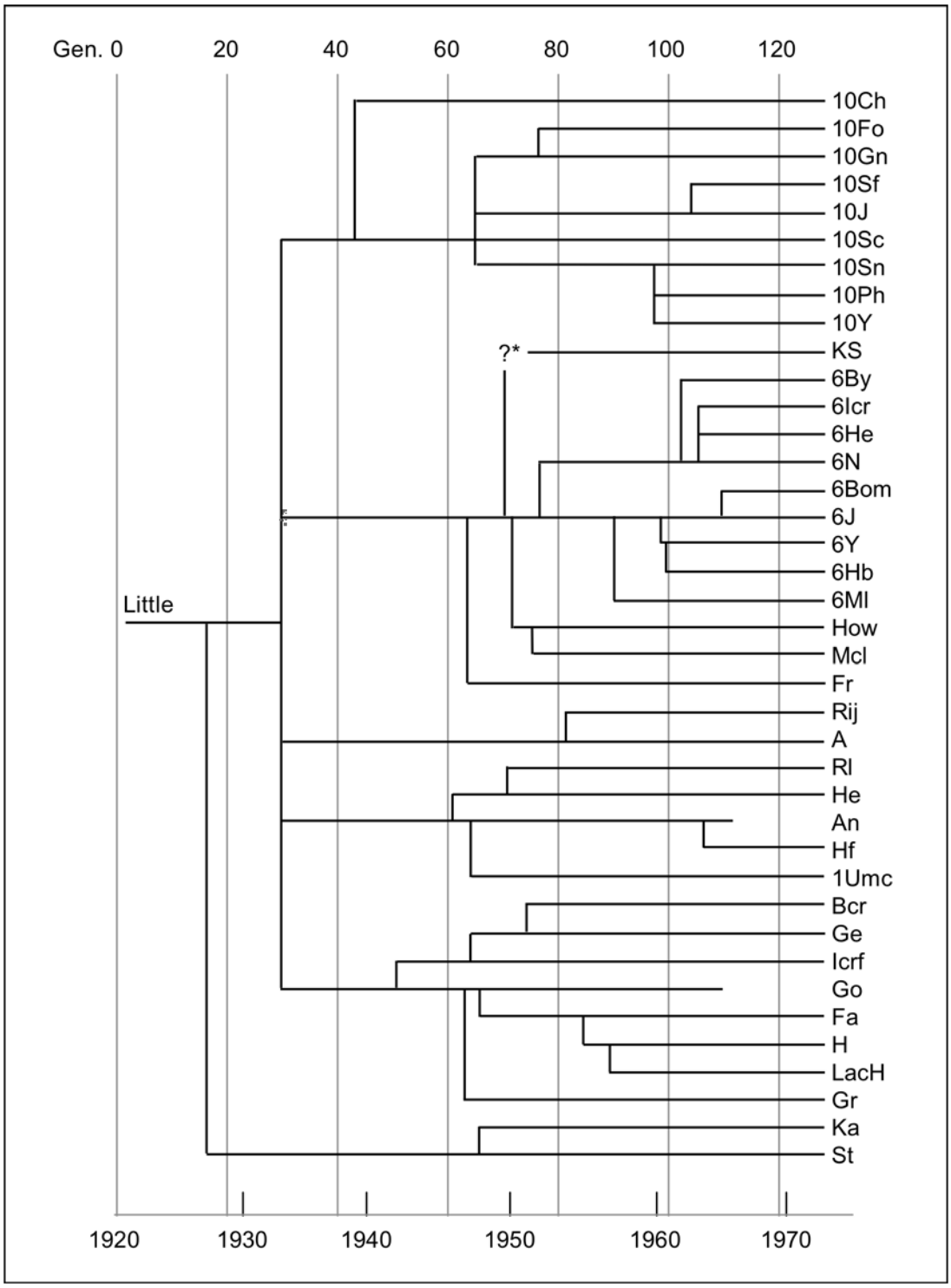
(Bailey, 1978)

Figure C.5. Substrains of the C3H strain.



(Bailey, 1978)

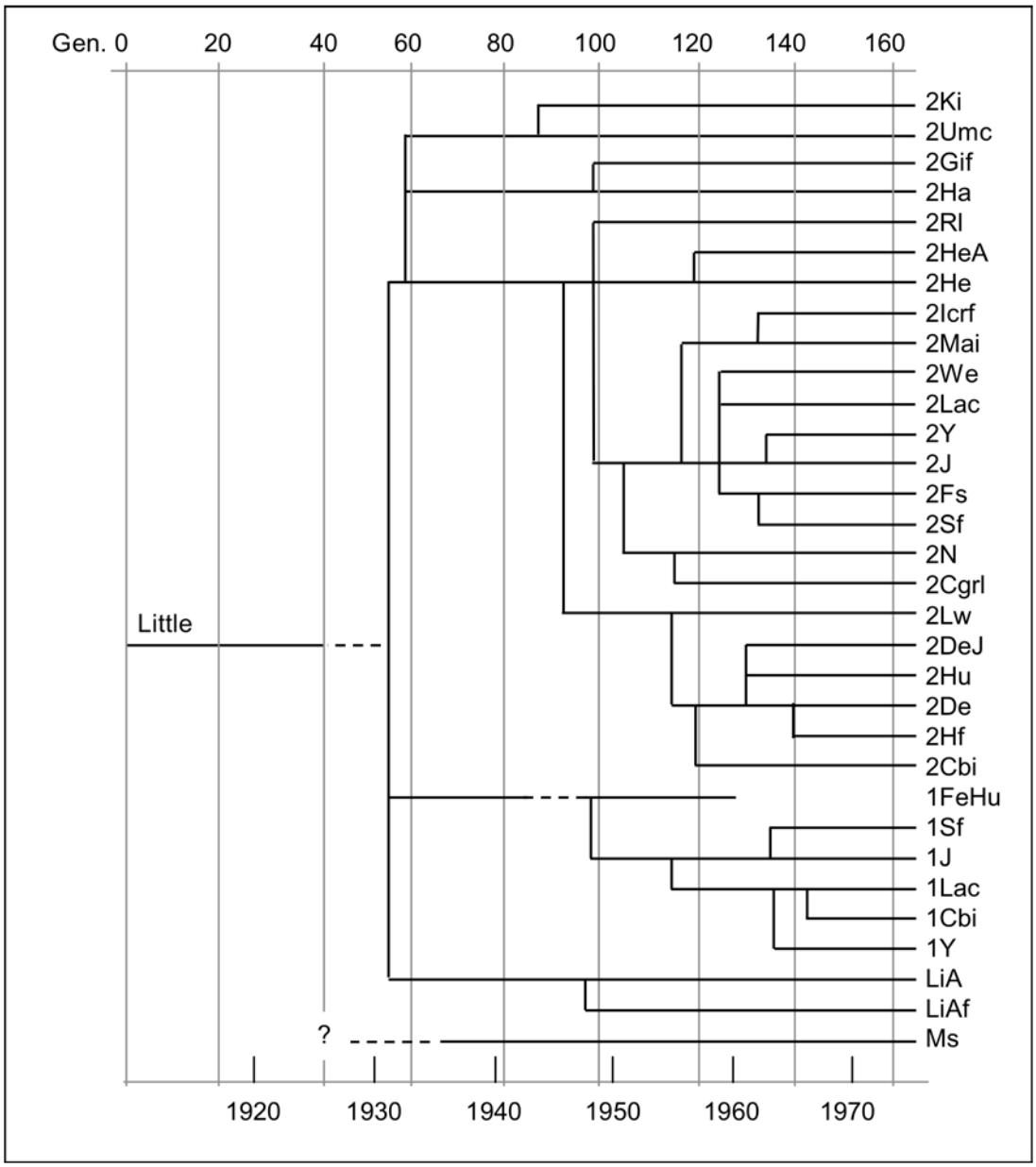
Figure C.6. Substrains of the C57BL strain.



(Bailey, 1978)
*The KS substrain is primarily C57BL/6 with contaminations from DBA/2 and BTBR strains (Petkov *et al*., 2004). The KS substrain was sent to The Jackson Laboratory to help restore the C57BL/6 stock following the fire in 1947.

For a poster that illustrates the history and development of today's C57BL/6 substrains, visit www.jax.org/jaxmice/jaxnotes/512/512s.html.

Figure C.7. Substrains of the DBA strain.



(Bailey, 1978)

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Appendix D: Commonly-Used Inbred Strains and Substrains of JAX® Mice—Genes and Research Applications

Carol Linder, Barbara Witham

Over 400 different inbred strains are currently used in biomedical research (Festing, 1998). Table D.1 summarizes the most commonly used parental strains, JAX® Mice substrains, mutant alleles of interest, and provides a guide to their application. For more information about research applications of JAX® Mice strains (including references), see the Find JAX® Mice website at www.jax.org/jaxmice/findmice.

Table D.1. Genetic characteristics and research applications of commonly-used inbred strains available from The Jackson Laboratory.

Parental strain	Substrains, JAX® Mice (stock number)	Genes of interest	Research applications
129	129P1/ReJ (001137) 129P3/J (000690) 129P3/JEmsJ (002357) 129P4/RrRkJ (001198) 129S1/SvImJ (002448) 129T2/SvEmsJ (002065) 129X1/SvJ (000691)	*Tyr*$^{c\text{-}ch}$ or *Tyr*^{c} (not 129S1/SvImJ) *Oca2*^{p}/*Oca2*^{p} (not 129S1/SvImJ) *A*^{w}/*A*^{w} *Cdh23*^{ahl} *Disc1*^{del} *Poli*^{d}	• Cancer (low background incidence of testicular teratomas) • General purpose • Neurodevelopmental defects (callosal agenesis, incomplete penetrance); neuronal and cognition defects, *Disc1*^{del}) • Polymerase iota deficient (*Poli*^{d}) • Sensorineural (early hearing loss, *Cdh23*^{ahl}) • Targeted mutagenesis (ES cell lines)
A	A/J (000646) A/HeJ (000645) A/WySnJ (000647)	*Tyr*^{c} *Tyrp1*^{b} *a/a* *Hc*^{0} *Cdh23*^{ahl} *Dysf*^{prmd} (A/J)	• Cancer (high susceptibility to carcinogen-induced lung adenomas; useful for carcinogen testing; late onset mammary tumors) • Cardiovascular (relatively resistant to diet-induced atherosclerosis) • Developmental biology (low background incidence of cleft palate; high susceptibility to cortisone-induced cleft palate) • General purpose • Immunodeficiency (specific complement deficiency, *Hc*^{0}) • Immunology (hybridoma production) • Progressive muscular dystrophy (*Dysf*^{prmd}) • Susceptibility to hepatomas induced by infection with *Helicobacter hepaticus* • Sensorineural (early hearing loss, *Cdh23*^{ahl})
AKR	AKR/J (000648) AKR/CumJ (002720)	*Tyr*^{c} *Hc*^{0} *Soat1*^{ald} *Thy1*^{a}	• Cancer (high leukemia strain due to retroviral integration) • Cardiovascular (relatively resistant to diet-induced atherosclerosis) • Developmental biology (hematopoietic defects) • Diabetes and obesity (susceptible to diet-induced obesity) • Endocrine deficiency (adrenal cortex defects, hypothalamus/pituitary defects, *Soat1*^{ald}) • Note: relatively short lifespan

Table continued on next page.

Table D.1. Genetic characteristics and research applications of commonly-used inbred strains available from The Jackson Laboratory. (continued)

Parental strain	Substrains, JAX® Mice (stock number)	Genes of interest	Research applications
BALB/c	BALB/cJ (000651) BALB/cByJ (001026) BALB/cGaJ (001905) BALB/cGrRkJ (000921) BALB/cWtEiJ (001311)	*Tyr*^{c} *Tyrp1*^{b} *A/A* *Mdmg1*$^{BALB/cBy}$ (BALB/cByJ) *Hld* (BALB/cJ, BALB/cByJ) *Cdh23*^{ahl} (BALB/cByJ)	• General purpose • Cancer research (late onset mammary gland tumors) • Cardiovascular (relatively resistant to diet-induced atherosclerosis) • Developmental biology research (hermaphroditism: sex chromosome chimerism [BALB/cWtEiJ]) • Immunology research (production of monoclonal antibodies and hybridomas) • Autoimmunity (experimental allergic encephalomyelitis (EAE), [BALB/cByJ and BALB/cWtEiJ]) • Neurobiology (callosal agenesis, incomplete penetrance; high anxiety [BALB/cJ]; hippocampal lamination defect, *Hld*) • Sensorineural (early hearing loss, *Cdh23*^{ahl})
CBA	CBA/CaH-T(14 ;15)6Ca/J (000655) CBA/CaHN-*Btk*^{xid}/J (001011) CBA/CaJ (000654) CBA/J (000656)	*Pde6b*^{rd1} (CBA/J) *A/A* *Btk*^{xid} (CBA/CaHN-*Btk*^{xid}/J)	• Cancer (mammary tumor development, hepatomas, lymphomas) • Cardiovascular (relatively resistant to diet-induced atherosclerosis [CBA/J]) • Diabetes and obesity [CBA/CaJ] • Immunology (human X-linked immunodeficiency and B cell defects [CBA/CaHN-*Btk*^{xid}/J]; experimental autoimmune thyroiditis [CBA/J]) • General purpose • Metabolism (exocrine pancreatic enzyme deficiency [CBA/J]) • Research tools (T6 translocation is a marker to distinguish host from donor cells [CBA/CaH-T(14;15)6/CaJ])
C3H	C3H/HeJ (000659) C3H/HeJSxJ (001824) C3H/HeOuJ (000635) C3H/HeSnJ (000661) C3HeB/FeJ (000658) C3HfB/BiJ (001908)	*Pde6b*^{rd1} *A/A* *MMTV*- *Tlr4*$^{Lps-d}$ (C3H/HeJ)	• Cancer (mammary tumor development enhanced by presence of exogenous mouse mammary tumor virus, MMTV. Note: Either embryo transfer or neonatal fostering eliminates MMTV; hepatomas) • Cardiovascular (susceptible to dystrophic cardiac calcinosis) • Dermatology (surgical model for alopecia areata alopecia [C3H/HeJ]) • General purpose • Immunodeficiency and inflammation (*Tlr4*$^{Lps-d}$ [C3H/HeJ]) • Sensorineural (retinal degeneration, *Pde6b*^{rd1}) Note: Because of an inversion involving 20% of Chr 6 in C3H/HeJ and probably in C3H/HeJSxJ, they are not useful for mapping loci in this region on Chr 6 (Akeson *et al*., 2006).

Table continued on next page.

Table D.1. Genetic characteristics and research applications of commonly-used inbred strains available from The Jackson Laboratory. (continued)

Parental strain	Substrains, JAX® Mice (stock number)	Genes of interest	Research applications
C57BL	C57BL/6J (000664) C57BL/6ByJ (001139) C57BL/6JEiJ (000924) C57BL/10J (000665) C57BL/10ScSnJ (000476) C57BL/10SxJ (001822) C57BL/10SnJ (000666) C57BL/10WtRkJ (001197) C57BL/10ScNJ (003752) C57BLKS/J (000662)	*a/a* *Tlr4*$^{Lps\text{-}d}$ (C57BL/10ScNJ) *Cdh23*^{ahl} (C57BLKS/J and C57BL/6J) *Gluchos1*$^{C57BL/6J}$(C57BL/6J) *Gluchos2*$^{C57BL/6J}$(C57BL/6J) *Gluchos3*$^{C57BL/6J}$(C57BL/6J) *Nnt*$^{C57BL/6J}$(C57BL/6J)	• Background for histocompatibility congenics (C57BL/10ScSnJ, C57BL/10SxJ, C57BL/10SnJ, and C57BL/10WtRkJ) • Cardiovascular (susceptible to diet-induced atherosclerosis; resistant to dystrophic cardiac calcinosis [C57BL/6]) • Developmental (eye defects, hematopoietic defects, skeletal defects [C57BL/6J]) • Diabetes and obesity (susceptible to diet-induced obesity, hyperglycemia, hyperinsulemia, and insulin resistance, multiple QTLs for glucose homeostasis, *Gluchos* and *Nnt* [C57BL/6J]) • General purpose • Immunology and inflammation (*Tlr4*$^{Lps\text{-}d}$ [C57BL/10ScNJ]) • Neurobiology (preference for alcohol and morphine [C57BL/6J]) • Research tools (background for transgenes, spontaneous, and targeted mutations [C57BL/6]) • Sensorineural (early hearing loss. *Cdh23*^{ahl})
DBA	DBA/1J (000670) DBA/1LacJ (001140) DBA/2J (000671) DBA/2BiJ (001907) DBA/2DeJ (000052) DBA/2HaSmnJ (000973)	*Cdh23*^{ahl} *Hc*^{0} (DBA/2) *Gpnmb* (DBA/2J) *a/a* *Tyrp1*^{b} *Tyrp1*^{isa} (DBA/2J) *Myo5a*^{d}	• Autoimmunity (collagen-induced arthritis [not DBA/2]) • Cardiovascular (low susceptibility to diet-induced atherosclerosis; susceptible to dystrophic cardiac calcinosis [DBA/2]) • DBA/2J often contrasted with C57BL/6J because of many polymorphic differences between the two strains • General purpose (DBA/1J, DBA/2J) • Immunodeficiency (specific complement deficiency, *Hc*^{0}) • Neurobiology (epilepsy, audiogenic seizures [DBA/2J]; extreme intolerance to alcohol and morphine [DBA/2J]) • Sensorineural (early hearing loss, *Cdh23*^{ahl}; glaucoma, *Gpnmb*^{R150X}, *Tyrp1*^{isa})
FVB	FVB/NJ (001800)	*Hc*^{0} *Pde6b*^{rd1} *Tyr*^{c} *Fv1*^{b}	• General purpose • Immunodeficiency (specific complement deficiency, *Hc*^{0}) • Transgenic production (large male pronuclei, good breeder) • Sensorineural (retinal degeneration, *Pde6b*^{rd1})

Table continued on next page.

Table D.1. Genetic characteristics and research applications of commonly-used inbred strains available from The Jackson Laboratory. (continued)

Parental strain	Substrains, JAX® Mice (stock number)	Genes of interest	Research applications
NOD	NOD/ShiLtJ (001976)	*Hc*^{0} *Tyr*^{c} *Cdh23*^{ahl}	• Autoimmunity (unique MHC haplotype [*H2*^{g7} = *K*^{d}, *Aa*^{d}, *Ab*^{g7}, *E*^{null}, *D*^{b}]) • Developmental (hematopoietic defects) • Immunodeficiency (defective APC immunoregulatory functions; defects in the regulation of the T lymphocyte repertoire; defective NK cell function; defective cytokine production from macrophages; specific complement deficiency, *Hc*^{0}) • Sensorineural (early hearing loss, *Cdh23*^{ahl}) • Type 1 diabetes • Wound healing (delayed and impaired)
NZB	NZB/BlNJ (000684)	*Hc*^{0} *a/a*	• Autoimmunity • Cardiovascular (relatively resistant to diet-induced atherosclerosis) • F1 hybrid (NZBWF1/J [100008]) between NZB/BlNJ and NZW/LacJ is widely used as a model for autoimmune disease resembling human systemic lupus erythematosus • Immunodeficiency (specific complement deficiency, *Hc*^{0})
SJL	SJL/J (000686)	*Dysf*^{im} *Pde6b*^{rd1} *Tyr*^{c}	• Autoimmunity (experimental allergic encephalomyelitis [EAE]) • Cancer (reticulum cell sarcomas, Hodgkin's disease) • Cardiovascular (relatively resistant to diet-induced atherosclerosis) • Muscular dystrophy, limb-girdle (type 2B) • Sensorineural (retinal degeneration, *Pde6b*^{rd1})
SWR	SWR/J (000689)	*Hc*^{0} *Pde6b*^{rd1} *Tyr*^{c}	• Autoimmunity (EAE) • Cancer (high incidence of lung and mammary gland tumors in aging mice) • General purpose • Immunodeficiency (specific complement deficiency, *Hc*^{0}) • Metabolic disease (nephrogenic diabetes insipidus with increasing age) • Sensorineural (retinal degeneration, *Pde6b*^{rd1})

(Modified from Linder, 2006.)

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For a complete list of references and more details, see the individual JAX® Mice datasheets, accessible at www.jax.org/jaxmice/query.

Appendix E: Coat Color Alleles for Popular Strains of JAX® Mice

This appendix includes several tables for reference purposes:

- Table E.1. Coat color genes and alleles: current and old symbols, current names.
- Table E.2. Common coat color genes and alleles for laboratory mice—related biological systems and relative dominance.
- Table E.3. Coat color phenotypes and genotypes for common strains of JAX® Mice.

For information about the genetics of mouse coat color, see 2.G, "Coat color genetics." For information about all the coat color genes and alleles, visit www.informatics.jax.org.

Table E.1. Coat color genes and alleles: current and old symbols, current names.

Current symbol		Old symbol	Name
Gene:	*a*	Unchanged	Nonagouti
Allele:	*a*	Unchanged	Nonagouti (black or brown)
	A or +	Unchanged	Agouti (wild-type), black hair with a subapical yellow band
	a^{e}	Unchanged	Extreme nonagouti
	a^{t}	Unchanged	Black and tan
	A^{w}	Unchanged	White-bellied agouti
	A^{y}	Unchanged	Yellow
Gene:	*Tyrp1*	b	Tyrosinase-related protein 1
Allele:	*Tyrp1 or* $Tyrp1^{+}$	B or +	Black (wild-type)
	$Tyrp1^{B\text{-}lt}$	?	Light
	$Tyrp1^{b}$	b	Brown
Gene:	*Tyr*	c	Tyrosinase
Allele:	*Tyr or* Tyr^{+}	C or +	Pigmented (wild-type)
	$Tyr^{c\text{-}ch}$	c^{ch}	Chinchilla (light grayish-brown)
	$Tyr^{c\text{-}e}$	?	Extreme dilution
	Tyr^{c}	c	Albino
Gene:	*Myo5a*	d	Myosin Va
Allele:	*Myo5a or* $Myo5a^{+}$	D or +	Non-dilute (wild-type)
	$Myo5a^{d}$	d	Dilute
Gene:	*Oca2*	*p*	Oculocutaneous albinism II
Allele:	$Oca2^{+}$	+	Normal pigment (wild-type)
	$Oca2^{p}$	*p*	Pink-eyed dilution

Table E.2. Common coat color genes and alleles for laboratory mice—related biological systems and relative dominance.

Gene		Allele			
Symbol, name	**Chr**	**Symbol**	**Name**	**Pleiotropic effects on…**	**Relative dominance**
a nonagouti	1	*a*	Nonagouti (black or brown)		$A^y > A^w > A > a$ $A > a^t$ *(dorsal)* $a^t > A$ *(ventral)*
		A or +	Agouti (wild-type) (black hair with a subapical yellow band)		
		a^e	Extreme nonagouti	Growth/size, skeleton	
		a^t	Black and tan	Craniofacial, vision/eye, growth/size, hearing/vestibular/ear	
		A^w	White-bellied agouti	Anemia, endocrine/exocrine	
		A^y	Yellow	Obesity, adipose, behavior, growth/size, homeostasis, liver/biliary, digestive/alimentary, renal/urinary	
Tyrp1 tyrosinase-related protein 1	4	*Tyrp1* or $Tyrp1^+$	Black (wild type)		$Tyrp1^+ > Tyrp1^b$ $Tyrp1^b$ / $Tyrp1^b$ adds brown tones to other coat colors
		$Tyrp1^{B\text{-}lt}$	Light	Hearing/vestibular/ear	
		$Tyrp1^b$	Brown	Vision/eye	
Tyr tyrosinase	7	*Tyr* or Tyr^+	Pigmented (wild-type)		$Tyr^+ > Tyr^c$, $Tyr^{c\text{-}ch}$, $Tyr^{c\text{-}e}$ Tyr^{c*}/ Tyr^{c*} compound recessive alleles epistatically mask other coat color genes and alleles, and make eyes pink.
		$Tyr^{c\text{-}ch}$	Chinchilla		
		$Tyr^{c\text{-}e}$	Extreme dilution		
		Tyr^c	Albino	Neurological degeneration lethality/prenatal-perinatal, homeostasis, nervous system	
Myo5a myosin Va	9	*Myo5a* or $Myo5a^+$	Non-dilute (wild-type)		$Myo5a^+ > Myo5a^d$ $Myo5a^d$ /$Myo5a^d$ dilutes effects of other coat color genes
		$Myo5a^d$	Dilute	Blood clotting disorders; vision/eye	
Oca2 oculocutaneous albinism II (old *p*, pink-eyed dilution)	7	*Oca2 or* $Oca2^+$	Wild-type		$Oca2^+ > Oca2^p$ $Oca2^p$/$Oca2^p$ dilutes effects of other coat color genes, and makes eyes pink.
		$Oca2^p$	Pink-eyed dilution	Vision/eye	
Ednrb endothelin receptor type B	14	$Ednrb^s$	Piebald	Growth/size	
Mc1r melanocortin 1 receptor	8	$Mc1r^{E\text{-}tob}$	Tobacco darkening		
Mlph melanophilin (old *ln*)	1	$Mlph^{ln}$	Leaden		
U umbrous	unk	n/a	Umbrous (darkens agouti mice)		Additive with wild-type (*U*/*U* darkens more than *U*/+)

unk = unknown

Table E.3: Coat color phenotypes and genotypes for common strains of JAX® Mice.

Strain	Coat color phenotype	Allele at common coat color loci					Other coat color loci
		a **(Chr2)**	***Tyrp1*** **(Chr4)**	***Oca2*** **(Chr7)**	***Tyr*** **(Chr7)**	***Myo5a*** **(Chr9)**	
129P1/ReJ (001137)	Pink-eyed, white-bellied chinchilla	A^w	+	$Oca2^p$	$Tyr^{c\text{-}ch}$	+	
129P3/J (000690)	Pink-eyed, white-bellied, light chinchilla	A^w	+	$Oca2^p$	$Tyr^{c\text{-}ch}/Tyr^c$	+	
	Albino	A^w	+	$Oca2^p$	Tyr^c/Tyr^c	+	
129S1/SvImJ (002448)	White-bellied agouti	A^w	+	+	+	+	
129T2/SvEms/J (002065)	White-bellied agouti chinchilla	A^w	+	+	$Tyr^{c\text{-}ch}$	+	
129X1/SvJ (000691)	Pink-eyed, white-bellied, light chinchilla	A^w	+	$Oca2^p$	$Tyr^{c\text{-}ch}/Tyr^c$	+	
	Albino	A^w	+	$Oca2^p$	Tyr^c/Tyr^c	+	
A/HeJ (000645)	Albino	a	$Tyrp1^b$	+	Tyr^c	+	
A/J (000646)	Albino	a	$Tyrp1^b$	+	Tyr^c	+	
A/WySnJ (000647)	Albino	a	$Tyrp1^b$	+	Tyr^c	+	
AEJ/GnLe/J (000199)	Dark black	a^e/a^e	+	+	+	+	
	White-bellied agouti	$A^{w\text{-}J}/a^e$	+	+	+	+	
AKR/J (000648)	Albino	a	+	+	Tyr^c	+	*hid**
AU/SsJ (000649)	Black	a	+	+	+	+	*U*
B6.129P2-*Apoe*^{tm1Unc}/J (002052)	Black	a	+	+	+	+	
B6D2F1/J (100006)	Black	a	$Tyrp1^b/+$	+	+	$Myo5a^d/+$	
BALB/cByJ (001026)	Albino	A	$Tyrp1^b$	+	Tyr^c	+	
CByJ.RBF-Rb(8.12) 5Bnr/J (001802)	Albino	A	$Tyrp1^b$	+	Tyr^c	+	
BALB/cJ (000651)	Albino	A	$Tyrp1^b$	+	Tyr^c	+	
BDP/J (000652)	Pink-eyed fawn	a	$Tyrp1^b$	$Oca2^p$	+	$Myo5a^d$	
BTBR T^+ *tf*/J (002282)	Black and tan	a^t	+	+	+	+	*tf**
BUB/BnJ (000653)	Albino	a	+	+	Tyr^c	+	
BXSB/MpJ (000740)	White-bellied agouti, affected	A^w	+	+	+	+	
C3H/HeOuJ (000635)	Agouti	A	+	+	+	+	
C3H/HeSnJ (000661)	Agouti	A	+	+	+	+	
C3HeB/FeJ (000658)	Agouti	A	+	+	+	+	
C57BL/6ByJ (001139)	Black	a	+	+	+	+	
C57BL/6J (000664)	Black	a	+	+	+	+	
C57BL/10J (000665)	Black	a	+	+	+	+	
C57BL/10SnJ (000666)	Black	a	+	+	+	+	

* *hid (hair interior defect) and tf (tufted)* affect hair morphology or distribution, but not hair color.

Table continued on next page.

Table E.3: Coat color phenotypes and genotypes for common strains of JAX® Mice. (continued)

Strain	Coat color phenotype	Allele at common coat color loci					Other coat color loci
		a (Chr2)	*Tyrp1* (Chr4)	*Oca2* (Chr7)	*Tyr* (Chr7)	*Myo5a* (Chr9)	
C57BLKS/J (000662)	Black	*a*	+	+	+	+	
C57BR/cdJ (000667)	Brown	*a*	*Tyrp1*^{b}	+	+	+	
C57L/J (000668)	Leaden (grey)	*a*	*Tyrp1*^{b}	+	+	+	*Mlph*^{ln}
C58/J (000669)	Black	*a*	+	+	+	+	
CAST/EiJ (000928)	Agouti	*A*	+	+	+	+	
CBA/CaH-T(14;15)6Ca/J (000655)	Agouti	*A*	+	+	+	+	
CBA/CaHN-*Btk*^{xid}/J (001011)	Agouti	*A*	+	+	+	+	
CBA/CaJ (000654)	Agouti	*A*	+	+	+	+	
CBA/J (000656)	Agouti	*A*	+	+	+	+	
CE/J (000657)	Greyish white	*A*^{w}	+	+	*Tyr*$^{c\text{-}e}$	+	
CXB7/ByJ (000357)	Black	*a*	+	+	+	+	
CZECHII/EiJ (001144)	White-bellied agouti	*A*^{w}	+	+	+	+	
DBA/1J (000670)	Dilute brown	*a*	*Tyrp1*^{b}	+	+	*Myo5a*^{d}	
DBA/1LacJ (001140)	Dilute brown	*a*	*Tyrp1*^{b}	+	+	*Myo5a*^{d}	
DBA/2DeJ (000052)	Dilute brown	*a*	*Tyrp1*^{b}	+	+	*Myo5a*^{d}	
DBA/2J (000671)	Dilute brown	*a*	*Tyrp1*^{b}	+	+	*Myo5a*^{d}	
DW/J *Mlph*^{ln} *Pou1f1*^{dw}/J (000643)	Agouti leaden	*A*	+	+	+	+	*Mlph*^{ln}
FVB/NJ (001800)	Albino	*A*	+	+	*Tyr*^{c}	+	
HRS/J (000673)	Without hair	*A*	*Tyrp1*^{b}	+	*Tyr*^{c}	*Myo5a*^{d}	*Hr*^{hr}/*Hr*^{hr}*
	Albino, unaffected	*A*	*Tyrp1*^{b}	+	*Tyr*^{c}	*Myo5a*^{d}	*Hr*^{hr}/+
I/LnJ (000674)	Pink-eyed dilute brown, piebald (spotted)	*a*	*Tyrp1*^{b}	*Oca2*^{p}	+	*Myo5a*^{d}	*Ednrb*^{s}
JF1/Ms (003720)	Black spotted white coat, black eyes	*a*	+	+	+	?	*Ednrb*^{s}
KK/HlJ (002106)	Albino	*a*	+	+	*Tyr*^{c}	+	
LG/J (000675)	Albino	*a*	+	+	*Tyr*^{c}	+	
LP/J (000676)	White-bellied agouti, piebald	*A*^{w}	+	+	+	+	*Ednrb*^{s}
LT/SvEiJ (006252)	Grey-brown	*a*	*Tyrp1*$^{B\text{-}lt}$	+	+	+	
MA/MyJ (000677)	Albino	*A*	+	+	*Tyr*^{c}	+	
MOLF/EiJ (000550)	White-bellied agouti	*A*^{w}	?	+	+	?	
MRL/MpJ (000486)	Albino, unaffected	*a*	+	+	*Tyr*^{c}	+	
MSM/Ms (003719)	White-bellied agouti	*A*^{w}	?	+	+	?	

*Although *Hr*^{hr} (hairless) does not affect coat color, it does affect appearance.

Table continued on next page.

Table E.3: Coat color phenotypes and genotypes for common strains of JAX® Mice. (continued)

Strain	Coat color phenotype	Allele at common coat color loci					Other coat color loci
		a (Chr2)	*Tyrp1* (Chr4)	*Oca2* (Chr7)	*Tyr* (Chr7)	*Myo5a* (Chr9)	
NOD.CB17-*Prkdc*^{scid}/J (001303)	Albino	*A*	+		*Tyr*^{c}	+	
NOD/ShiLtJ (001976)	Albino	*A*	+		*Tyr*^{c}	+	
NON/ShiLtJ (002423)	Albino	*A*	+		*Tyr*^{c}	+	
NOR/LtJ (002050)	Albino	*A*	+		*Tyr*^{c}	+	
NZB/BlNJ (000684)	Black	*a*	+	+	+	+	
NZW/LacJ (001058)	Albino	*A*	*Tyrp1*^{b}	*Oca2*^{p}	*Tyr*^{c}	+	
P/J (000679)	Pink-eyed fawn	*a*	*Tyrp1*^{b}	*Oca2*^{p}	+	*Myo5a*^{d}	
PERA/EiJ (000930)	Agouti	*A*	+	+	+	+	
PL/J (000680)	Albino	*A*	+		*Tyr*^{c}	+	
PWK/PhJ (003715)	Agouti	*A*	+	+	+	+	
RBF/DnJ (000726)	Albino	?	+	+	*Tyr*^{c}	+	*Mc1r*$^{E\text{-}tob}$
RF/J (000682)	Albino	*a*	+	+	*Tyr*^{c}	+	
RHJ/Le (000266)	Albino	*a*	*Tyrp1*^{b}	+	*Tyr*^{c}	+	
RHJ/LeJ (001591)	Without hair	*a*	*Tyrp1*^{b}	+	*Tyr*^{c}	+	*Hr*$^{rh\text{-}J}$/*Hr*$^{rh\text{-}J}$†
	Albino, unaffected	*a*	*Tyrp1*^{b}	+	*Tyr*^{c}	+	*Hr*$^{rh\text{-}J}$/+
RIIIS/J (000683)	Albino	*A*	+	+	*Tyr*^{c}	+	
SEA/GnJ (000644)	Light brown agouti	*A*	*Tyrp1*^{b}	+	+	*Myo5a*^{d}	
SEC/1GnLeJ (000270)	Pink-eyed chinchilla	*a*	*Tyrp1*^{b}	+	*Tyr*$^{c\text{-}ch}$	+	
SJL/J (000686)	Albino	*A*	+	*Oca2*^{p}	*Tyr*^{c}	+	
SM/J (000687)	White-bellied agouti	*A*^{w}/*a*	+	+	+	+	
	Black	*a/a*	+	+	+	+	
SPRET/EiJ (001146)	White-bellied agouti	*A*^{w}	+	+	+	+	
ST/bJ (000688)	Albino	*a*	*Tyrp1*^{b}		*Tyr*^{c}	+	
SWR/J (000689)	Albino	*A*	+	+	*Tyr*^{c}	+	
WSB/EiJ (001145)	Agouti with head blaze, variable belly spotting, grayish coat‡	*A*	+	+	+	+	

†Although *Hr*$^{rh\text{-}J}$ (rhino) does not affect coat color, it does affect appearance.

‡Although the color pattern often appears to be white-bellied agouti, and is designated as such in some earlier publications, the non-agouti gene has the *A* allele. The combination of frequent belly spotting and a coat dilution can give the illusion of a white-bellied agouti phenotype.

Appendix F: Histocompatibility Haplotypes and Loci

This appendix includes several tables related to histocompatibility haplotypes and loci:

Table F.1. *H2* haplotypes for standard strains: allelic designations

Haplo-	Allelic designations for the *H2* complex*										
	Class 1a	Class II				Class III	Class 1a		Class 1b		
type	*K*	*Aβ*	*Aα*	*Eβ*	*Eα*	*C4b*	*D*	*L*	*Qa-2*	*T18*	*Qa-1*
a	*k*	*k*	*k*	*k*	*k*	*d*	*d*	*d*	^	*a*	*a*
b	*b*	*b*	*b*	*b*†	^	*b*	*b*	*b*‡	*a*	*b*	*b*
bc	*b*	*b*	*b*	*b*†	^	*b*	*b*	*b*‡	*a*	*f*	*b*
d	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	^	*c*	*b*
f	*f*	*f*	*f*	*f*	*f*	*f*	*f*	*f*	*b*	*d*	*b*
j	*j*	*j*	*j*	*j*	*j*	*j*	*b*	*b*‡	*a*	*d*	*b*
k	*k*	*k*	*k*	*k*	*k*	*k*	*k*	^	*b*	*b*	*b*
k2	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*b*	*a*	*a*
g7	*d*	*g7*	*d*	*g7*†	^	*d*	*b*	^	*a*	*b*	*a*
gx	*d*	*g7*	*d*	*g7*†	^	#	*dx*	#	#	#	#
m	*k*	*k*	*k*	*k*	*k*	*k*	*q*	*q*	*a*	*a*	*a*
nb1	*b*	*nb1*	*nb1*	*k*	*k*	#	*b*	#	#	#	#
p	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*b*	*e*	*a*
q	*q*	*q*	*q*	*q*†	^	*q*	*q*	*q*	*a*	*b*	*b*
q2	*q*	*q*	*q*	*q*†	^	*q*	*q*	*q*	*a*	*a*	*a*
r	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*b*	*b*	*b*
s	*s*	*s*	*s*	*s*†	^	*s*	*s*	*s*	*a*	*b*	*b*
s2	*s*	*s*	*s*	*s*†	^	*s*	*s*	*s*	*a*	*a*	*a*
u	*u*	*u*	*u*	*u*	*u*	*u*	*d*	*d*	^	*a*	*a*
v	*v*	*v*	*v*	*v*	*v*	*v*	*v*	*v*	*a*	*b*	*b*
z	*u*	*u*	*u*	*u*	*u*	*z*	*z*	*z*	*b*	*b*	*b*

^ null

unknown

**Aβ* may be designated *Ab*; *Aα* may be designated *Aa*; *Eβ* may be designated *Eb*. *Eα* may be designated *Ea*. The former designation for *C4b* was *S*.

†The Eβ chain is produced, but is not expressed on the cell surface because there is no Eα chain to pair with.

‡The D^b-linked L^b allele is a functionally null variant.

Table F.2.i. *H2* haplotypes for inbred strains.

Strain	Stock number	Haplo-type
129P1/ReJ	001137	*b*
129P3/J	000690	*bc**
129P3/JEmsJ	002357	*b*
129P4/RrRkJ	001198	*b*
129S1/SvImJ	002448	*b*
129T2/SvEms	002064	*b*
129T2/SvEmsJ	002065	*b*
129X1/SvJ	000691	*bc***
A/HeJ	000645	*a*
A/J	000646	*a*
A/WySnJ	000647	*a*
AKR/CumJ	002720	*k*
AKR/J	000648	*k*
ALR/LtJ	003070	*gx*
ALS/LtJ	003072	*nb1*
AU/SsJ	000649	*q*
B6 x IDH2/EiJ	002543	*b*
BALB/cBy	000650	*d*
BALB/cByJ	001026	*d*
BALB/cGaJ	001905	*d*
BALB/cGrRkJ	000921	*d*
BALB/cHeA	001255	*d*†
BALB/cJ	000651	*d*
BALB/cWtEiJ	001311	*d*
BDP/J	000652	*p*
BPH/2J	003005	*d*
BPL/1J	003006	*b*
BPN/3J	003004	*d*
BRVR/WrDvJ	001891	*k*
BTBR T^{+} *tf*/J	002282	*b*
BUB/BnJ	000653	*q2*‡
C3H/HeJ	000659	*k*
C3H/HeJBirLtJ	005972	*k*
C3H/HeJSxJ	001824	*k*
C3H/HeOuJ	000635	*k*
C3H/HeSn	000474	*k*
C3H/HeSnJ	000661	*k*
C3HeB/FeJ	000658	*k*
C3HfB/BiJ	001908	*k*
C57BL/6By	000663	*b*
C57BL/6ByJ	001139	*b*
C57BL/6J	000664	*b*
C57BL/6JEiJ	000924	*b*
C57BL/10J	000665	*b*
C57BL/10ScNJ	003752	*b*
C57BL/10ScSnJ	000476	*b*
C57BL/10SnJ	000666	*b*
C57BL/10SxJ	001822	*b*
C57BL/10WtRkJ	001197	*b*
C57BLKS/J	000662	*d*
C57BR/cdJ	000667	*k2*‡
C57L/J	000668	*bc*§
C58/J	000669	*k2*‡
CBA/CaGnLeJ	001143	*k*
CBA/CaH-T(14;15)6Ca/J	000655	*k*
CBA/CaHN Btk^{xid}/J	001011	*k*
CBA/CaJ	000654	*k*
CBA/J	000656	*k*
CE/J	000657	*k*
DA/HuSnJ	000660	*qp*
DBA/1J	000670	*q*
DBA/1LacJ	001140	*q*
DBA/2BiJ	001907	*d*
DBA/2DeJ	000052	*d*
DBA/2HaSmnJ	000973	*d*
DBA/2J	000671	*d*
DBA/8BiDsmJ	002860	*q*
DDY/EFrkJ	002814	unk
DDY/JclSidSeyFrk	002243	*s*
DW/J $Mlph^{ln}$ $Pou1f1^{dw}$/J	000643	*b*
EL/EFrkJ	002813	unk
EL/SuzSeyFrkJ	001956	*b*
FL/1ReJ	000023	*k*
FL/4ReJ	000025	*k*
FVB/NJ	001800	*q*
FVB/NMob	001491	*q*
HRS/J	000673	*k*
HTG/GoSfSnJ	000556	*g*
I/LnJ	000674	*j*
IDH2/Ei	000633	*b*†
KK/HlJ	002106	*b*
LDH2/EiJ	001266	*b*
LG/J	000675	d
LLC.A/CkcJ	001200	unk
LP/J	000676	bc*
LT/SvEi	003588	d
MA/MyJ	000677	k
MEV-W/TyJ	001856	unk
MRL/MpJ	000486	k
MY/HuLeJ	000265	k
NH/KiPtJ	003091	*k*
NOD/ShiLt	001289	*g7*
NOD/ShiLtJ	001976	*g7*
NON/ShiLtJ	002423	*nb1*
NOR/LtJ	002050	*g7*
NU/J	002019	*q*
NZB/BlNJ	000684	*d2**
NZL/LtJ	005067	*z*
NZM391/J	003108	*z*
NZM2410/J	002676	*z*
NZO/HlLtJ	002105	*z*
NZW/LacJ	001058	*z*
NZW/Osu	003560	*z*
P/J	000679	*p*
PL/J	000680	*u*
PN/nBSwUmabJ	005052	*q*
PRO/1AReJ	000173	*b*
PRO/ReJ	000059	*b*
RBF/DnJ	000726	unk
RF/J	000682	*k*
RHJ/LeJ	001591	*d*
RIII/DmMobJ	001088	*r*
RIIIS/J	000683	*r*
SB/LeJ	000269	*b*
SEA/GnJ	000644	*d*
SENCARA/PtJ	002746	*q*
SENCARB/PtJ	002747	*q*
SENCARC/PtJ	002748	*q*
SI/Col $Tyrp1^{b}$ $Dnahc11^{iv}$/J	001045	*d*
SJL/Bm	001902	*s*
SJL/J	000686	*s2*‡
SM/J	000687	*v*
SOD1/EiJ	001224	*k*
ST/bJ	000688	*k*
SWR/Bm	001900	*q*
SWR/J	000689	*q2*‡
WLC/MorJ	002600	*unk*
YBR/EiJ	000933	*d*

* (Fischer Lindahl K, 1997)
** (Kumanovics *et al*., 2002)
† The Jackson Laboratory carries this strain only as a background genotype for a mutation or for creation of an F1 hybrid.
‡ (Fischer Lindahl K, 1997; Shen FW, 1982)
§ (Shen FW, 1982)

Table F.2.ii. *H2* haplotypes for F1 hybrids.

Strain	Stock number	Haplo-type
B6129PF1/J	100492	*b/bc*
B6129PF1/J -$A^{w\text{-}J}/A^{w}$	100409	*b/bc*
B6129PF2/J	100903	*b/bc*
B6129SF1/J	101043	*b*
B6129SF2/J	101045	*b*
B6AF1/J	100002	*b/a*
B6C3F1/J	100010	*b/k*
B6C3FeF1/J *a/a*	001022	*b/k*
B6CBACaF1/J -$A^{w\text{-}J}/A$	001201	*b/k*
B6CBAF1/J	100011	*b/k*
B6D2F1/J	100006	*b/d*
B6EiC3SnF1/J	001875	*b/k*
B6EiD2F1/J	003550	*b/d*
B6SJLF1/J	100012	*b/s*
C3D2F1/J	100004	*k/d*
C3FeB6F1/J	100016	*k/b*
C3FeB6F1/J $A/A^{w\text{-}J}$	001203	*k/b*
CAF1/J	100003	*d/a*
CB6F1/J	100007	*d/b*
CByB6F1/J	100009	*d/b*
CByD2F1/J	100015	*d*
CSJLF1/J	100019	*d/s*
NZBWF1/J	100008	*d/z*
PLSJLF1/J	100299	*u/s*

Table F.2.iii. *H2* haplotypes for recombinant inbred strains.

Strain	Stock number	Haplo-type
58NXL8/TyJ	000564	unk
129XAE/SvJ	001258	unk
129XAM/SvJ	001263	unk
AKXD1/TyJ	001005	*k*
AKXD2/TyJ	000776	*d*
AKXD3/TyJ	000959	*k*
AKXD6/TyJ	000777	*k*
AKXD7/Ty	001016	*k*
AKXD9/TyJ	000763	*d*
AKXD10/TyJ	001017	*k*
AKXD11/TyJ	001003	*k*
AKXD13/TyJ	000765	*d*
AKXD14/TyJ	000779	*d*
AKXD15/TyJ	000954	*k*
AKXD16/TyJ	000958	*d*
AKXD18/TyJ	001093	*d*
AKXD20/TyJ	001001	*d*
AKXD21/TyJ	001062	*k*
AKXD22/TyJ	000947	*d*
AKXD23/TyJ	000780	*d*
AKXD24/TyJ	000969	*d*
AKXD25/TyJ	000949	*k*
AKXD27/TyJ	000764	*k*
AKXD28/TyJ	000957	*k*
AKXL5/Ty	000048	*b*
AKXL6/TyJ	000087	*k*
AKXL6A/TyJ	000757	unk
AKXL7/Ty	000324	*b*
AKXL8/Ty	000101	*k*
AKXL9/Ty	000325	*b*
AKXL12/Ty	000326	*b*
AKXL13/TyJ	000089	*k*
AKXL16A/TyJ	000404	unk
AKXL17/TyJ	000088	*b*
AKXL17A/TyJ	000748	*b*
AKXL19/Ty	000086	*b*
AKXL21/TyJ	000782	*k*
AKXL24/TyJ	000046	*b*
AKXL29/TyJ	000042	*b*
AKXL37/TyJ	000044	*b*
AKXL38/TyJ	000328	*k*
AKXL38A/TyJ	000747	unk
AKXL39/TyJ	003699	unk
AKXL43/TyJ	003700	unk
AKXL46/TyJ	003701	unk
AXB1/PgnJ	001673	*a*
AXB2/PgnJ	001674	*a*
AXB4/PgnJ	001676	*b*
AXB5/PgnJ	001677	*a*
AXB6/PgnJ	001678	*b*
AXB8/PgnJ	001679	*a*
AXB10/PgnJ	001681	*b*
AXB11/Pgn	001682	*a*
AXB12/PgnJ	001683	*a*
AXB13/PgnJ	001826	*b*
AXB13a/PgnJ	001684	*b*
AXB15/PgnJ	001685	*a*
AXB19/PgnJ	001687	*b*
AXB19a/PgnJ	001686	*b*
AXB19b/PgnJ	001688	*b*
AXB23/PgnJ	001690	*b*
AXB24/PgnJ	001691	*a*
BRX58N1/TyJ	000753	unk
BRX58N7/TyJ	000752	unk
BRX58N8/TyJ	000698	unk
BRX58N9/TyJ	000756	unk
BRX58N11/TyJ	000750	unk
BRX58N13/TyJ	000749	unk
BXA1/PgnJ	001692	*b*
BXA2/PgnJ	001693	*b*
BXA4/PgnJ	001694	*a*
BXA7/PgnJ	001696	*b*
BXA8/PgnJ	001697	*a*
BXA11/PgnJ	001699	*b*
BXA12/PgnJ	001700	*a*
BXA13/PgnJ	001701	*b*
BXA14/PgnJ	001702	*b*
BXA16/PgnJ	001703	*b*
BXA17/PgnJ	001704	*a*
BXA24/PgnJ	001710	*b*
BXA25/PgnJ	001711	*a*
BXA26/PgnJ	001999	*a*
BXD1/TyJ	000036	*d*
BXD2/TyJ	000075	*b*
BXD5/TyJ	000037	*d*
BXD6/TyJ	000007	*d*
BXD8/TyJ	000084	*b*
BXD9/TyJ	000105	*d*
BXD11/TyJ	000012	*d*
BXD12/TyJ	000045	*d*
BXD13/TyJ	000040	*b*
BXD14/TyJ	000329	*b*
BXD15/TyJ	000095	*b*
BXD16/TyJ	000013	*d*
BXD18/TyJ	000015	*d*

Table continued on next page.

Table F.2.iii. *H2* haplotypes for recombinant inbred strains. (continued)

Strain	Stock number	Haplo-type
BXD19/TyJ	000010	*b*
BXD20/TyJ	000330	*b*
BXD21/TyJ	000077	*d*
BXD22/TyJ	000043	*d*
BXD23/TyJ	000098	*b*
BXD24a/TyJ	005243	*d*
BXD24b/TyJ	000031	*d*
BXD25/Ty	000081	*d*
BXD25/TyJRwwJ	006255	*d*
BXD27/TyJ	000041	*d*
BXD28/TyJ	000047	*d*
BXD29/TyJ	000029	*b*
BXD30/Ty	000073	*d*
BXD31/TyJ	000083	*d*
BXD32/TyJ	000078	*d*
BXD33/TyJ	003222	*d*
BXD34/TyJ	003223	*d*
BXD36/TyJ	003225	*d*
BXD38/TyJ	003227	*d*
BXD39/TyJ	003228	*b*
BXD40/TyJ	003229	*d*
BXD42/TyJ	003230	*b*
BXH2/Ty1BedJ	002632	*k*
BXH2/TyJ	000034	*k*
BXH4/TyJ	000011	*b*
BXH6/TyJ	000038	*k*
BXH7/TyJ	000014	*k*
BXH8/TyJ	000076	*b*
BXH9/TyJ	000008	*b*
BXH10/TyJ	000032	*b*
BXH11/TyJ	000039	*b*
BXH12/Ty	000080	*k*
BXH14/TyJ	000009	*k*
BXH19/TyJ	000033	*b*
BXH20/KccJ	003784	*b*
BXH22/KccJ	003786	*b*
BXJ2/TyJ	000096	unk
BXSB/MpJ	000740	*b*
CX8B/EiJ	001568	unk
CX8D/EiJ	001569	unk
CX8G/EiJ	001566	unk
CX8I1/EiJ	001570	unk
CX8I2/EiJ	001571	unk
CX8M/EiJ	001550	unk
CX8N/EiJ	001532	unk
CXB1/ByJ	000351	*d*
CXB2/ByJ	000352	*b*
CXB3/ByJ	000353	*b*
CXB4/ByJ	000354	*d*
CXB5/ByJ	000355	*b*
CXB6/ByJ	000356	*b*
CXB7/ByJ	000357	*b*
CXB8/HiAJ	001629	*b*
CXB9/HiAJ	001630	K^b, D^d
CXB10/HiAJ	001631	*b*
CXB11/HiAJ	001632	*b*
CXB12/HiAJ	001633	*d*
CXB13/HiAJ	001634	*b*
CXJ1/SlkJ	001577	*d*
CXJ3/SlkJ	001578	*d*
CXJ4/SlkJ	001579	*s*
CXJ6/SlkJ	001580	*s*
CXJ8/SlkJ	001581	*d*
CXJ9/SlkJ	001582	*d*
CXJ15/SlkJ	001583	*s*
LXB3/TyJ	000754	unk
LXPL/2TyJ	000303	unk
LXPL6/TyJ	000307	unk
LTXBJ/NaJ	002109	unk
LTXBO/SvJ	000402	unk
NX129-1TyJ	001193	unk
NX129-10/TyJ	000948	unk
NX129-18/TyJ	001539	unk
NXSMC/EiJ	001651	*d*
NXSMD/EiJ	001652	*d*
NXSME/EiJ	001653	*d*
NXSMF/EiJ	001654	*v*
NXSMI/EiJ	001655	*v*
NXSML/EiJ	001656	*v*
NXSMN/EiJ	001657	*v*
NXSMP/EiJ	001659	*d*
NXSMQ/EiJ	001660	*v*
NXSMT1/EiJ	001661	*v*
NXSMT2/EiJ	001662	*d*
NXSMU/EiJ	001663	*v*
NXSMW/EiJ	001665	*d*
NXSMX/EiJ	001666	*v*
NXSMZ/EiJ	001667	*v*
SWXJ2/BmJ	001072	*s*
SWXJ3/BmJ	001073	*s*
SWXJ4/BmJ	001074	*q*
SWXJ5/BmJ	001075	*q*
SWXJ6/BmJ	001076	*s*
SWXJ7/BmJ	001077	*q*
SWXJ8/BmJ	001078	*s*
SWXJ9/BmJ	001079	*q*
SWXJ10/BmJ	001080	*q*
SWXJ11/BmJ	001081	*s*
SWXJ12/BmJ	001082	*s*
SWXJ13/BmJ	001083	*q*
SWXJ14/BmJ	001084	*s*
SWXL4/TyJ	000074	*b*
SWXL12/TyJ	000332	unk
SWXL15/TyJ	000334	*q*
SWXL16/TyJ	000335	*b*
SWXL17/TyJ	000336	*q*

Table F.2.iv. *H2* haplotypes for recombinant congenic strains.

Strain	Stock number	Haplo-type
B6cC3-1/KccJ	003787	*b*
CBcNO6/LtJ	002349	*k*
CBcNO7A/LtJ	003052	*k*
CBcNO7B/LtJ	003053	*k*
CBcNO7C/LtJ	003054	*k*
CBcNO7D/LtJ	003055	*k*
NOcCB1/LtJ	002348	*g7*
NONcNZO1/Lt	003668	*nb1*
NONcNZO3/Lt	003670	*z*
NONcNZO4/Lt	003671	*nb1*
NONcNZO5/LtJ	004455	*z*
NONcNZO6/Lt	003673	*nb1*
NONcNZO8/Lt	003675	*z*
NONcNZO10/LtJ	004456	*nb1*

Table F.2.v. *H2* haplotypes for chromosome substitution (CS or consomic) strains.

Strain	Stock number	Haplo-type
129S1/SvImJ-Chr $Y^{C57BL/6J}$/NaJ	005548	*b*
A/J-Chr $Y^{C57BL/6J}$/NaJ	005546	*a*
BALB/cByJ-Chr $Y^{C57BL/6By}$/J	001452	*d*
C3.SW/Lt-Chr $Y^{C3HeB/FeChp}$/J	002110	*b*
C3.SW/Lt-Chr Y^{SW}/J	002111	*b*
C57BL/6J-Chr $1^{PWD/Ph}$/ForeJ	005259	*b*
C57BL/6J-Chr $2^{PWD/Ph}$/ForeJ	005995	*b*
C57BL/6J-Chr $3^{PWD/Ph}$/ForeJ	005518	*b*
C57BL/6J-Chr $4^{PWD/Ph}$/ForeJ	006226	*b*
C57BL/6J-Chr $5^{PWD/Ph}$/ForeJ	005260	*b*
C57BL/6J-Chr $6^{PWD/Ph}$/ForeJ	005261	*b*
C57BL/6J-Chr $7^{PWD/Ph}$/ForeJ	005996	*b*
C57BL/6J-Chr $9^{PWD/Ph}$/ForeJ	005262	*b*
C57BL/6J-Chr $11.1^{PWD/Ph}$/ForeJ	005997	*b*
C57BL/6J-Chr $11.2^{PWD/Ph}$/ForeJ	005998	*b*
C57BL/6J-Chr $11.3^{PWD/Ph}$/ForeJ	006372	*b*
C57BL/6J-Chr $12^{PWD/Ph}$/ForeJ	005263	*b*
C57BL/6J-Chr $13^{PWD/Ph}$/ForeJ	005519	*b*
C57BL/6J-Chr $14^{PWD/Ph}$/ForeJ	005264	*b*
C57BL/6J-Chr $15^{PWD/Ph}$/ForeJ	005265	*b*
C57BL/6J-Chr $16^{PWD/Ph}$/ForeJ	005266	*b*
C57BL/6J-Chr $17^{PWD/Ph}$/ForeJ	005267	unk
C57BL/6J-Chr $18^{PWD/Ph}$/ForeJ	005268	*b*
C57BL/6J-Chr $19^{PWD/Ph}$/ForeJ	005269	*b*
C57BL/6J-Chr $X.1^{PWD/Ph}$/ForeJ	005762	*b*
C57BL/6J-Chr $X.3^{PWD/Ph}$/ForeJ	006227	*b*
C57BL/6J-Chr $Y^{PWD/Ph}$/ForeJ	005270	*b*
C57BL/6J-Chr $1^{A/J}$/NaJ	004379	*b*
C57BL/6J-Chr $2^{A/J}$/NaJ	004380	*b*
C57BL/6J-Chr $3^{A/J}$/NaJ	004381	*b*
C57BL/6J-Chr $4^{A/J}$/NaJ	004382	*b*
C57BL/6J-Chr $5^{A/J}$/NaJ	004383	*b*
C57BL/6J-Chr $6^{A/J}$/NaJ	004384	*b*
C57BL/6J-Chr $7^{A/J}$/NaJ	004385	*b*
C57BL/6J-Chr $8^{A/J}$/NaJ	004386	*b*
C57BL/6J-Chr $9^{A/J}$/NaJ	004387	*b*
C57BL/6J-Chr $10^{A/J}$/NaJ	004388	*b*
C57BL/6J-Chr $11^{A/J}$/NaJ	004389	*b*
C57BL/6J-Chr $12^{A/J}$/NaJ	004390	*b*
C57BL/6J-Chr $13^{A/J}$/NaJ	004391	*b*
C57BL/6J-Chr $14^{A/J}$/NaJ	004392	*b*
C57BL/6J-Chr $15^{A/J}$/NaJ	004393	*b*
C57BL/6J-Chr $16^{A/J}$/NaJ	004394	*b*
C57BL/6J-Chr $17^{A/J}$/NaJ	004395	*a*
C57BL/6J-Chr $18^{A/J}$/NaJ	004396	*b*
C57BL/6J-Chr $19^{A/J}$/NaJ	004397	*b*
C57BL/6J-Chr $X^{A/J}$/NaJ	004398	*b*
C57BL/6J-Chr $Y^{A/J}$/NaJ	004399	*b*
C57BL/6J-Chr $2^{C3H/HeJ}$/J	006357	*b*
C57BL/6J-Chr $11^{C3H/HeJ}$/J	006358	*b*
C57BL/6J-Chr $Y^{129S1/SvImJ}$/NaJ	005547	*b*

Table F.2.vi. *H2* haplotypes for conplastic strains

Strain	Stock number	Haplo-type
C57BL/6J-$mt^{A/J}$/NaJ	005545	*b*
C57BL/6J-$mt^{PWD/Ph}$/ForeJ	005761	*b*
NOD/Lt-$mt^{ALR/Lt}$/MxLt	005332	*g7*

Table F.3. *H2* haplotypes for H2-congenic strains: allelic designations.

Haplo-type	Strain	Stock number	Allelic designations for the H2 complex*										
			Class Ia	Class II				Class III	Class Ia		Class Ib		
			K	*Aβ*	*Aα*	*Eβ*	*Eα*	*C4b*	*D*	*L*	*Qa-2*	*T18*	*Qa-1*
a	B10.A-*H2*^{a} *H2-T18*^{a}/SgSnJ	000469	*k*	*k*	*k*	*k*	*k*	*d*	*d*	*d*	^	*a*	*a*
ar1	B10.LG-*H2*^{ar1}/J	001894	*d*	#	#	#	#	#	#	#	#	#	#
ar1	C3.LG-*H2*^{ar1}/CkcCyJ	000440	*d*	#	#	#	#	#	#	#	#	#	#
as1	B10.S-*H2*^{as1}(8R)/J	001760	*k*	*k*	*k*	*k/s*	*s*	*s*	*s*	*s*	*a*	*b*	*b*
b	AK.B6-*H2*^{b}/J	002090	*b*	*b*	*b*	*b*†	^	*b*	*b*	^	*a*	*b*	*b*
b	BKS.B6-*H2*^{b}/J	001041	*b*	*b*	*b*	*b*†	^	*b*	*b*	^	*a*	*b*	*b*
b	BRVR.B10-*H2*^{b}/J	001892	*b*	*b*	*b*	*b*†	^	*b*	*b*	^	*a*	*b*	*b*
b	C.B10-*H2*^{b}/LilMcdJ	001952	*b*	*b*	*b*	*b*†	^	*b*	*b*	^	*a*	*b*	*b*
b	D1.LP-*H2*^{b} *H2-T18*$^{b?}$/SnJ	000435	*b*	*b*	*b*	*b*†	^	*b*	*b*	*b*‡	*a*	*b*	*b*
b	NOD.B10Sn-*H2*^{b}/J	002591	*b*	*b*	*b*	*b*†	^	*b*	*b*	^	*a*	*b*	*b*
bc	A.BY-*H2*^{bc} *H2-T18*^{f}/SnJ	000140	*b*	*b*	*b*	*b*†	^	*b*	*b*	*b*‡	*a*	*f*	*b*
bc	C3.SW-*H2*^{b}/SnJ	000438	*b*	*b*	*b*	*b*†	^	*b*	*b*	*b*‡	*a*	*f*	*b*
bm1	B6.C-*H2*^{bm1}/By	000368	*bm1*	*b*	*b*	*b*†	^	*b*	*b*	^	*a*	*b*	*b*
bm1	B6.C-*H2*^{bm1}/ByJ	001060	*bm1*	*b*	*b*	*b*†	^	*b*	*b*	^	*a*	*b*	*b*
bm2	B6.C-*H2*^{bm2}/ByJ	000364	*bm2*	*b*	*b*	*b*†	^	*b*	*b*	^	*a*	*b*	*b*
bm3	C57BL/6J-*H2*^{bm3}/EgJ	001156	*bm3*	*b*	*b*	*b*†	^	*b*	*b*	^	*a*	*b*	*b*
bm4	B6.C-*H2*^{bm4}/ByJ	000369	*bm4*	*b*	*b*	*b*†	^	*b*	*b*	^	*a*	*b*	*b*
bm5	C57BL/6Kh-*H2*^{bm5}/KhEgJ	001157	*bm5*	*b*	*b*	*b*†	^	*b*	*b*	^	*a*	*b*	*b*
bm7	B6.C-*H2*^{bm7}/KhEgJ	001158	*bm7*	*b*	*b*	*b*†	^	*b*	*b*	^	*a*	*b*	*b*
bm10	B6.C-*H2*^{bm10}/KhEgJ	001160	*bm10*	*b*	*b*	*b*†	^	*b*	*b*	^	*a*	*b*	*b*
bm11	B6.C-*H2*^{bm11}/KhEgJ	001161	*bm11*	*b*	*b*	*b*†	^	*b*	*b*	^	*a*	*b*	*b*
bm12	B6(C)-*H2-Ab1*^{bm12}/KhEgJ	001162	*b*	*bm12*	*b*	*b*†	^	*b*	*b*	^	*a*	*b*	*b*
bm14	B6By(CBy)-*H2-D1*^{bm14}/(HZW42)ByJ	000145	*b*	*b*	*b*	*b*†	^	*b*	*bm14*	^	*a*	*b*	*b*
bm23	B10.D2-*H2*^{bm23}/EgJ	001163	*bm23*	*b*	*b*	*b*†	^	*b*	*b*	^	*a*	*b*	*b*
bp5	B10.F-*H2*^{bp5}/(14R)J	001823	*b*	*b*	*b*	*b*†	^	*p*	*p*	*p*	*b*	#	#
bq1	B10.MBR-*H2*^{bq1}/SxEgJ	001154	*b*	*k*	*k*	*k*	*k*	*k*	*q*	*q*	*a*	*a*	*a*
d	B6.C-*H2*^{d}/bByJ	000359	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	^	*d*	*b*
d	B6.C-*H2*^{d} *Mdmg1*$^{BALB/cBy}$/aByJ	000360	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	^	*d*	*b*
d	B10.D2-*H2*^{d}/n2SnJ	000462	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	^	*d*	*b*
d	B10.D2-*Hc*^{0} *H2*^{d} *H2-T18*^{c}/o2SnJ	000460	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	^	*c*	*d*
d	B10.D2-*Hc*^{0} *H2*^{d} *H2-T18*^{c}/oSnJ	000461	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	^	*c*	*b*
d	B10.D2- *Hc*^{1} *H2*^{d} *H2-T18*^{c}/nSnJ	000463	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	^	*c*	*b*
d	BRVR.D2-*H2*^{d}/J	001893	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	^	*d*	*b*
d	D1.C-*H2*^{d} *H2-T18*^{c}/SnJ	000437	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	^	*c*	*b*
dm1	B10.D2-*H2*^{dm1}/EgJ	001164	*d*	*d*	*d*	*d*	*d*	*d*	*dm1*	*dm1*	#	#	#

Table continued on next page.

Table F.3. H2 haplotypes for *H2*-congenic strains: allelic designations. (continued)

Haplo-type	Strain	Stock number	Allelic designations for the H2 complex*										
			Class Ia	Class II				Class III	Class Ia		Class Ib		
			K	*Aβ*	*Aα*	*Eβ*	*Eα*	*C4b*	*D*	*L*	*Qa-2*	*T18*	*Qa-1*
dm2	BALB/c-*H2^{dm2}*/KhEgJ	001165	*d*	*d*	*d*	*d*	*d*	*d*	*d*	^	*b*	*c*	*b*
f	A.CA-*H2^{f} H2-T18^{a}*/SnJ	000472	*f*	*f*	*f*	*f*	*f*	*f*	*f*	*f*	*b*	*d*	*b*
f	B10.M-*H2^{f}*/nMobJ	001068	*f*	*f*	*f*	*f*	*f*	*f*	*f*	*f*	*b*	*d*	*b*
f	B10.M-*H2^{f} H2-T18$^{a?}$*/SnJ	000459	*f*	*f*	*f*	*f*	*f*	*f*	*f*	*f*	*b*	*d*	*b*
fm2	B10.M-*H2^{fm2}*/MobJ	000739	*f*	*f*	*f*	*f*	*f*	*f*	*fm2*	*f*	#	#	#
g	B10.HTG-*H2^{g}*/2CyJ	001012	*d*	*d*	*d*	*d*	*d*	*d*	*b*	^	*a*	*b*	*b*
g	B10.HTG-*H2^{g}*/3CyJ	000999	*d*	*d*	*d*	*d*	*d*	*d*	*b*	^	*a*	*b*	*b*
g	C3H.HTG-*H2^{g} H2-T18$^{b?}$*/SnJ	000443	*d*	*d*	*d*	*d*	*d*	*d*	*b*	^	*a*	*b*	*b*
g3	B10.D2-*H2^{g3}*/(103R)EgJ	001151	*d*	*d*	*d*	*d*	*d*	*d*	*b*	^	*a*	*b*	*b*
g6	B6.C-*H2^{g6}*/J	001429	*d*	*d*	*d*	*d*	*d*	*d*	*b*	#	#	#	#
g7	B6.NOD-(*D17Mit21-D17Mit10*)/LtJ	003300	*d*	*g7*	*d*	*g7*†	^	*d*	*b*	^	*a*	*b*	*a*
g7	NON.NOD-*H2^{g7}*/LtJ	001627	*d*	*g7*	*d*	*g7*†	^	*d*	*b*	^	*a*	*b*	*a*
h2	B10.A-*H2^{h2}*/(2R)SgSnJ	000468	*k*	*k*	*k*	*k*	*k*	*d*	*b*	^	*a*	*b*	*b*
h4	B10.A-*H2^{h4}*/(4R)SgDvEgJ	001150	*k*	*k*	*k*	*k/b*	*b*	*b*	*b*	^	*a*	*b*	*b*
i3	B10.A-*H2^{i3}*/(3R)SgDvEgJ	001149	*b*	*b*	*b*	*b/k*	*k*	*d*	*d*	*d*	*a*	*a*	*a*
i5	B10.A-*H2^{i5} H2-T18^{a}*/(5R)SgSnJ	000467	*b*	*b*	*b*	*b/k*	*k*	*d*	*d*	*d*	*a*	*a*	*a*
i7	B10.D2-*H2^{i7}*/(107R)EgJ	001153	*b*	*b*	*b*	*b*	*b*	*b*	*d*	*d*	*a*	*c*	*b*
ia	B10.D2-*H2^{ia}*/(106R)EgJ	001152	*b*	*b*	*b*	*b*	*b*	*b*	*d*	*d*	*a*	*c*	*b*
j	B10.WB-*H2^{j} H2-T18^{b}*/SnJ	000445	*j*	*j*	*j*	*j*	*j*	*j*	*b*	*b*‡	*a*	*b*	*b*
j	C3.JK-*H2^{j} H2-T18^{b}*/SnJ	000441	*j*	*j*	*j*	*j*	*j*	*j*	*b*	*b*‡	*a*	*b*	*b*
k	B6.AK-*H2^{k}*/FlaEgJ	001148	*k*	*k*	*k*	*k*	*k*	*k*	*k*	^	*b*	*b*	*b*
k	B6.AK-*H2^{k}*/J	001895	*k*	*k*	*k*	*k*	*k*	*k*	*k*	^	*b*	*b*	*b*
k	C.C3-*H2^{k}*/LilMcdJ	001951	*k*	*k*	*k*	*k*	*k*	*k*	*k*	^	*b*	*b*	*b*
k2	B10.BR-*H2^{k} H2-T18^{a}*/SgSnJ	000465	*k*	*k*	*k*	*k*	*k*	*k*	*k*	^	*b*	*a*	*a*
kp1	B10.P-*H2^{kp1}*/(10R)SgJ	001825	*k*	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*b*	#	#
m	AK.M-*H2^{m} H2-T18^{a}*/nSnJ	000470	*k*	*k*	*k*	*k*	*k*	*k*	*q*	*q*	*a*	*a*	*a*
m	B10.AKM-*H2^{m} H2-T18^{a}*/SnJ	000466	*k*	*k*	*k*	*k*	*k*	*k*	*q*	*q*	*a*	*a*	*a*
nb1	NOD.NON-*H2^{nb1}*/LtJ	001626	*b*	*nb1*	*nb1*	*k*	*k*	#	*b*	#	#	#	#
o2	C3H-*H2^{o2} C4^{b}*/SfSnJ	000473	*d*	*d*	*d*	*d*	*d*	*d*	*k*	*k*	*b*	*b*	*b*
p	C3.NB-*H2^{p} H2-T18$^{c?}$*/SnJ	000439	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*b*	*e*	*a*
pa	B10.Y-*H2^{pa} H2-T18^{c}*/SnJ	000444	#	#	#	#	#	#	#	#	#	*e*	*a*
pb1	B10.F-*H2^{pb1}*/(13R)J	001818	*p*	*p*	*p*	*p*	*p*	*b*	*b*	^	*a*	*b*	*b*
q	B10.D1-*H2^{q}*/SgJ	002024	*q*	*q*	*q*	*q*†	^	*q*	*q*	*q*	*a*	*b*	*b*
q	NOD.SW-*H2^{q}*/J	002032	*q*	*q*	*q*	*q*†	^	*q*	*q*	*q*	*a*	*b*	*b*
qp1	B10.DA-*H2^{qp1} H2-T18^{b}*/(80NS)/SnJ	000464	*q*	*q*	*q*	*q*	*q*	*q*	*s*	*s*	*a*	*b*	*b*

Table continued on next page.

Table F.3. *H2* haplotypes for H2-congenic strains: allelic designations. (continued)

Haplo-type	Strain	Stock number	Allelic designations for the H2 complex*										
			Class Ia	Class II				Class III	Class Ia		Class Ib		
			K	*Aβ*	*Aα*	*Eβ*	*Eα*	*C4b*	*D*	*L*	*Qa-2*	*T18*	*Qa-1*
qp1	D1.DA-*H2*^{qp1}/SnJ	000436	*q*	*q*	*q*	*q*	*q*	*q*	*s*	*s*	*a*	*b*	*b*
r	B10.RIII-*H2*^{r}/(71NS)nMobJ	001069	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*b*	*b*	*b*
r	B10.RIII-*H2*^{r} *H2-T18*^{b}/(71NS)SnJ	000457	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*b*	*b*	*b*
r	LP-RIII-*H2*^{r} *H2-T18*^{b}/SnJ	000434	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*b*	*b*	*b*
s	A.SW-*H2*^{s} *H2-T18*^{b}/SnJ	000471	*s*	*s*	*s*	*s*†	^	*s*	*s*	*s*	*a*	*b*	*b*
s	B10.S-*H2*^{s}/SgMcdJ	001953	*s*	*s*	*s*	*s*†	^	*s*	*s*	*s*	*a*	*b*	*b*
sm1	B10.S-*H2*^{sm1}/(12R)SgJ	001817	*s*	*s*	*s*	*s*	*s*	*s*	*s*	#	#	#	#
t1	A.TL-*H2*^{t1}/SfDvEgMobJ	001067	*s*	*k*	*k*	*k*	*k*	*k*	*d*	*d*	*a*	*c*	*b*
t2	A-TH-*H2*^{t2}/SfDvEgMobJ	001066	*s*	*s*	*s*	*s*	*s*	*s*	*d*	*d*	*a*	*a*	*a*
t4	B10.S-*H2*^{t4}/(9R)/J	001650	*s*	*s*	*s*	*s/k*	*k*	*d*	*d*	*d*	*a*	*a*	*a*
u	B10.PL-*H2*^{u} *H2-T18*^{a}/(73NS)SnJ	000458	*u*	*u*	*u*	*u*	*u*	*u*	*d*	*d*	^	*a*	*a*
v	B10.SM *H2*^{v} *H2-T18*^{b}/(70NS)Sn-*cw*/J	000456	*v*	*v*	*v*	*v*	*v*	*v*	*v*	*v*	*a*	*b*	*b*
y1	B10.AQR-*H2*^{y1}/KljMcdJ	001954	*q*	*k*	*k*	*k*	*k*	*s*	*s*	*s*	*a*	*a*	*a*
y2	B10.T-*H2*^{y2}/(6R)SgDvEgJ	001155	*q*	*q*	*q*	*q*†	^	*q*	*d*	*d*	*a*	*a*	*a*

^ null

unknown

**Aβ* may be designated *Ab*; *Aα* may be designated *Aa*; *Eβ* may be designated *Eb*. *Eα* may be designated *Ea*. The former designation for *C4b* was *S*.

†The Eβ chain is produced, but is not expressed on the cell surface because there is no Eα chain to pair with.

‡The *D*^{b}-linked *L*^{b} allele is a functionally null variant.

Table F.4. Minor histocompatibility loci for inbred and congenic strains.

Locus	Chr	Allele	Strain	Stock number	Congenics	
					Donor strain	Backcrosses (N)
H1	7	*a*	B10.D2-*H1*^{a}/(58N)SnJ	000425	D2.WA/Sn	9
			BDP/J	000652		
			C3H/HeSnJ	000661		
			CBA/CaJ	000654		
			CBA/J	000656		
			DBA/1J	000670		
			DBA/2J	000671		
			WC/ReJ-*Kitl*^{Sl}/J	000693		
		b	129X1/SvJ	000691		
			A/WySnJ	000647		
			B6.C-*Tyr*^{c} *H1*^{b} *Hbb*^{d}/ByJ	000383	BALB/cBy	15
			B10.129P-*H1*^{b} *Hbb*^{d} *Tyr*^{c} *Ea7*^{a}/(5M)oSnJ	000409	129P/Sn	11
			B10.129P-*H1*^{b} *Tyr*^{c} *Hbb*^{d}/(5M)nSnJ	000418	129P/Sn	11
			B10.C-*H1*^{b} *Hbb*^{d} *Tyr*^{c}/(41N)SnJ	000432	BALB/c	8
			BALB/cByJ	001026		
			C3.K-*H1*^{b}/nSnJ	000413	Non-inbred	11
		c	C57BL/6ByJ	001139		
			C57BL/6J	000664		
			C57BL/10Sn/J	000666		
			C57BR/cdJ	000667		
			C57L/J	000668		
			C58/J	000669		
		d	P/J	000679		
		e	WB/ReJ *Kit*^{W}/J	000692		
		f	CE/J	000657		
H3	2	*a*	C57BL/6J	000664		
			C57BL/10J	000665		
			C57BLKS/J	000662		
			C57BR/cdJ	000667		
			C57L/J	000668		
			WB/ReJ *Kit*^{W}/J	000692		
			WC/ReJ-*Kitl*^{Sl}/J	000693		
		b	129P3/J	000690		
			129X1/SvJ	000691		
			B10.LP-*H3*^{b}/Sn	000421	LP/J	13
			B10.LP-*H3*^{b} *H13*^{b}/(36NS)Sn	000422	LP/J	11
			B10.UW-*H3*^{b} *we Pax1*^{un} *a*^{t}/SnJ	000419	UW/Le	8
			CE/J	000657		
			CeH/HeSnJ	000661		
			C58/J	000669		

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Table F.4. Minor histocompatibility loci for inbred and congenic strains. (continued)

Locus	Chr	Allele	Strain	Stock number	Congenics	
					Donor strain	Backcrosses (N)
H3 (continued)	2	*b*	DBA/1J	000670		
			DBA/2J	000671		
			LP/J	000676		
			SWR/J	000689		
		c	B6.C-*H3*^{c}/ByJ	001286	BALB/c	5
			B10.C-*H3*^{c}/SnJ	000429	BALB/c	11
			B10.C-*H3*^{c} *H13*$^{?}$ *A*/(28NX)SnJ	000433	BALB/c	8
			BALB/cByJ	001026		
		d	B10.KR-*H3*^{d}/SnJ	000424	KR/Di	11
		e	B10.PA-*Pldn*^{pa} *H3*^{e} *a*^{t}/SnJ	000477	Wild-derived	34
*H4**	(see *H46–H47*)					
H7	9	*a*	129X1/SvJ	000691		
			BDP/J	000652		
			C57BL/6J	000664		
			C57BL/10SnJ	000666		
			C57BLKS/J	000662		
			C57BR/cdJ	000667		
			C57L/J	000668		
			C58/J	000669		
			DBA/1J	000670		
			DBA/2J	000671		
		b	A/HeJ	000645		
			A/WySnJ	000647		
			B6.C-*H7*^{b}/By *Kit*$^{W\text{-}50J}$/J	000560	BALB/cBy(-*H7*^{b})	19
			B10.C-*H7*^{b}/(47N)SnJ	000430	BALB/c	7
			BALB/cByJ	001026		
			C3H/HeSnJ	000661		
			DA/HuSnJ	000660		
H8	14	*a*	C57BL/6J	000664		
			C57BL/10SnJ	000666		
			C57BR/cdJ	000667		
			C57L/J	000668		
			C58/J	000669		
		b	129X1/SvJ	000691		
			B10.D2-*H8*^{b}/(57N)SnJ	000426	D2.WA/Sn	8
			DBA/1J	000670		
			DBA/2J	000671		
			LP/J	000676		
	14	*c*	B6.C-*H8*^{c}/(HW96)ByJ	000113		
			BALB/cByJ	001026	BALB/cBy	15

Table continued on next page.

Table F.4. Minor histocompatibility loci for inbred and congenic strains. (continued)

Locus	Chr	Allele	Strain	Stock number	Congenics	
					Donor strain	Backcrosses (N)
H9	unk	*a*	A/HeJ	000645		
			A/WySnJ	000647		
			C3H/HeSnJ	000661		
			C57BL/10SnJ	000666		
			C57BR/cdJ	000667		
			C57L/J	000668		
			CE/J	000657		
			PL/J	000680		
			WB/ReJ *Kit^{w}*/J	000692		
		b	B10.C-*H9^{b}*/(45N)SnJ	000431	BALB/c	9
			BALB/cByJ	001026		
			CBA/CaJ	000654		
			CBA/J	000656		
			DA/HuSn	000660		
			LP/J	000676		
H10	unk	*a*	C57BL/10SnJ	000666		
		b	129P3/J	000690		
			B10.129P-*H10^{b}*/(9M)SnJ	000132	129/J	7
H11	unk	*a*	C57BL/10/SnJ	000666		
		b	129P3/J	000690		
			B10.129P-*H11^{b}*/(10M)SnJ	000416	129/J	8
		?	B10.D2-*H11$^{?}$*/(55N)SnJ	000428	D2.WA/Sn	10
H12	unk	*a*	A/WySnJ	000647		
			BDP/J	000652		
			C3H/HeSnJ	000661		
			C57BL/6J	000664		
			C57BL/10SnJ	000666		
			C57BR/cdJ	000667		
			C57L/J	000668		
			C58/J	000669		
			CE/J	000657		
			DBA/1J	000670		
			DBA/2J	000671		
			PL/J	000680		
		b	129X1/SvJ	000691		
			B10.129P-*H12^{b}*/(6M)SnJ	000417	129/J	7
			SWR/J	000689		

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Table F.4. Minor histocompatibility loci for inbred and congenic strains. (continued)

Locus	Chr	Allele	Strain	Stock number	Congenics	
					Donor strain	Backcrosses (N)
H13	2	*a*	A/HeJ	000645		
			A/WySnJ	000647		
			AKR/J	000648		
			C57BL/6J	000664		
			C57BL/10SnJ	000666		
			C57BLKS/J	000662		
			C57BR/cdJ	000667		
			C57L/J	000668		
			CBA/CaJ	000654		
			CBA/J	000656		
			DA/HuSn	000660		
			RF/J	000682		
			SWR/J	000689		
			WC/ReJ-*Kitl*^{Sl}/J	000693		
		b	129X1/SvJ	000691		
			B10.CE-*H13*^{b} *A*^{w}/(30NX)SnJ	000427	CE/J	9
			B10.LP-*H13*^{b} *A*^{w}/Sn	000420	LP/J	11
			B10.LP-*H3*^{b} *H13*^{b}/(36NS)Sn	000422	LP/J	9
			C58/J	000669		
			CE/J	000657		
			DBA/1J	000670		
			DBA/2J	000671		
			LP/J	000676		
		c	C3H/HeSnJ	000661		
		d	SM/J	000687		
		?	B10.C-*H3*^{c} *H13*$^{?}$ *A*/(28NX)SnJ	000433	probably KR/Di for *H1*	8
		?	B10.KR-*H13*$^{?}$ *A*/SnJ	000423	KR/Di	11
H15	4	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H15*^{c}/(HW13J)ByJ	000382	BALB/cBy	11
			BALB/cByJ	001026		
H16	4	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H16*^{c}/(HW13K)ByJ	000381	BALB/cBy	11
			BALB/cByJ	001026		
H17	12	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H17*^{c}/(HW14)ByJ	000130	BALB/cBy	17
			BALB/cByJ	001026		
H18	4	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H18*^{c}/(HW17)ByJ	000380	BALB/cBy	12
			BALB/cByJ	001026		

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Table F.4. Minor histocompatibility loci for inbred and congenic strains. (continued)

Locus	Chr	Allele	Strain	Stock number	Congenics	
					Donor strain	Backcrosses (N)
H19	7	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H19*^{hc}/(HW20)ByJ	000135	BALB/cBy	11
			BALB/cByJ	001026		
H20	4	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H20*^{c}/ByJ	000379	BALB/cBy	17
			BALB/cByJ	001026		
H21	4	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H21*^{c}/ByJ	000378	BALB/cBy	17
			BALB/cByJ	001026		
H22	7	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H22*^{c} *Gpi1*^{a}/(HW38)ByJ	000377	BALB/cBy	12
			BALB/cByJ	001026		
H23	3	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H23*^{c}/(HW53)ByJ	000376	BALB/cBy	15
			BALB/cByJ	001026		
H24	7	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H24*^{c} *Gpi1*^{a}/(HW54)ByJ	000123	BALB/cBy	16
			BALB/cByJ	001026		
H25	a	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H25*^{c}/(HW65)ByJ	000114	BALB/cBy	15
			BALB/cByJ	001026		
H26	unk	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H26*^{c}/ByJ	000375	BALB/cBy	18
			BALB/cByJ	001026		
H27	unk	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H27*^{c}/ByJ	000374	BALB/cBy	15
			BALB/cByJ	001026		
H28	3	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H28*^{c} *If1*^{h}/(HW110)dBy	000116	BALB/cBy	15
			B6.C-*H28*^{c} *If1*^{l}/(HW81)aByJ	000146	BALB/cBy	16
			B6.C-*H28*^{c} *If1*^{l}/(HW94)bByJ	000384	BALB/cBy	15
			B6.C-*H28*^{c} *If1*^{l}/(HW97)cByJ	000142	BALB/cBy	15
			BALB/cByJ	001026		
H29	8	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H29*^{c}/(HW88)ByJ	000373	BALB/cBy	15
			BALB/cByJ	001026		
H30	15	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H30*^{c}/(HW105)ByJ	000138	BALB/cBy	14
			BALB/cByJ	001026		

Table continued on next page.

Table F.4. Minor histocompatibility loci for inbred and congenic strains. (continued)

Locus	Chr	Allele	Strain	Stock number	Congenics	
					Donor strain	Backcrosses (N)
H31	17	*a*	A/J	000646		
		b	C57BL/6J	000664		
H32	17	*a*	A/J	000646		
		b	C57BL/6J	000664		
H34	12	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H34*^{c}/(HW22)ByJ	000136	BALB/cBy	16
			BALB/cByJ	001026		
H35	1	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H35*^{c}/ByJ	000143	BALB/cBy	16
			BALB/cByJ	001026		
H36	unk	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H36*^{c}/ByJ	000372	BALB/cBy	15
			BALB/cByJ	001026		
H37	3	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H37*^{c}/(HW106)ByJ	000371	BALB/cBy	14
			BALB/cByJ	001026		
H38	3	*b*	C57BL/6ByJ	001139		
		c	B6.C-*H38*^{c}/By-*Kit*$^{W\text{-}56J}$/J	000495	B6.C*H38*^{c}/(HW119)ByJ	22p
			B6.C-*H38*^{c}/(HW119)ByJ	000370	BALB/cBy	18
			BALB/cByJ	001026		
*H46–H47**	7	*a*	AKR/J	000648		
			C3H/HeSnJ	000661		
			C57BL/6J	000664		
			C57BL/10J	000665		
			C57BL/10SnJ	000666		
			C57BLKS/J	000662		
			C57BR/cdJ	000667		
			C57L/J	000668		
			C58/J	000669		
			CBA/CaJ	000654		
			CBA/J	000656		
			SJL/J	000686		
		b	129P3/J	000690		
			129X1/SvJ	000691		
			B10.129P-*H46*^{b} *H47*^{b}/(21M)Sn	000414	129P3/J	15
			BDP/J	000652		
H51	19	†	B6.C3Fe-*H51 Hps1*^{ep}/ByJ†	000050	C3HeB/FeJ	20
		†	C3HeB/FeJ	000658		
		‡	C57BL/6ByJ	001139		

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Table F.4. Minor histocompatibility loci for inbred and congenic strains. (continued

Locus	Chr	Allele	Strain	Stock number	Congenics	
					Donor strain	Backcrosses (N)
H52	5	†	B6By.C-*H52 Fgf5*^{go}/J†	000795	BALB/cBy	11
		†	BALB/cByJ	001026		
		‡	C57BL/6ByJ	001139		
H54		†	B6.Cg-S*gk3*^{fz} *H54 Mlph*^{ln}/+ *H54* +/J†	000112	C57BR/cdJ	11
		†	C57BR/cdJ	000667		
		‡	C57BL/6J	000664		
H61	9	*b*	B6.C-*H61*^{b} *Me1*^{a}/(HW23)By	000137	BALB/cBy	15
		b	BALB/cByJ	001026		
		§	C57BL/6ByJ	001139		
HX	X	*b*	C57BL/6ByJ	001139		
		c	BALB/cByJ	001026		
HY	Y	*a*	A/J	000646		
		b	C57BL/6J	000664		

*H46 and H47, formerly designated H4, are linked (Davis and Roopenian, 1990).

†Minor histocompatibility loci have been identified for *H51*, *H52*, and *H54*, but allele names have not been assigned. The congenic strain and the donor strain (both designated by †) share an allele that differs from the allele in the background strain (designated by‡). (Bailey DW and Bunker HP, 1972)

‡See footnote for †.

§For *H61*, the allele name in C57BL/6ByJ has not been assigned. This allele, however, differs from the *b* allele in BALB/cByJ.

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Appendix G: Equivalencies of Human Age to Life Phases of Mice

Kevin Flurkey, Joanne M. Currer, David E. Harrison

Mice have virtually the same organs and tissues as humans, despite the considerable difference in shape and size. Not surprisingly, the developmental, maturational, and aging schedule for these organs and tissues also has the same sequence, although the timing is very different. In this appendix we provide comparisons of the developmental, maturational, and aging rates as well as age equivalencies between mice and humans.

Age equivalencies and relative rates of development

The relative rates of pre- and post-natal development, maturation, and aging in mice and humans differ greatly at different life history stages. In a detailed study of prenatal age equivalencies, Otis and Brent (1950) compared 147 anatomical markers of development (such as appearance of the first somites, anterior limb buds, and first intestinal villi) in mice and humans. Ages ranged from 1 day post conception (dpc) in both species to 16.5 dpc in mice and to the equivalent, 85 dpc, in humans. Their analysis suggests that gestation can be divided into three phases that are developmentally equivalent between mice and humans. Within each phase, the ratio of developmental rates (the time it takes for each marker to appear) between mice and humans is remarkably consistent; however, between phases, this ratio differs dramatically.

- Conception to implantation. Pre-implantation cleavage occurs at about the same rate in mice and humans. Implantation occurs at 4.5 dpc in mice and 6 dpc in humans.
- Implantation through formation of most organs (4.5 to 14.5 dpc in mice; 6 to 45 dpc in humans). This is the phase when almost all the organs are formed. During this phase, developmental markers are expressed about four times faster in mice than in humans. At the end of this phase, an abrupt change occurs as development shifts to a phase characterized more by cell proliferation.
- Formation of organs through organ differentiation. Otis and Brent (1950) reported data only for the first two days after this third phase begins (14.5 to 16.5 dpc in mice, equivalent to about 45 to 85 dpc in humans). The phase continues, however, as tissues and organs that are necessary for independent life grow and complete their differentiation. In mice, this phase continues even after birth—until about day 12 postnatal, which is developmentally equivalent to birth in humans. During organ differentiation, developmental markers appear about 15–20 times earlier in mice than in humans.

Parturition as a developmental marker

Often developmental timing is "anchored" to birth—birth completes gestation and initiates postnatal development. However, among mammalian species, the developmental stage of the fetus at birth varies considerably. For example, in contrast to humans, mice are blind, deaf, and hairless when they are born. It is not until mice are about 12 days old that they are developmentally comparable to humans at birth.

Following completion of generalized tissue differentiation at the end of tissue expansion, subsequent maturational markers consist almost entirely of markers that are dependent on maturation of the gonads and sex hormones. Laboratory mice are sexually mature at about 30–37 days of age. If we use 12 years as an age at which most humans are capable of reproducing (National Institutes of Health, 2008), the maturational equivalency rate is about 200 times faster in mice than humans (based on 12–34 days of age in mice, 0–12 years of age in humans).

After sexual maturation, few biologically timed events occur at precise ages; age-related change for almost all the biomarkers of aging (collagen cross-linking and wound healing, for example) is more progressive and continuous than for developmental markers. If we consider the age at which females can no longer reproduce (around 450 days for genetically mixed populations of mice [Klebanov *et al.*, 2001] and 50 years for humans) and maximum lifespan as representative markers, mice age about 30 times faster than humans throughout adulthood.

Table G.1 provides a summary of the above information.

Table G1. Comparison of developmental and aging rates between mice and humans.

Developmental or aging stage	Age range		Relative rate of development or aging in mice compared to humans
	Mice	Humans	
Conception to implantation	0 to 4 days post conception (dpc)	0 to 5 dpc	Starts about the same.
Implantation through formation of most organs	4.5 to 14.5 dpc	5 to 45 dpc	Increases to about 4 times faster in mice.
Formation of organs through organ differentiation	14.5 dpc to 12 days old (postnatal)	45 dpc to birth*	Increases to about 15 to 20 times faster in mice.
Organ differentiation to sexual maturity	12 to about 34 days old	Birth to about 12 years old	Increases to about 200 times faster in mice.
Sexual maturity through adulthood	34 days old and older	12 years old and older	Diminishes to about 30 times faster in mice.

*Human gestation is about 266 days.

Life phases of adult mice

Following sexual maturity, we have very few age-specific milestones to help us characterize stages of aging. But for some types of research, such as pathology studies and aging and lifespan studies, we identify three stages of adult maturation and aging.

Mature adult: 3–6 months of age

Mature adulthood refers to a period when mice are mature but not yet affected by senescence. Although mice are sexually mature by 35 days, relatively rapid maturational growth continues for most biological processes and structures until about 90 days. Thus, the youngest mice in the mature adult group should be at least three months old. The upper age limit is six months, because after that age mice might exhibit some age-related change. (As an example, female mice are retired from breeding at eight months because litter size diminishes.) However, if, for a specific biomarker, measurements are known to be stable through adulthood until 12 months, adult mice as old as 12 months may be used as the "normal" control.

Middle age: 10–15 months of age

Middle age refers to a period during which senescent changes can be detected for some, but not all, biomarkers of aging. For the middle-aged group, mice should be at least 10 months old. Senescence processes that begin in younger adults (for example, collagen cross linking and accumulation of activated/memory T cells) often can be detected by then. The upper age limit for the middle-aged group is typically 15 months because, at this age, most biomarkers still have not changed to their full extent and some have not yet started changing.

Old age: 18 months–death

Old age refers to a period when senescent changes can be detected in almost all biomarkers in all animals. For studies of senescence, for the old-aged group, mice should be at least 18 months old. The upper limit is 22–26 months, depending on the timing of the onset of disease for a specific genotype. Past that age, even in robust F1 hybrids, undetected disease in a large subset of the population can produce misleading results.

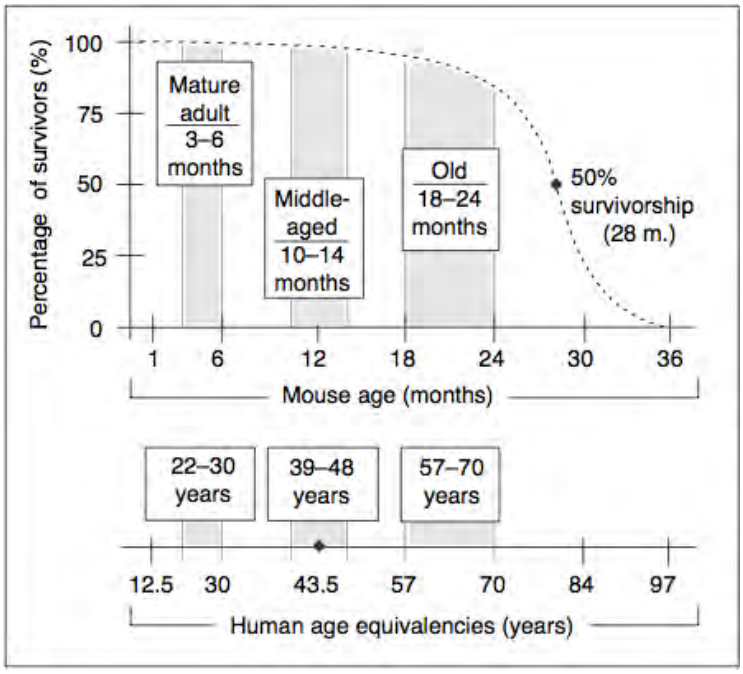
In cross-sectional studies, thorough necropsies are absolutely essential to determine the presence of a concurrent disease that might interfere with results. It is important to note that, in cross-sectional studies, the upper boundary for the old group should be adjusted for each genotype based on relative survivorship and age-specific incidence of disease. A general guideline for the upper age limit for biomarker studies in any genotype is the age at 85–90% survivorship.

Of course, mice older than 26 months can be used to study terminal processes of senescence and age-related pathology.

Comparison of life phases between mice and humans

Using a survival curve for C57BL/6J (000664) mice, Figure G.1 compares the life phases of this genotype with those of humans.

Figure G.1. Life phases for C57BL/6J mice, human equivalencies, and maturational comparisons.



C57BL/6J survivorship data are based on a large cohort of mice (150 males and 150 females). Comparison of life phases is based on decades of work on maturation and aging by D. E. Harrison, The Jackson Laboratory. (Modified from Flurkey *et al*., 2007).

Genotype differences among mice

The guidelines presented in this appendix are similar for most inbred strains and F1 hybrids. However, it is important that researchers recognize strain variation. For details about specific strains of mice, refer to the Mouse Phenome Database (MPD) at www.jax.org/phenome.

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Appendix H: Transfer of a Mutant or Variant Allele to a New Genetic Background by Phenotypic Selection

Creating congenic mice is straightforward when a genetic marker is available. However, researchers often wish to create a congenic strain when a mutant or variant allele is identifiable only by phenotype. This is typically the case for spontaneous mutants or for strain differences that segregate as a single locus (which indicates that a single gene is involved). Techniques to create congenic strains based strictly on phenotypic selection have been used since the 1940s (Table H.1). These techniques also make it possible to transfer mutations with a lethal or infertile phenotype. Note that these procedures are for the actual backcrossing and development of the strain. Once the mutation has been transferred to the new background, the new strain is maintained—and controls are chosen—using the same strategies provided in Table 3.8, "Expansion and maintenance breeding schemes for models with single-locus mutations."

Table H.1. Using phenotypic selection to create a congenic mouse.

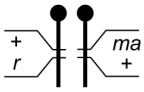
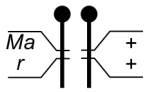
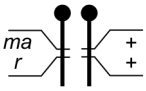
For this type of mutant or variant allele…	… use this breeding scheme.	Comments
A dominant allele (*M*)	Backcross matings using heterozygotes: 1. Backcross a heterozygote (*M*/+) to the inbred recipient strain (+/+). *Offspring*: (*M*/+), (+/+) 2. Repeat step 1 for generations N2–N10. 3. To expand the line or maintain the allele on this background, see Table 3.8.	• This breeding pattern can continue indefinitely. • With sibling mating, the line should be "refreshed" occasionally by backcrossing to the inbred recipient strain to prevent substrain divergence. • This is the same scheme used to transfer an allele or gene that can be genotyped.
A recessive allele (*m*) when the homozygote is viable and fertile	Backcross-intercross (sometimes called cross-intercross) matings using homozygotes: 1. Backcross a homozygote (*m*/*m*) to the inbred recipient strain (+/+). *Offspring*: (*m*/+) 2. Intercross heterozygote (*m*/+) offspring. *Offspring*: (*m*/*m*), (*m*/+), (+/+) 3. Select homozygous (*m*/*m*) offspring. 4. Repeat steps 1–3 until N10. 5. After N10, either incross siblings or backcross to the inbred recipient strain. OR To expand the line or maintain the allele on this background, see Table 3.8.	• With sibling mating, the line should be "refreshed" occasionally by backcrossing to the recipient inbred strain to prevent substrain divergence.
A recessive allele (*m*) that produces sterility or is lethal in homozygotes	Backcross-intercross (sometimes called cross-intercross) matings using heterozygotes: 1. Backcross a heterozygote (*m*/+) to the inbred recipient strain (+/+). *Offspring*: (*m*/+), (+/+) 2. Intercross siblings (test mating). *Offspring*: (*m*/*m*), (*m*/+), (+/+) 3. Identify breeding pairs that produced (*m*/*m*) mutants. These are heterozygote carriers (*m*/+). 4. Repeat steps 1–3 until N10. 5. To expand the line or maintain the allele on this background, see Table 3.8.	• In the test mating intercross, for mutations that are fully penetrant, 1 of 4 sibling matings will produce mutant offspring. • Ovarian transplants may be used to propagate the strain once the mutation has been moved to the recipient inbred strain after N7 (to ensure histocompatibility).

M = dominant mutant allele; *m* = recessive mutant allele; + = wild-type allele.

Appendix I: Using a Balanced Stock to Carry a Recessive Mutation That Is Sterile or Lethal, Including Embryonic Lethal

Stocks carrying recessive mutations that cause sterility or lethality, including embryonic lethality, are a challenge to maintain and expand. Today, researchers often use direct genotyping and, occasionally, ovarian transplants into histocompatible hosts. These are good strategies for producing a maximal quantity of mutant offspring. However, when a genetic probe is unavailable, researchers still use a breeding method based on classical genetics. This method—using a balanced stock of double heterozygotes—incorporates a marker gene (coat color, for example) that is so closely linked (or balanced) with the mutant gene that they rarely recombine. A major disadvantage of using a balanced stock is the time and effort it takes to produce it initially. But once a balanced stock is available, it provides a way to carry a mutation and generate mutants and controls that can be even more efficient than direct genotyping. Table I.1 describes the marker and double heterozygote options available with a balanced stock.

Table I.1. Balanced stocks: marker and double heterozygote breeding options.

To…	…when the marker allele is…	…maintain the balanced stock using this breeding strategy	Implications regarding identification of offspring
distinguish carriers from non-carriers by sight to efficiently propagate the strain	recessive	 Double heterozygotes in *repulsion*	The mice used to set up new breeding pairs (double heterozygotes in repulsion) will *not* express either the marker or the mutation. (See Figure I.1.)
	dominant	 Double heterozygotes in *coupling*	The mice used to set up new breeding pairs (double heterozygotes in coupling) will express the marker but *not* the mutation. (See Figure I.2.)
distinguish mutants by sight before the mutant phenotype is expressed	recessive	 Double heterozygotes in *coupling*	The mice used to set up new breeding pairs (double heterozygotes in coupling) will *not* express either the marker or the mutation—carriers and non-carriers cannot be distinguished. (See Figure I.3.)

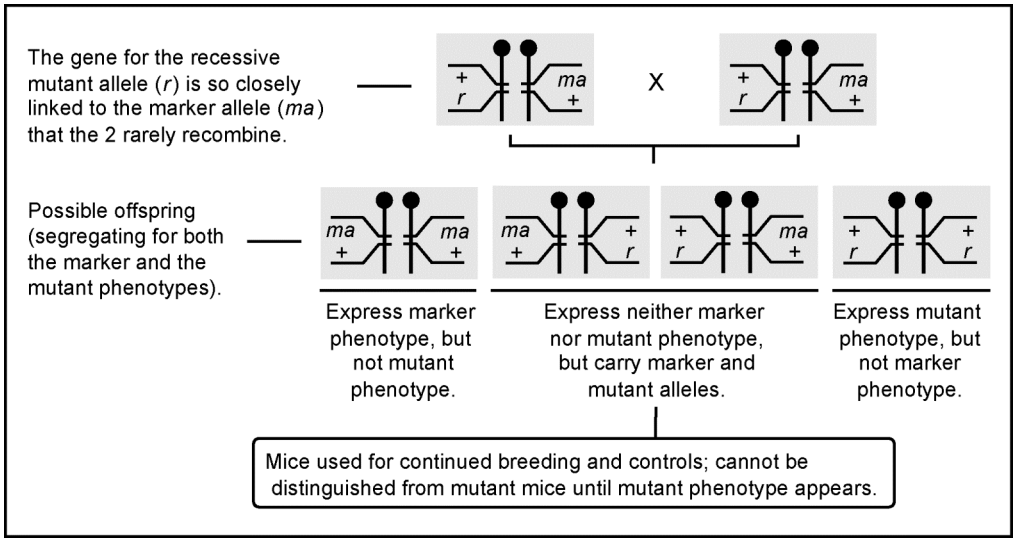
ma = recessive marker allele; *Ma* = dominant marker allele; *r* = recessive mutant allele.

A note about conventions…	Coupling	Repulsion
In this appendix, we chose to represent linked dihybrid genotypes using vertical lines for each genotype so that we could more clearly specify the allelic composition at each locus. The commonly used graphic and typographic conventions are shown at the right. The examples are for mice with linked genotypes *ma*/+ and *r*/+.	*ma* *r* + + OR *ma* r/+ +	*ma* + + *r* OR *ma* +/+ *r*

Offspring of double heterozygotes in repulsion with a recessive marker: new breeders that express neither the marker nor the mutant phenotype

Figure I.1 illustrates the results of breeding double heterozygotes in repulsion with a recessive marker allele. The offspring that are double heterozygotes in repulsion are "normal" appearing. They do not express either the marker or the mutant phenotype, but they are expected to carry both recessive alleles. They are used to create the next generation and are also used as controls, but they cannot be distinguished by sight from the offspring that express the mutant phenotype until the mutant phenotype appears.

Figure I.1. Illustration of balanced stock with double heterozygotes in repulsion—recessive marker allele.



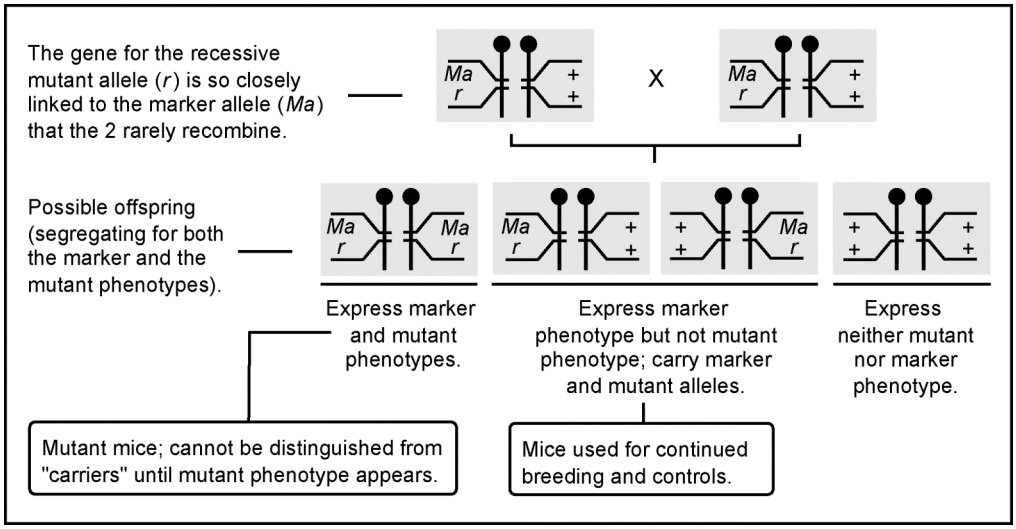
ma = recessive marker allele; *r* = recessive mutant allele.

The double heterozygotes in repulsion will be produced at a frequency that is dependent on the linkage between the marker gene and the gene of interest. For example, if the genes are 7 cM apart, 93% of the mice that express neither the marker nor the recessive phenotype will be double heterozygotes in repulsion. When these mice are bred, 86% (0.93 x 0.93) of the matings will produce the expected phenotypic ratios in the offspring (Green, 1966). Only the heterozygotes in repulsion from these breeding pairs should be used to propagate the line. (It is important to note that the few wild-type animals that result from crossovers should not be used for breeding.)

Offspring of double heterozygotes in coupling with a dominant marker: new breeders that express the marker phenotype but not the mutant phenotype

Figure I.2 illustrates the results of breeding double heterozygotes in coupling when the marker allele is dominant. The offspring that are double heterozygotes in coupling express the dominant marker phenotype. They do not express the recessive mutant phenotype, but they carry a recessive allele. These offspring are used to create the next generation and are also used as controls, but they cannot be distinguished by sight from the offspring that express the mutant phenotype until the mutant phenotype appears.

Figure I.2. Illustration of balanced stock with double heterozygotes in coupling—dominant marker allele.

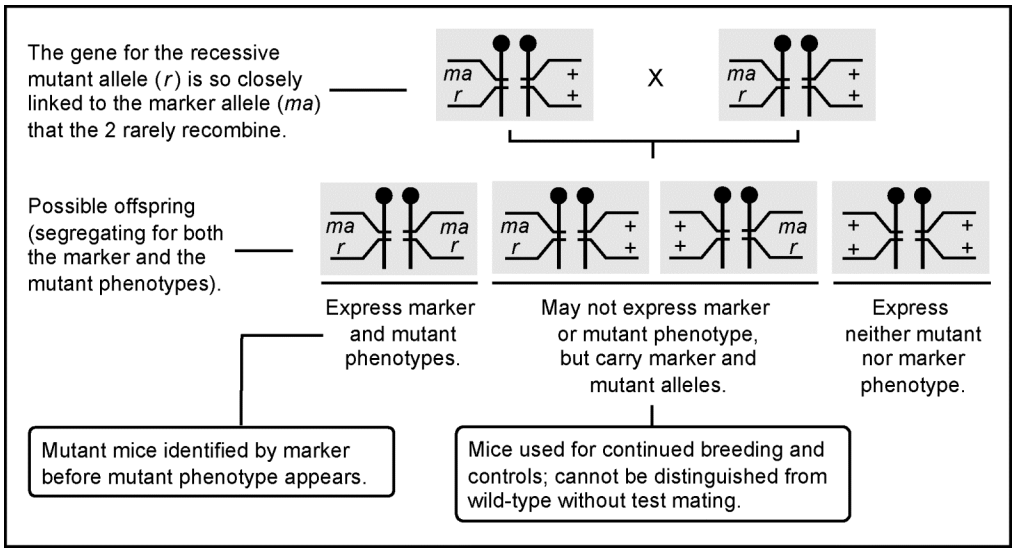


Ma = dominant marker allele; *r* = recessive mutant allele.

Offspring of double heterozygotes in coupling with a recessive marker: mutant mice that express the marker phenotype before expression of the mutant phenotype

Figure I.3 illustrates the results of breeding double heterozygotes in coupling when the marker allele is recessive. The offspring that express the marker phenotype also express the mutant phenotype. These mutant mice can be identified as soon as the marker phenotype appears, which could be well before the mutant phenotype appears. The offspring that are double heterozygotes in coupling do not express either the marker or mutant phenotypes. They are carriers of both recessive alleles, but they cannot be distinguished by sight from the wild-type offspring. Although either of these genotypes can be used as controls, only the double heterozygotes in coupling can be used to propagate the strain. These mice must be identified by test mating. Breeder pairs that do not produce a mouse with the marker phenotype within the first 13 offspring should be discarded.

Figure I.3. Illustration of balanced stock with double heterozygotes in coupling—recessive marker allele.

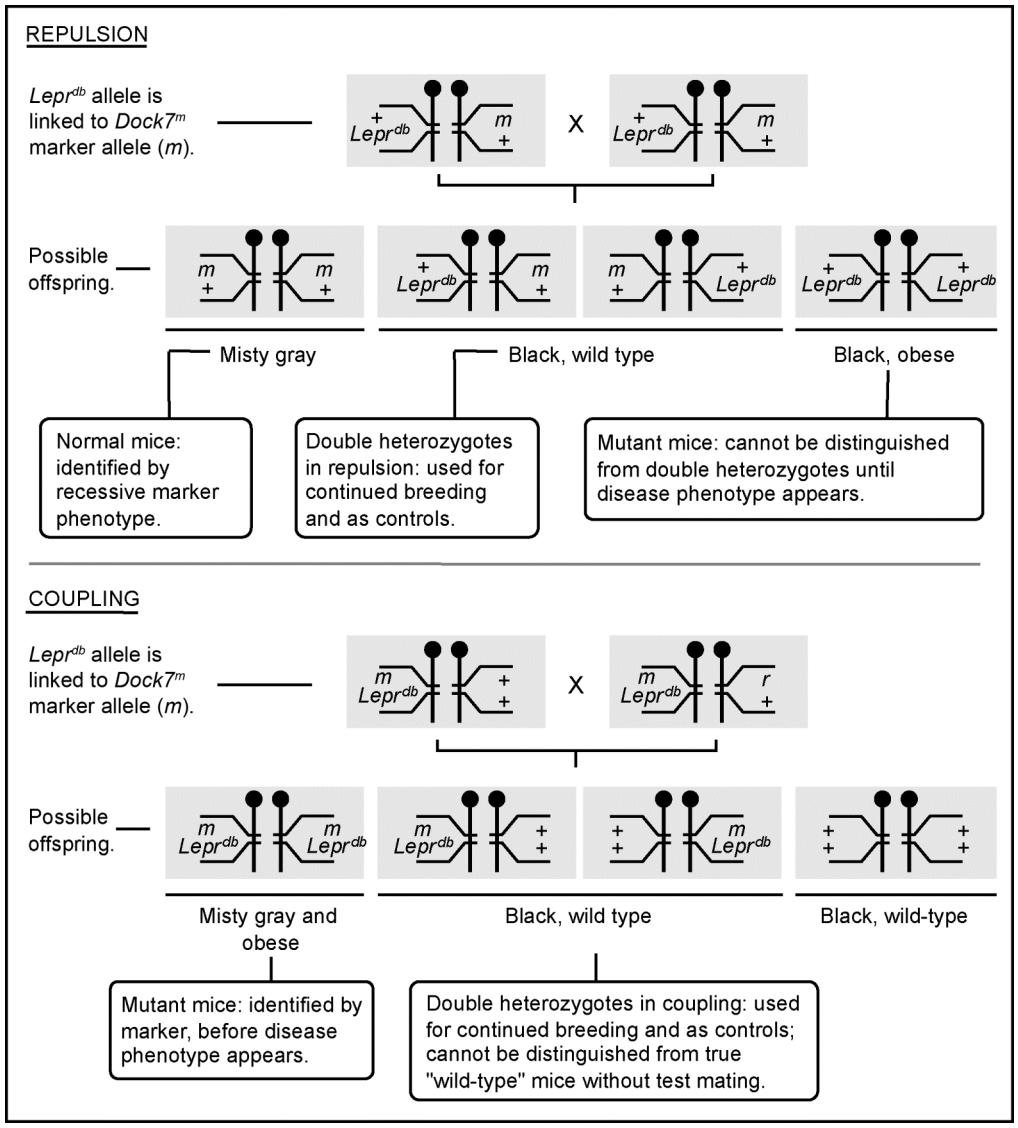


ma = recessive marker allele; *r* = recessive mutant allele.

Example of balanced stock: BKS.Cg-*Dock7*^{m} +/+ *Lepr*^{db} (000642)

An example of a strain of JAX® Mice maintained as a balanced stock is BKS.Cg-*Dock7*^{m} +/+ *Lepr*^{db}/J (000642), in which the *Dock7*^{m} allele is balanced with the *Lepr*^{db} diabetes mutant allele on Chr 4. *Lepr*^{db} homozygotes are sterile, and a balanced stock provides a way to breed mutant mice. We distribute both double heterozygotes in repulsion and double heterozygotes in coupling. Breed the heterozygotes in repulsion to produce the greatest number of carriers of the mutation. Breed the heterozygotes in coupling to produce mutant mice that can be identified by the marker before the disease phenotype appears. Figure I.4 illustrates the implications of breeding each genotype.

Figure I.4. BKS.Cg-*Dock7*^{m} +/+ *Lepr*^{db} (000642): implications of breeding double heterozygotes in repulsion and double heterozygotes in coupling.



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Appendix J: Cryopreservation

Cryopreservation is the freezing of biological tissues and organs at temperatures less than -80 C (-112 F) for the purpose of preservation and live recovery. Cryopreservation technology is a critical colony management tool used for assisted reproductive techniques (ARTs), minimization of genetic drift, and preservation of a strain or stock of mice, either to free up colony shelf space or to provide a way to restore a line in the event of a disaster.

Our Cryopreservation Laboratory provides services for internal and external investigators. We also present courses and seminars on cryopreservation, both at our campus and at other institutions. This appendix provides an overview of the cryopreservation process and our cryopreservation activities.

Where to get information about cryopreservation at The Jackson Laboratory…

JAX® Services:
Telephone:
1-800-422-6423 (North America)
1-207-288-6294 (International)
Email:
jaxservices@jax.org
Web:
www.jax.org/jaxservices/cryopreservation-and-recovery

Our Cryopreservation Laboratory:
www.jax.org/cryo

Training:
www.jax.org/courses

Overview of cryopreservation procedures

Cryopreservation of sperm, ovaries, and embryos requires specific protocols, which include specifications for the cryoprotectant media, time and temperature requirements, and techniques and procedures for tissue collection, freezing, and thawing. All cryopreservation protocols must be followed very precisely to help assure recovery of live tissue. For details of our cryopreservation program at The Jackson Laboratory, visit our Cryopreservation Laboratory website at www.jax.org/cryo.

Sperm

Although researchers have been cryopreserving sperm for many years, fertilization rates using thawed sperm have been quite low and inconsistent, especially for several popular strains of inbred mice such as C57BL/6J (000664) and FVB/NJ (001800). Now, however, researchers at The Jackson Laboratory have developed a patented technique that has significantly improved fertilization rates to 50 percent or greater for these and other strains (JAX® NOTES, 2006, 2008).

Cryopreservation of sperm is most often used for single mutations on common inbred backgrounds, most transgenics, and strains developed using homologous recombination.

Sperm cryopreservation is now a viable alternative at The Jackson Laboratory.

Historically, the use of sperm cryopreservation was limited by poor recovery rates. Researchers at The Jackson Laboratory are funded to study ways to improve cryopreservation methods. In the spring of 2006, we developed patent pending techniques that greatly improve the recovery of frozen sperm for strains for which recovery was poor (Ostermeier *et al.*, 2008). This advancement enhances the application of sperm cryopreservation as a colony management tool.

Freezing techniques

The epididymides and vas deferentia are removed from the mouse and placed in cryoprotectant medium. Sperm are released into the medium and then frozen.

Thawing techniques

Frozen samples are thawed rapidly by removing them from liquid nitrogen storage and placing them into a warm water bath until all ice crystals are melted (approximately two minutes). Immediately, morphology and motility are evaluated. Thawed sperm should be used as soon as possible for *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI).

Considerations

- Progeny will be heterozygotes or a mix of heterozygotes and wild-type.
- Yield from cryopreserved sperm is high—10^7 sperm per male.
- Recovery of cryopreserved sperm from F1 and outbred strains is excellent; recovery from inbred strains is much better with improved techniques.

Ovaries

Cryopreservation of ovaries is an alternative when embryo freezing cannot be used or is not cost effective. It also is a way to preserve the genotype of a female when you are not ready to breed her or do not have sperm available for IVF or ICSI.

It takes longer to produce live mice from frozen ovaries than from frozen sperm or embryos. This is because the female must recover for one to two weeks after the ovarian transplant before being set up for mating. Then time must be allowed for her to become pregnant.

Cryopreservation of ovaries is most often used for mice with single mutations on a common inbred background, most transgenics, and strains developed using homologous recombination.

Freezing techniques

Ovaries are removed and placed in a Petri dish containing medium. Each ovary is divided into two to four segments. The segments are transferred to cryoprotectant solution and then into cryotubes. Ovaries are then cooled and frozen.

Thawing techniques

The protocol for thawing frozen ovaries or ovary segments involves rehydrating in fresh medium. Following thawing, the ovarian tissue can be used immediately in ovarian transplantation or for IVF.

Considerations

- Progeny will be heterozygotes or a mix of heterozygotes and wild-type.
- Cryopreservation of ovaries does not preserve Chr Y.
- Yield from cryopreserved ovaries is low—approximately 20–50 live born per ovarian donor.
- Reliability of recovery of cryopreserved ovaries is questionable.
- For ovary transplantation, recipient females must be histocompatible with the ovary donor.

Embryos

For many years at The Jackson Laboratory, we used the method of Wittingham, Liebo and Mazur (1972), for which embryos are frozen in cryovials using dimethyl sulfoxide (DMSO) as a cryoprotectant. Since 2002, we have used the method of Renard and Babinet (1984), which uses semen straws for storage and propylene glycol (PrOH) as the cryoprotectant. Using the PrOH method, we can cryopreserve 2-cell embryos, pack straws more densely in the freezer, and, due to the chemistry of PrOH, use a less-expensive controlled rate freezer. This method has one major disadvantage: because of the reduced thermal mass, straws must be handled with care to avoid inadvertent warming and damage.

Cryopreservation of embryos is useful when preservation of the entire genome is necessary, such as for strain preservation or for preservation of multiple, unlinked loci.

Freezing technique

Oocytes are removed from super-ovulated females and fertilized *in vitro*. Embryos are screened carefully for abnormalities. Then they are loaded into medium-filled straws and frozen.

Thawing technique

Embryos are thawed and transferred into the oviducts of pseudopregnant recipients.

Considerations

- Cryopreservation of embryos is an inexpensive, simple, reliable, technique.
- Compared to other techniques, the yield of live pups per donor is relatively low—less than 15 live born per oocyte donor (seven per oviduct).

Advantages of cryopreservation

Cryopreservation has several distinct advantages for colony managers.

Colony management issues

- Cryopreserve a strain to free up colony shelf space. Balance the cost of cryopreservation and storage against the cost of maintaining the live strain and the risk of losing the strain.
- Cryopreserve a rescued stock or a rare stock you cannot afford to lose.
- Cryopreserve a stock for backup to insure against catastrophic loss—from genetic or pathogenic contamination or natural disaster.

For any of these applications, storing additional frozen tissue off-site provides extra security.

Purchasing cryopreserved embryos rather than live mice…

Sometimes, purchase of cryopreserved embryos is a preferable alternative to shipment of live mice. This strategy is as applicable to collaborators sharing mice as it is to researchers purchasing mice from a supplier.

Frozen tissue generally is not subject to the same importation restrictions as live animals are. The frozen embryos are germ free. Also, shipping costs are less. Of course, the researcher must have the appropriate facilities and expertise to thaw and implant the embryos successfully.

Genetic issues

- Cryopreserve a strain to minimize genetic drift. By refreshing your breeding stock with cryopreserved embryos every 10 generations, you effectively reduce the number of generations in the breeding lineage and greatly minimize genetic drift.
- Cryopreserve a transgenic strain after you have established that the transgene is in the animal and working and, again, after you have completed any additional genetic manipulations. If you subsequently lose the transgene through breeding, you can restore the stock with the appropriate frozen embryos.
- Cryopreserve stocks that must be maintained with complex breeding schemes. Because complex breeding schemes increase the chance for breeding errors, cryopreserved embryos are valuable insurance against losing the strain or stock.

Cryopreservation resources at The Jackson Laboratory

The Cryopreservation Laboratory

The Jackson Laboratory has maintained a successful cryopreservation program—cryopreserving and reconstituting strains—for more than two decades. We have cryopreserved more than 3,000 lines as sperm, ovaries, or embryos. We recover more than 400 lines per year. Our cryopreservation laboratory comprises a group of researchers dedicated to improving cryopreservation techniques and training the scientific community in those techniques. For more information, visit www.jax.org/cryo.

Cryopreservation facilities

At The Jackson Laboratory we store cryopreserved stocks in liquid nitrogen (-196 C [-320.8 F]) in a secure facility on our campus. We also have backup storage at a remote site.

Features of all of our cryopreservation services. We…

- Store frozen material in multiple tanks at two locations.
- Incorporate numerous quality controls, including test thaws of control embryos.
- Continuously monitor all tanks with an alarm system and we regularly monitor visually.
- Provide an annual report detailing the number of straws and balance of storage time remaining on your contract.
- Offer three options at the end of the contract period. You can
  - pay for additional storage,
  - instruct us to destroy the embryos, or
  - release the embryos to The Jackson Laboratory and authorize us to store and distribute them.

Cryopreservation-based JAX® Services

To meet specific needs of colony managers, both within and outside The Jackson Laboratory, we bundle cryopreservation services in many different configurations, customized to meet program objectives. Scheduling is an important component of these services. The length of time it takes to recover a frozen stock of mice depends on which tissues are cryopreserved, the availability of frozen material, and the yield and viability of the embryos produced. For most recovery projects, live mice can typically be recovered within 15 weeks of order placement.

We can also deploy teams of reproductive specialists to your facility to cryopreserve large numbers of strains. This service is ideally suited for institutions or investigators with large rederivation projects or substantial backlogs of strains that must be cryopreserved. On site, our teams can routinely cryopreserve 120 strains or more in a week.

For an overview of our JAX® Services related to cryopreservation, refer to Chapter 18, "JAX® Services." Or contact us as indicated on the first page of this appendix.

Cryopreservation training programs

At The Jackson Laboratory, we provide cryopreservation training using several different strategies. Cryopreservation is a component of our Colony Management Workshop, which we offer multiple times each year. We also offer workshops, both at The Jackson Laboratory and at other institutions, specifically tailored to the techniques of cryopreservation and associated ARTs. Topics are tailored to the audience, but options include the following: cryopreservation strategies, mouse reproductive biology, basic cryobiology, sperm cryo (lecture and lab), *in vitro* fertilization (lecture, lab, troubleshooting), slow-rate freezing (lecture and lab), repository operations, and embryo vitrification (lecture and lab). For information about cryopreservation training, visit our courses and conferences website at www.jax.org/courses.

Other sources of information about cryopreservation

Davisson MG, Taft RA. 2006. Strategies for managing an ever increasing mutant mouse repository. *Brain Res*. 1091:225–257.

Byers SL, Payson SJ, Taft RA. 2005. Performance of ten inbred mouse strains following assisted reproductive technologies (ARTs). *Theriogenology*. 65:1716–1726.

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Sztein JM, Sweet H, Farley J, Mobraaten LE. 1998. Cropreservation and orthotopic transplantation of mouse ovaries: new approach in gamete banking. *Biol Reprod*. 58:1071–1074.

Taft RA, Davisson M, Wiles MV. 2006. Know thy mouse. *Trends Genet*. 22:659–653.

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Ostermeier GC, Wiles MV, Farley JS, Taft RA. 2008. Conserving, distributing and managing genetically modified mouse lines by sperm cryopreservation. *PLoS ONE*. 3(7):e2792. doi:10.1371/journal.pone.0002792.

Renard JP, Babinet C. 1984. High survival of mouse embryos after rapid freezing and thawing inside plastic straws with 1-2 propanediol as cryoprotectant. *J Exp Zool*. 230:443–448.

Whittingham DG, Leibo SP, Mazur P. 1972. Survival of mouse embryos frozen to -196 degrees and -269 degrees C. *Science*. 178:411–414.

JAX® NOTES. 2006. Reliable new sperm cryopreservation service developed at The Jackson Laboratory. *JAX® NOTES*. 504:1–2.

JAX® NOTES. 2008. New sperm cryopreservation milestone: 1,000 strains cryopreserved and successfully recovered. *JAX® NOTES*. 508:3.

Appendix K: Donating or Submitting a Strain of Mice to The Jackson Laboratory

The Jackson Laboratory is committed—and publicly funded—to distribute strains of mice developed by researchers at The Jackson Laboratory and elsewhere. We have accepted thousands of strains of mice that were donated by investigators and that are currently maintained live or cryopreserved. Due to funding constraints, the number of strains our Repository can accept in a year are limited. To supplement our strain donation program, we also offer a strain distribution program. This appendix provides overviews of both programs.

Strain donation

Following submission and approval of a strain donation from a researcher, we rederive the strain to produce SPF mice at The Jackson Laboratory and then house and distribute mice from our facility—with no cost to the investigator who donated the strain. If the donating investigator elects, we will provide up to three rederived breeder pairs to the donator (presuming that mice are available), also at no charge. Strain donation provides a real cost savings to donating researchers, who can free themselves from the time and expense of maintaining a colony and shipping mice to colleagues. (These costs can be especially bothersome for strains not being actively used for research.)

Strain donation has two other important benefits for donating researchers:

- It provides a contingency source of the strain.
- It fulfills NIH obligations for investigators to share mice.

The stringent importation policy of The Jackson Laboratory: a major component of both programs.

Donated or sponsored strains are subject to the same rigorous importation procedures as all strains of mice that enter The Jackson Laboratory. We quarantine and rederive all incoming mice. We quarantine the SPF progeny until weaning, and we release pups only after they "pass" pathological evaluation by our Animal Health organization.

For further details on our animal health program and importation procedure, please refer to Chapter 7, "Animal Health—Preventing, Identifying and Eradicating Microbial Contamination." Or visit our Animal Health website at www.jax.org/jaxmice/genetichealth or our importation website at www.jax.org/imr/importhome.

Responsibilities of a donating investigator

All donating investigators submitting a strain to The Jackson Laboratory have specific responsibilities to the scientific community and The Jackson Laboratory. These responsibilities are described in the document, "Information for Donating Investigators," available online at www.jax.org/grc/strain_submission_form. We strongly recommend that donating investigators review this document thoroughly before submitting a strain to us for review.

Selection criteria

All strain donations must be approved by our Genetic Resources Committee (GRC), comprising at least 10 scientists with extensive expertise in many areas of biomedical research. Committee members use the following evaluation guidelines for each donated strain:

- Importance of its current use for research (based on publication history and current demand).
- Importance of its anticipated or potential future use.
- Difficulty of maintaining it relative to its scientific value.
- Difficulty of re-creating the strain relative to the time and effort required for its importation and preservation.
- In the case of a potentially valuable mutant strain with low current demand, existence and reliability of other resources that would ensure its survival.

Where to learn more

For full program details, visit www.jax.org/grc. You also can call Tech Support at 1-800-422-6423 (North America) or 1-207-288-5845 (International). Or email us at micetech@jax.org.

What to do if you want to expedite the donation process or if we decline the submission

Sometimes our scheduling and funding priorities delay importation and distribution of a new strain or preclude us from accepting a new strain. In either case, donating investigators can take advantage of our Sponsored Strain Distribution program. A program summary follows.

Sponsored Strain Distribution

Our Sponsored Strain Distribution program is one of our JAX® Services. It represents a way for The Jackson Laboratory to share the cost of distributing novel mouse strains with the investigators who developed the strains. Our program fulfills the requirements of the official "NIH policy on sharing of model organisms for biomedical research."

Registering a mutation…

One function of the Mutant Mouse Resource group at The Jackson Laboratory is to register mutations discovered or developed by investigators. Even if researchers don't want The Jackson Laboratory to distribute a mouse strain, they might want to register the mutation. To do so, email mmr@jax.org.

Program commitments include the following:

- Rederivation and cryopreservation of the strain; development of an archive.
- Adaptation or refinement of existing genotyping protocols followed by posting on our website (www.jax.org).
- Development of a strain data sheet for our JAX® Mice Database.
- Submission of strain information to the Mouse Genome Informatics (MGI) databases (www.informatics.jax.org).
- Promotion of the strain to the international research community via an email campaign and inclusion in JAX® Mice literature.

Selection criteria

Candidate strains must meet the following criteria:

- Details of the phenotype or construction of the strain or both must have been published in a peer-reviewed journal.
- A functional allele-specific, PCR-based assay must be available.
- The strain must have a practicable fertility rate.
- The submitter must have the permission of the strain's creator or owner to donate the strain.

Where to learn more

For details about Sponsored Strain Distribution, visit www.jax.org/jaxservices/sponsoreddistribution. You also can call our JAX® Services representatives at 1-800-422-6423 (North America) or 1-207-288-6294 (International). Or email us at jaxservices@jax.org.

Appendix L: Simplifying Power Analysis to Determine Sample Size

Kevin Flurkey, Joanne M. Currer, David E. Harrison

Power analysis is the quantitative way to determine the optimal number of mice for an experiment. Its use has become more widespread because the National Institutes of Health and other funding agencies now require formal justification for the number of mice proposed for an experiment.

This appendix provides background information on power analysis and a computational shortcut that simplifies its application.

Definitions of precision and power

Any experiment that requires a statistical test involves a three-way trade-off among sample size, precision, and power. Precision refers to the minimum difference between two groups that an experimenter considers to be important. Power is the likelihood of detecting that difference. With power analysis, you can determine the minimal number of mice (sample size) you will need in order to have a reasonable chance (power) of identifying a given difference (precision) between experimental groups.

How to calculate sample size using our computational shortcut

The basic steps to determine sample size are as follows:

1. Choose the probabilities of a type I error (a false positive) and a type II error (a false negative) that are acceptable to you.
2. Choose whether you will conduct a two-tailed (bidirectional) test or a one-tailed (unidirectional) test.
3. With your choices from Steps 1 and 2, use our computational tool to solve the power analysis equation for sample size (n).
4. For a small sample size ($n \leq 25$ mice), make a final adjustment to n.

Below are the details for each step.

1. Choose the probabilities of type I and type II errors.

Type I errors

Type I errors are false positives. They occur when a statistical test indicates that two groups differ, even though there is no real difference. α is the probability of a type I error.

The conventionally accepted level for α is 0.05. When a larger value, such as 0.10, is used for α, results are generally considered "suggestive, but not definitive." Smaller values of α may be used when the cost of a false positive is particularly high.

Type II errors

Type II errors are false negatives. They occur when a real difference between two groups is not detected (i.e., the statistical test generates a false negative result). β is the probability of a type II error. The power of a test (i.e., the chance of detecting a given difference) is stated by the formula $1-\beta$.

Typically, β values of 0.10 or 0.20 are used. The greater tolerance for type II errors, compared to type I errors, reflects a general view that errors of *omission* are less serious than errors of *commission*. If a negative finding (i.e., a finding of no difference between the groups) is particularly important for the conclusions of a study, a β value of 0.10, or even 0.05, should be used. Whenever a finding of no difference between the groups is important, written results should include a statement of the precision of the test at a given power (for example, "In the present experiment a difference between the groups of at least 2 gm in body weight would have been detected 90% of the time.").

Can both error types occur for a single statistical test?

If a statistical test indicates that the means of the groups differ, the potential for only a type I error exists. If a statistical test does *not* indicate that the means of the groups differ, the potential for only a type II error exists. Thus, type I and type II errors cannot occur at the same time. However, when determining an appropriate sample size, power analysis must take into account the potential for either error type. (At the planning stage of the experiment, you do not know the results, and it is the results that determine whether you are vulnerable to type I or type II errors.)

2. Choose whether you will conduct a two-tailed or a one-tailed test.

Two-tailed test

For a two-tailed test, the direction of the difference between the mean of the treatment group and the mean of the control group is not specified *a priori*. Either a positive or a negative difference can be accepted as significant.

One-tailed test

For a one-tailed test, the direction of the difference is set by the researcher before the experiment is run. Only a single direction is tested, and any difference in the opposite direction will not be accepted as significant. One-tailed tests require a smaller sample size for a given power.

How do error types relate to the directionality of the test?

For a type I error, the researcher can designate *a priori* whether a test will be one- or two-tailed. If no designation is made, a two-tailed test is assumed. For a type II error, only one-tailed tests are possible.

3. Solve the power analysis equation for sample size *n*.

a. Using Figure L.1, select the two-tailed or one-tailed version of the power analysis equation.

Figure L.1. Power analysis equations.

Two-tailed test	One-tailed test
$n = (Z_\alpha + Z_\beta)^2(2\sigma^2/\delta^2)$	$n = (Z_{2\alpha} + Z_\beta)^2(2\sigma^2/\delta^2)$
Z_α = standardized score for α	$Z_{2\alpha}$ = standardized score for 2α
Z_β = standardized score for β	Z_β = standardized score for β
σ = population standard deviation	σ = population standard deviation
$\lvert\delta\rvert$ = minimum difference regarded as important	δ = minimum difference regarded as important

$(Z_\alpha + Z_\beta)^2$ $(Z_{2\alpha} + Z_\beta)^2$	"Multipliers" for two- and one-tailed tests. We've simplified the power analysis calculation by providing a computational tool (Table L.1) that uses your α and β choices to provide the multiplier value.
σ	The standard deviation for the population. This value is usually estimated from preliminary or previously published studies. It is the only parameter in power analysis that is estimated, rather than designated, by the researcher. Therefore, it is important to get as many estimates as possible to derive the most representative value for σ.
s	The standard deviation for the sample. Note that when using the power analysis equation to calculate sample size, s is substituted for σ.
δ	The precision of the experiment, i.e., the minimum difference between the means of the experimental group and the control group that is regarded as important. This value is set by the researcher based on experience and resource limitations.

b. Use Table L.1—our computational tool—to select the multiplier.

Table L.1 provides the multipliers that incorporate the standard Z scores for specific α and β values. Panel A is for two-tailed tests; Panel B is for one-tailed tests. The α and β values (in bold) and the standardized scores (in parentheses) are listed across the top row and down the left column. The intersecting cells represent the multipliers for those specific values.

Table L.1. Multipliers for various combinations of α and β

Panel A. Two-tailed test: multipliers for results of $(Z_\alpha + Z_\beta)^2$

α, (Z_α) / β, (Z_β)	**0.10**, (1.645)	**0.05**, (1.96)	**0.01**, (2.576)	**0.001**, (3.30)	**0.0001**, (3.8)
0.50, (0.0)	2.7	3.8	6.6	10.9	14.4
0.20, (0.842)	6.2	7.9	11.7	17.2	21.6
0.10, (1.283)	8.6	10.5	14.9	21.0	25.8
0.05, (1.645)	10.8	13.0	17.8	24.5	29.6

Panel B. One-tailed test: multipliers for results of $(Z_{2\alpha} + Z_\beta)^2$

α, ($Z_{2\alpha}$) / β, (Z_β)	**0.10**, (1.283)	**0.05**, (1.645)	**0.01**, (2.326)	**0.001**, (3.09)	**0.0001**, (3.7)
0.50, (0.0)	1.6	2.7	5.4	9.5	13.7
0.20, (0.842)	4.5	6.2	10.0	15.5	20.6
0.10, (1.283)	6.6	8.6	13.0	19.1	24.8
0.05, (1.645)	8.6	10.8	15.8	22.4	28.6

These tables adapted from Snedecor GW and Cochran GW. (1980), "Statistical Methods," 7th ed. Table 6.14.1, p. 104. The Iowa State University Press, Ames, IA.

c. Use the multiplier, substitute your δ value and your *s* value (estimate of σ), and solve the power analysis equation. Round any fractional result to the next highest integer.

$$n = multiplier * (2s^2/\delta^2)$$

The total number of mice in the study is 2*n* (the treatment group plus the control group).

4. Make final adjustments for a small sample size.

When the sample size per group is small (less than 25), add a correction factor to *n*. For α = 0.10 or 0.05, the sample size should be *n* + 1. For α = 0.01, the sample size should be *n* + 2. The correction factor is the same for both two-tailed and one-tailed tests.

Example of a calculation for a two-tailed test

The problem: We need to determine the number of mice per group (n) required for a study. Our criteria are as follows:

- We decided that the minimum difference that we consider important ($|\delta|$) is equal to the standard deviation (s) of our outcome variable. For our example, both $|\delta|$ and s equal 5.
- We chose the probability of a type I error (α) to be 0.05.
- We chose the probability of a type II error (β) to be 0.20 (power equals 80% probability).

The solution:

$$n = (Z_\alpha + Z_\beta)^2(2s^2/\delta^2) = multiplier * (2s^2/\delta^2)$$

1. Using Table L.1, Panel A, we selected the probability value of α (0.05) from the top row and β (0.20) from the left column. The intersecting cell contains the value of the multiplier $(Z_\alpha + Z_\beta)^2$. $(Z_{0.05} + Z_{0.20})^2 = multiplier = 7.9$
2. We substituted our $|\delta|$ and s values and completed the calculation. $n = 7.9 * (2(5^2)/5^2)$; $n = 15.8$; $n = 16$
3. We made the adjustment for a small sample size. n adjusted = 16 + 1 = 17 mice

This result can be generalized: For a two-tailed comparison of two groups, when the minimum difference considered important equals the standard deviation, if you want an 80% chance of detecting the difference, you need 17 mice per group.

Note: For a one-tailed test, we would have first specified the direction of the hypothesis we were testing. Then we would have used $(Z_{2\alpha} + Z_\beta)^2$ as the multiplier. The solution would be 6.2 * $(2(5^2)/5^2)$ = 12.4; n adjusted = 13 + 1 = 14 mice.

Additional considerations when comparing more than 2 groups

The formulae for calculating sample size that we present here are appropriate only for the comparison of two groups; power analysis is more complex for other experimental designs. However, these formulae may be used for any experimental design that can be divided into pair-wise comparisons. To do so, you should adjust the Z_α value for the number of comparisons before calculating the multiplier. For example, if your experimental design can be partitioned into four, two-tailed comparisons of two groups each, a Bonferroni adjustment would give α = 0.05 / 4 = 0.0125. Z_α would be the standardized score for α = 0.0125, which is 2.50 (obtained from the standard normal distribution).

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Snedecor GW and Cochran GW. 1980. *Statistical Methods*, 7th Edition. The Iowa State University Press, Ames, IA.

Appendix M: Courses and Educational Programs

Clarence Cook Little founded The Jackson Laboratory in 1929. Since 1924, Little had been running student programs on the current site of The Jackson Laboratory, and in 1931 he organized the first formal summer student program, thus establishing a powerful link between our research and education initiatives. Today, in conjunction with our research programs, we offer numerous courses and educational programs here at our campus in Bar Harbor and at partnering institutions around the world. Following is a brief overview of our programs.

Where to get more information…

Upcoming courses and conferences at The Jackson Laboratory:
www.jax.org/courseseducation

Conferences we will be attending:
www.jax.org/booth/index

Mailing list signup form:
www.jax.org/courses/signup_form

Postdoctoral opportunities

Postdoctoral opportunities at The Jackson Laboratory provide research experience under the tutelage of a faculty member. Postdoctoral associates also participate in seminars, meetings, courses, and workshops; apply for fellowship grants; present results to in-house interest groups; and publish the results of their research in peer reviewed journals. Our staff works closely with our postdoctoral associates, which normally number around 50, to ensure that their research experience at The Jackson Laboratory is as productive and as successful as possible. For details, visit www.jax.org/research/opportunities/postdoc.

The Jackson Laboratory also offers a special postdoctoral training program for veterinarians. This three-year program offers mentor-based training in normal mammalian development and in animal models of human disease. For details, visit www.jax.org/research/opportunities/vet.

Postdocs rate our program at The Jackson Laboratory…

On an annual basis *TheScientist.com* conducts an international survey to determine which research institutions and universities provide the best experience for postdoctoral associates. In 2008, The Jackson Laboratory was ranked #9 among U. S. research institutions (Scheff, 2008).

Courses and conferences

The Courses and Conferences Program seeks to promote both research and resource missions of The Jackson Laboratory by offering a wide array of courses, conferences, and workshops designed to educate and train members of the scientific community in the use of the laboratory mouse as a platform for discovery research in biomedicine. The Office of Courses and Conferences develops and administers programs that bring large numbers of outside investigators to the Laboratory each year, enabling collaboration and productive discourse between Jackson Laboratory scientific staff and the global research community. The Office also oversees and otherwise administers traveling workshops designed to train scientists how to more efficiently use the scientific resources provided by The Laboratory.

The Jackson Laboratory is a thought leader in the development and application of mouse models for research on human disease, and has historically served as a gathering place for scientists from around the world. Currently, The Jackson Laboratory is recognized as one of the only institutions of its kind to offer a courses and workshops program focusing on the use of the laboratory mouse and related resources in basic and translational research.

In addition to programs hosted on-site in Bar Harbor, the Office of Courses and Conferences administers a wide range of highly focused practical workshops at participating institutions around the world. These hands-on workshops are specifically designed to promote the use of mouse resources by making training more accessible to investigators and their staff who may not be able to travel to Bar Harbor.

Following are highlights of several of our most popular, regularly held courses and conferences. For more information about courses and conferences, visit www.jax.org/courses.

Short Course on Medical and Experimental Mammalian Genetics

The two-week Short Course on Medical and Experimental Mammalian Genetics (the "Short Course") has been held at The Jackson Laboratory every summer since 1960. The course was organized by Drs. Victor McKusick and John Fuller as a collaboration between The Jackson Laboratory and Johns Hopkins University. The initial goal was to improve genetics education in medical schools (Ledger, 1999). Over the years, the objective has broadened to include presentation to the public of the latest developments in both experimental animal and human genetics.

Speakers include faculty members from The Jackson Laboratory and Johns Hopkins University as well as prominent scientists from other institutions throughout the world. The Short Course continues to be one of our most highly regarded courses.

A few words about one of the originators of the Short Course, Dr. Victor A. McKusick…

Dr. Victor A. McKusick, one of the co-founders of the Short Course, was a recipient of the National Medal of Science in 2002. In 2008 he was awarded the prestigious Japan Prize for Medical Genetics and Genomics for his role in the establishment and development of the field of medical genetics.

We are proud of the long and productive relationship we have had with Dr. McKusick, and in the important role he has played in helping us educate the medical community and investigators about the latest directions and discoveries in biomedical research.

Short Course on Experimental Models of Human Cancer

The Jackson Laboratory offers a variety of other residential "short courses" that are disease focused and designed for advanced predoctoral and postdoctoral students as well as established investigators. A typical example is The Short Course on Experimental Models of Human Cancer, which focuses on the mouse as an experimental model for translational cancer research. Over a period of eight days, students are exposed to a mix of formal lectures on genetics, genetic tools, translational models for cancer research, stem cells, and epigenetics. Hands-on workshops and tutorials include genome informatics; mouse colony management; necropsy, gross pathology tumor histopathology, and *in vivo* imaging.

The course is held in a retreat-like setting and is limited to 35 participants to ensure a supportive learning atmosphere with exceptional interaction between students and faculty. Course participants and faculty reside at the historic Highseas Conference Center in dormitory style housing, consisting of shared bedrooms and bathrooms. Highseas overlooks Frenchman Bay on the rocky coast of Maine and is adjacent to beautiful Acadia National Park. Meals are prepared by our resident chef.

Colony Management: Principles and Practices and other practical workshops

We offer many hands-on workshops designed to train scientists in the practical aspects of using the laboratory mouse and related resources in their research. Colony Management: Principles and Practices is one of our most popular workshops and is offered several times throughout the year, both at our Bar Harbor facility and at other institutions. This workshop provides training in the theory and practice of maintaining mouse colonies for production and research. Topics include genetics of the laboratory mouse, nomenclature, animal health, genetic quality control, breeding strategies, general husbandry, cryopreservation, and disaster prevention.

Educational programs

Educational programs at The Jackson Laboratory are tailored to scientists at various levels of their career—from talented high school students investigating genetic diseases to experienced researchers defining the cutting edge of genomic research. Programs include the following:

Summer student program

The Jackson Laboratory summer student program is one of the most significant ways in which we immerse high school and undergraduate students in scientific research and the responsibilities of independent work.

Our training department works with high school and university administrators to solicit applications and select students who can most benefit from this unique opportunity of independent research under the mentorship of a research scientist. Although participants learn about techniques and fundamentals of biology, the emphasis of the program is on methods of discovery and communication of new knowledge, not mastery of established facts. At the end of the program, students present their results at the annual Summer Student Symposium, attended by their mentors, peers, parents, and The Jackson Laboratory community.

The summer student program is about much more than research. Students spend between eight to ten weeks in Bar Harbor, where they reside together dormitory style at Highseas, a seaside Georgian retreat located on campus and overlooking beautiful Frenchman Bay. An important component of the summer student program experience is the opportunity for intellectual and social interaction between other students and Faculty. The program is renowned for the opportunities it provides to exceptional science students and currently claims at least two Nobel Laureates among its alumni.

For details about the program, visit www.jax.org/education/summerstudent.

The success of our summer student program…

The Jackson Laboratory summer student program is one of the accomplishments about which we are most proud. Since its inception in 1931, more than 2,000 students have participated in the program. Several students have done quite well.

Two graduates of our program, David Baltimore and Howard M. Temin, were awarded the Nobel Prize in Medicine or Physiology for their discoveries concerning the interaction between tumor viruses and the genetic material of the cell. In Dr. Baltimore's Nobel Prize autobiography, he states that his interest in biology began when he attended our summer student program as a high school student in 1952 (Baltimore, 1976). Dr. Temin writes in his autobiographical entry that his specific interest in biological research was focused by several summers he spent as part of our summer program, between 1949 and 1952 and again in 1955 (Temin, 1976).

But it isn't just Nobel Prize winners who have benefited from our Summer Student Program. We are especially proud of our program graduates who have returned home and opened doors to possibilities they did not even know existed before their summer in Bar Harbor.

College academic year program

Our college academic year program is a way for undergraduate students to spend an academic year working full-time on an independent research project under the sponsorship of a faculty member of The Jackson Laboratory. Some participants are previous summer students who want to continue their research programs. Others want "wet lab" experience that is not a part of their normal undergraduate curriculum. Others are participants in educational programs that require specific research experience. Academic credit is provided in accordance with the policy of the student's college or university.

For more information, visit www.jax.org/education/academicyear.

Predoctoral opportunities

The Jackson Laboratory participates in a graduate program in conjunction with the Graduate School of Biomedical Sciences (GSBS) at The University of Maine. The program consists of six tracks: cell and molecular biology, functional genomics/interdisciplinary studies, neurobiology, cell and molecular biology, toxicology, and biomedical engineering.

For more information, visit www.jax.org/education/predoc.

Other academic programs

- Master of Science in Teaching program, for secondary science and mathematics teachers participating in the University of Maine Center of Science and Mathematics Education Research. For more information, visit www.jax.org/education/mst.
- High school internship, for local Maine high school students. For more information, visit www.jax.org/education/hs-internship.

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Appendix N: Sources of Information about Laboratory Mice

Following are a list of web addresses for information accessible online and a list of print resources that researchers at The Jackson Laboratory have found particularly useful.

Information available at The Jackson Laboratory website: www.jax.org

The Jackson Laboratory website (www.jax.org) provides a wide variety of information related to laboratory mice, research conducted using them, and the relationship of this research to human health. Following is a list of some of the information available at the time this book was printed. We have included direct web address for your convenience. We regularly evaluate and update information, so we recommend browsing www.jax.org frequently.

Web-based information related specifically to JAX® Mice.

JAX® Mice database	www.jax.org.jaxmice/query
JAX® Mice and Services	www.jax.org/jaxmice
Courses and educational programs	www.jax.org/courseseduation/index
Animal health and genetic quality	www.jax.org/jaxmice/geneticheath
Our repositories	www.jax.org/jaxmice/findmice/repository
Research being conduced by our faculty	www.jax.org/research/faculty
Relevance of our research to human health	www.jax.org/advances/index
New discoveries and news about The Jackson Laboratory; registration for email notification of updates (RSS feed) and JAX enews.	www.jax.org/news/index

Printed material, PDF files, and email newsletters related specifically to JAX® Mice, all available at www.jax.org/jaxmice/literature.

Catalogs and general literature:

- Most Popular JAX® Mice Strains Catalog
- Complete 2007–2009 JAX® Mice Catalog
- JAX® Services June 2008–May 2009 Catalog
- 2007–2009 Price List (domestic)
- 2007–2009 Price List (international)

Resource manuals (in-depth educational guides on our mouse models and other tools:

- Autoimmune disease
- Breeding strategies
- Cancer
- Cardiovascular
- Genetic background
- Infectious disease
- Neurobiology
- Research tools
- Sensorineural
- Type 2 diabetes and obesity

Posters and calendar:

- JAX® Mice coat colors
- 14-day pups (appearance at 3–14 days old)
- Divergence of C57BL6 substrains
- Choosing immunodeficient JAX® Mice
- 2009 Courses and Conferences calendar

Email newsletter subscriptions:

- JAX® Mice News email biweekly newsletter
- DIO (diet-induced obesity) weekly newsletter
- JAX® NOTES email quarterly newsletter
- Special J offers (limited offers for JAX® Mice)

For more information…	
Mouse Genome Informatics (MGI)	Web: www.informatics.jax.org Email: mgi-help@informatics.jax.org Phone: 1-207-288-6445
Customer Service	Web: www.jax.org/jaxmice/orders Email: orderquest@jax.org Phone: 1-800-422-6423 (United States) 1-207-288-5845 (International)
Technical Support	Web: www.jax.org/jaxmice/micetech Email: micetech@jax.org Phone: 1-800-422-6423 (United States) 1-207-288-5845 (International)
JAX® Services	Web: www.jax.org/jaxservices Email: jaxservices@jax.org Phone: 1-800-422-6423 (United States) 1-207-288-6294 (International)

General online resources and print resources

Online books and other information related to laboratory mice in general, available at www.informatics.jax.org

On-line books: • *Biology of the Laboratory Mouse*. 1968. Green EL, ed. • *Mouse Genetics, Concepts and Applications*. 1995. Silver L. • *The Anatomy of the Laboratory Mouse*. Cook M. 1965. • *The Coat Colors of Mice, A Model for Mammalian Gene Action and Interaction*. 1979. Silvers WK. • *Origins of Inbred Mice*. 1978. Morse HC III, ed.	www.informatics.jax.org (More Resources menu option)
Festing's characteristics of inbred mice and rats	www.informatics.jax.org/external/festing/search_form.cgi
Poster of "Genealogies of mouse inbred strains"	www.informatics.jax.org/mgihome/genealogy

Print resources

- *Guide for the Care and Use of Laboratory Animals*. National Resource Council. 1996. National Academy Press. Washington DC.
- *The Laboratory Mouse*. Hedrich HJ (ed). 2004. Elsevier, London.
- *The Laboratory Mouse*. Vol. 8. Laboratory Animal Pocket References. Suckow M, Danneman P, Brayton C. 2000. CRC Press. Boca Raton FL.
- *The Mouse in Biomedical Research,* 2nd edition. Fox JG, *et al*. (eds). 2007. American College of Laboratory Animal Medicine Series. Academic Press. NY.

Ordering JAX® Mice

Web: www.jax.org/jaxmice/orders
Email: orderquest@jax.org
Phone: 1-800-422-6423 (United States)
1-207-288-5845 (International)

Appendix O: General Biological Information about Laboratory Mice

Diploid chromosome number	40
Adult weight (females and males)	20–40 g
Newborn weight	0.75–1.5 g
Food consumption	1.5 g/10 g body weight (~3–6 g/day)
Water consumption	1.5 ml/10 g body weight (~3–6 ml/day)
Rectal temperature	36–39 C
Heart rate	310–800 bpm
Blood volume	0.6–0.8 ml/10 g body weight
Weaning age	18–28 days
Sexual maturity	28–60 days
Estrous cycle	4–5 days
Gestation	18–21 days
Litter size	2–12 pups
Post-partum estrus	Fertile
Reproductive lifespan (female)	Terminates at 6–12 months
Reproductive lifespan (male)	Terminates at 12–14 months
Lifespan	1.5–3 years

Several places in this handbook provide more specific detail:

- Table 4.1, "Reproductive performance for selected strains of JAX® Mice," page 138.
- Table 13.1, "Reproductive characteristics for most inbred strains of laboratory mice," page 242.
- Appendix G, "Equivalencies of Human Age to Life Phases of Mice," page 329.

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