# 1

# Summary of Currently Available Mouse Models

*Ami Ito, Namiko Ito, Kimie Niimi, Takashi Arai, and Eiki Takahashi*

*RIKEN Brain Science Institute, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan*

## 1.1 Introduction

It is estimated that laboratory mice comprise more than 80% of the animals used in research. Researchers have nearly 100 years of experience working with mice in the field in settings such as commercial rodent production sites, laboratories, academic institutions, and pharmaceutical companies and have also performed resource studies. Mice require relatively little space and do not have complex dietary needs. They are easy to physically manipulate and relatively inexpensive compared to other laboratory animals. Although this is problematic with some species, mice can be inbred, which enables inclusion of littermates as controls and production of large number of animals. Production of inbred strains means that their backgrounds can be defined. Given ideal conditions, mice can produce at least four generations in a year, meaning that multiple generations can be produced rapidly and followed for experimental purposes. As small mammals, they have a limited lifespan, which facilitates aging and multigenerational studies.

Mice were the first mammals after humans to have their genome sequenced [1]. As there is approximately 85% homology between the mouse and human genomes, any given gene is most likely present in both the mouse and human genomes and will generally have a similar function [1]. This allows mice to serve as models of many human conditions and, more importantly, allows us to study basic mammalian genetics and other conserved systems in mammalian cells.

The study of mutant mice has evolved from the exploration of the collections of the spontaneous coat color mutants maintained by nineteenth century mouse fanciers to the advent of several methods of directed manipulation of the mouse genome. A further explanation for the dominance of the mouse in research is the robustness of their embryos. These may be cryopreserved and cultured from the one-cell stage to the post-implantation stage. Advances in molecular technologies have improved our ability to create mouse models of human

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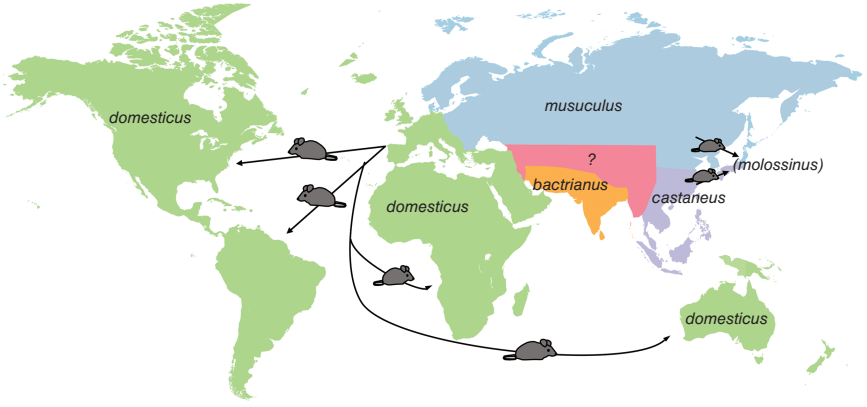


**Figure 1.1** Mice used in the laboratory are derived from a variety of sources.

disease by means of transgenesis, targeted mutagenesis, and the CRISPR/Cas9 system. These mutations continue to provide valuable research tools for the study of the functions of genes within the entire organism. Today, there are repositories of genetically mutant mice located around the world that provide scientists with access to models of many diseases, contributing substantially to our understanding of both basic biological pathways and disease mechanisms.

This chapter describes the history of laboratory mice; the types of mouse strains available; the handling of mouse colonies, mouse cell lines, and strains; and the use of mouse models in preclinical studies.

## 1.2 Origin and History of Laboratory Mice

Mice currently used in the laboratory are domesticated animals. Laboratory mice are fatter, slower, less aggressive, and more amenable to handling than their wild-caught counterparts. Mice likely originated on the Indian subcontinent and spread throughout the world with agriculture and human movement [2] (Figure 1.1). Contemporary mice have genetic contributions from both *Mus musculus* ssp. *musculus* and *Mus musculus* ssp. *domesticus*, and evidence indicates that *Mus musculus* ssp. *molossinus* and *Mus musculus* ssp. *castaneus* made smaller contributions. Therefore, mice should not be referred to by their species name but rather as laboratory mice or by a specific strain or stock name. Additionally, some recently developed laboratory mouse strains are derived wholly from other *Mus* species or other subspecies, such as *Mus spretus*.

The source of many of the mouse strains currently in use is the mouse colony established by Miss Abbie Lathrop (1868–1918) in her small white farmhouse in Granby, Massachusetts [3]. Dr. William E. Castle (1867–1962), a pioneer in mouse genetics, purchased some of Lathrop's mice and trained Dr. Clarence. C. Little (1888–1971), the founder of the Jackson Laboratory. Dr. Little bred

C57BL/6 from Lathrop's mouse number 57. The resulting C57BL/6 became the most frequently used strain of laboratory mice.

## 1.3 Laboratory Mouse Strains

### 1.3.1 Wild-Derived Mice

Wild-derived mice are descendants of mice originally caught in the wild. The available wild-derived strains are *M. musculus* ssp. *musculus*, *M. musculus* ssp. *domesticus*, *M. musculus* ssp. *molossinus*, *M. musculus* ssp. *castaneus*, *Mus caroli*, *Mus hortulames*, *Mus praetextus*, *Mus pahari*, and *Mus spretus* (Table 1.1). Wild-derived mice are genetically distinct from common laboratory mice in a number of complex phenotypic characteristics and are valuable tools for genetic evolution and systematics research. They enable mapping of both the single-gene traits and quantitative trait loci (QTL) contributing to complex phenotypes.

**Table 1.1** Origin of wild-derived inbred strains.[a)]

| Species | Geographic origin | Strain |
|---|---|---|
| *M. musculus* ssp. *musculus* | Kunratice, Czech Republic | PWD/PhJ |
| | Lhotka, Czech Republic | PWK/PhJ |
| *M. musculus* ssp. *domesticus* | California, USA | CALB/RkJ |
| | Lewes, Delaware, USA | LEWES/EiJ |
| | Ohio, USA | MOR/RkJ |
| | Tirano, Italy | TIRANO/Ei |
| | Monastir, Tunisia | WMP/PasDnJ |
| | Centreville, Queen Anne City, Maryland, USA | WSB/EiJ |
| | Zalende, Switzerland | ZALENDE/Ei |
| *M. musculus* ssp. *molossinus* | Japan | JF1/Ms |
| | Fukuoka, Kyushu, Japan | MOLC/RkJ, MOLD/RkJ |
| | | MOLF/EiJ, MOLG/DnJ |
| | Mishima, Shizuoka, Japan | MSM/Ms |
| *M. musculus* ssp. *castaneus* | Thailand | CASA/RkJ, CAST/EiJ |
| *M. caroli* | Thailand | *Mus caroli*/EiJ |
| *M. hortulanus* | Pancevo, Serbia | PANCEVO/EiJ |
| *M. pahari* | Thailand | *Mus pahari*/EiJ |
| *M. spretus* | Puerto Real, Cadiz Province, Spain | SPRET/EiJ |

*M., Mus.*

a) https://www.jax.org/search?q.

Source: Data from Jax Mice Database – Wild-derived Inbred Website.

### 1.3.2 Inbred Mice

Strains can be termed inbred if they have been mated brother × sister for 20 or more consecutive generations, and individuals of the strain can be traced to a single ancestral pair at the twentieth or subsequent generation. At this point, the individuals' genomes will, on average, have only 0.01 residual heterozygosity (excluding any genetic drift) and can, for most purposes, be regarded as genetically identical. Inbred mouse strains exhibit specific characteristics (Table 1.2) and provide a uniform genetic background for accurate phenotypic evaluation.

### 1.3.3 Hybrid Mice

Mice that are the progeny of two inbred strains, crossed in the same direction, are genetically identical and can be designated using uppercase abbreviations of the two parents (maternal strain listed first), followed by F1. Note that the reciprocal F1 hybrids are not genetically identical and their designations are, therefore, different.

**Examples**

D2B6F1: Mouse that is the offspring of a DBA/2N mother and a C57BL6/J father. A full F1 designation is (DBA/2N x C57BL/6J)F1.
B6D2F1: Mouse that is the offspring of a C57BL6/J mother and a DBA/2N father. A full F1 designation is (C57BL/6J x DBA/2N)F1.

For the sake of clarity, the full strain symbols of the above cases should be given in any publication when the hybrids or crosses are first referred to. If a hybrid were to be constructed using a substrain known to differ from the "standard" strain genetically and/or phenotypically, the substrain should be indicated in the hybrid symbol: e.g. C3H/HeSn = C3Sn. The approved abbreviations for common mouse strains are listed below:

129: 129 strains (may include subtype; e.g. 129S6 for strain 129S6/SvEvTac)
A: A strains, except for Heston substrains
A/He: A/He (Heston substrains)
AK: AKR strains
B6J: C57BL/6J substrains
B6N: C57BL/6N substrains
C: BALB/c strains
cBy: BALB/cBy (Bailey substrains)
cWt: BALB/cWt (Whitten substrains)
C3: C3H strains
CBA: CBA strains, except Carter substrains
CBA/Ca: CBA Carter substrains
D1: DBA/1 strains
D2: DBA/2 strains.

**Table 1.2** Inbred strain of mice.

| Strain | Comment | Substrains | Comment |
|---|---|---|---|
| 129 | 129 Strain has a high incidence of spontaneous testicular teratomas, although the incidence differs between substrains. They are widely used in the production of targeted mutations because of the availability of multiple embryonic stem (ES) cell lines derived from them. Major genetic variation exists between various sublines of the 129 "family." | 129S1/SvIm | |
| | | 129P3 | |
| | | 129X1/Sv | |
| | | 129S4/SvJae | |
| A | This strain is widely used to model cancer and for carcinogen testing given their high susceptibility to carcinogen-induced tumors. Other also uses this for hybridoma production for immunological research. | A | |
| AKR | AKR mice are useful in cancer, immunology, and metabolism research. | AKR | |
| BALB/c | BALB/c mice are used for the production of monoclonal antibodies. Mammary tumor incidence is normally low, but infection with mammary tumor virus by fostering to MMTV+ C3H mice dramatically increases tumor number and age of onset. BALB/c mice develop other cancers later in life, including reticular neoplasm, primary lung tumors, and renal tumors. | BALB/cBy | BALB/cBy was separated from the BALB/c strain in 1935. Rare spontaneous myoepitheliomas arising from myoepithelial cells of various exocrine glands have been observed in the BALB/cBy substrain. BALB/cBy has a deletion in the Qa2 subregion of the murine MHC. A deficiency of Acads (acyl-coenzyme A dehydrogenase, short chain) leads to severe organic aciduria. BALB/cByJ develops a fatty liver upon fasting or dietary fat challenge and become hypoglycemic after an 18-hour fast. |
| | | BALB/c | BALB/c is susceptibility to developing the demyelinating disease upon infection with Theiler's murine encephalomyelitis virus. The BALB/c substrain is also susceptible to *Listeria*, all species of *Leishmania*, and several species of *Trypanosoma* but is resistant to experimental allergic orchitis (EAO). |

**Table 1.2** (Continued)

| Strain | Comment | Substrains | Comment |
|---|---|---|---|
| C3H | C3H mice are used in a wide variety of research areas including cancer, infectious disease, sensorineural, and cardiovascular biology research. C3H substrains at The Jackson Laboratory are homozygous for the retinal degeneration 1 mutation (*Pde6b*$^{rd1}$), causing blindness by weaning age. | C3H/He | A spontaneous mutation in *Tlr4* occurred in C3H/HeJ at the lipopolysaccharide response locus (mutation in toll-like receptor 4 gene, *Tlr4*$^{Lps\text{-}d)}$ making C3H/HeJ mice more resistant to endotoxin. C3H/HeJ mice are highly susceptible to infection by Gram-negative bacteria such as *Salmonella enterica*. |
| | | C3H/HeOu | C3H/HeOuJ mice show high incidence of hepatoma. This strain does not carry mouse mammary tumor virus (MMTV), but virgin and breeding females may still develop some mammary tumors later in life. |
| C57BL/6 | C57BL/6 is the most widely used inbred strain. Although this strain is refractory to many tumors, it is a permissive background for maximal expression of most mutations. Five single nucleotide polymorphism (SNP) differences have been identified that distinguish C57BL/6J from C57BL/6N. | C57BL/6J | C57BL/6J is the first to have its genome sequenced. C57BL/6J mice are resistant to audiogenic seizures, have a relatively low bone density, and develop age-related hearing loss. C57BL/6J mice are also susceptible to diet-induced obesity, type 2 diabetes, and atherosclerosis. Macrophages from this strain are resistant to the effects of anthrax lethal toxin. |
| | | C57BL/6N | C57BL/6N is an NIH subline of C57BL/6. It was separated from C57BL/6J in 1951. This strain does not have the deletion in the Nnt gene that has been found in C57BL/6J. |
| CBA | CBA was developed in 1920 from a cross of a Bagg albino female and a DBA male and selected for a low mammary tumor incidence. | CBA/Ca | CBA/Ca mice are commonly used for leukemogenesis research because this strain has a low spontaneous incidence of leukemia while myeloid leukemia can readily be induced. CBA/Ca mice carry viral proteins Mtv8, Mtv9, and Mtv14. Male CBA/Ca mice develop a mild adult-onset diabetes–obesity syndrome that is characterized by hyperglycemia, hyperinsulinemia, and insulin resistance. Unlike the CBA/J substrain, CBA/CaJ mice do not carry the retinal degeneration 1 allele (*Pde6b*$^{rd1}$) mutation, and CBA/CaJ mice are not histocompatible with the CBA/J. |

| | | | |
|---|---|---|---|
| | | CBA | Unlike the CBA/Ca substrain, CBA mice is the only CBA substrain that carries the retinal degeneration 1 allele (*Pde6b*$^{rd1}$) mutation, which causes blindness by wean age. Renal tubulointerstitial lesions have been observed in this strain at a high frequency. |
| DBA | DBA is the oldest of all inbred strains of mice. In 1929–1930, crosses were made between substrains, and several new substrains were established, including substrains DBA/1 and DBA /2. DBA/1 and DBA/2 differ at least at the following loci: Car2, Ce2, Hc, H2, If1, Lsh, Tla, and Qa3. Differences between the substrains are probably too large to be accounted for by mutation and are probably because of substantial residual heterozygosity following the crosses between substrains. | DBA/1 | DBA/1J mice are used as a model for rheumatoid arthritis: immunization with type II collagen leads to the development of severe polyarthritis mediated by an autoimmune response. DBA/1J mice show an intermediate susceptibility to developing atherosclerotic aortic lesions on an atherogenic diet. In response to challenge, DBA/1J mice develop immune-mediated nephritis characterized by proteinuria, glomerulonephritis, and tubulointerstitial disease |
| | | DBA/2 | DBA/2J mice show low susceptibility to developing atherosclerotic aortic lesions, high-frequency hearing loss, susceptibility to audiogenic seizures, development of progressive eye abnormalities that closely mimic human hereditary glaucoma, and extreme intolerance to alcohol and morphine. |
| FVB | FVB/NJ are commonly used for transgenic injection because of the prominent pronuclei in their fertilized eggs and the large litter size. FVB/NJ mice are homozygous for the retinal degeneration 1 allele of Pde6brd1, resulting in blindness by wean age. | FVB/N | |

### 1.3.4 Outbred Stocks

Outbred stocks are genetically undefined and intentionally not bred with siblings or close relatives, as the purpose of an outbred stock is to maintain maximum heterozygosity. One advantage of using outbred stocks is lower cost because outbred stocks have a relatively long lifespan, are resistant to disease, and have high fecundity. Regarding the nomenclature of outbred stocks, the common strain root is preceded by the Laboratory Code of the institution holding the stock.

**Example**

Hsd:NIHS, The NIH Swiss maintained by Harlan (Hsd) outbred stocks.

### 1.3.5 Closed Colony

A closed colony has limited genetic diversity. All mating occurs among the colony members, and no animals are introduced into the colony from outside the stock from generation to generation. Animals are produced by "rotation breeding" using the Harlem system, in which one male is mated with six to seven females. In terms of the nomenclature of outbred stocks, the common strain root is preceded by the Laboratory Code of the institution holding the stock.

**Example**

Jcl:ICR refers to the ICR maintained by CLEA Japan, Inc. (Jcl), closed colony.

Closed colonies may be established to maintain a difficult mutation, where the desire is to maintain a reasonably uniform background, but poor mating performance prohibits use of sib-mating. Closed colony designations consist of the strain of origin and appropriately designated mutations (if applicable), followed by [cc] to indicate closed colony.

**Example**

BALB/cAnNTac-*Bmp4*$^{tm1Blh}$[cc], a closed colony of mice originating from the BALB/cAnNTac inbred strain carrying the *Bmp4*$^{tm1Blh}$ targeted mutation.

### 1.3.6 Congenic Mice

Congenic strains are produced by repeated backcrosses to an inbred (background) strain for at least 10 generations, with selection for a particular marker from the donor strain. Marker-assisted breeding or marker-assisted selection breeding, also known as "speed congenic," permits the production of congenic strains equivalent to 10 backcross generations in as few as five generations. At this point, the residual amount of unlinked donor genome in the strain is likely to be <0.01. Regarding the nomenclature of congenic mice, strains produced by repeated crosses onto an inbred strain are designated by the full or abbreviated (above) strain name of the background strain followed by a full stop (.), the abbreviated name of the donor strain, followed by a hyphen, and the gene symbol.

**Example**

B6.129P2-*Apoe*$^{tm3(APOE*4)Mae}$N12, this mouse, which was developed in the laboratory of Nobuya Maeda at the University of North Carolina, was created by targeting the murine *apoe* gene for replacement with the human APOE4 allele in 129P2 donor E14TG2a ES cells and injecting the targeted cells into blastocysts. Chimeras were backcrossed to C57BL/6 for further 11 generations (N12).

## 1.4 Mutant Mice

### 1.4.1 Spontaneous

Spontaneous mice are detectable in housing colonies. Mice bearing spontaneous mutations have provided a source of animal models for basic biomedical research and genetic diseases. Mutant mice are first recognized by an abnormal phenotype and are genetically analyzed using forward genetic approaches (i.e. analysis advancing from phenotype to gene). The advances of technological approaches, most notably the complete sequencing of the mouse genome [1], significantly shortened the time from mutant strain discovery to gene identification.

### 1.4.2 Transgenesis

Transgenesis involves introduction of known genes into the mouse genome at random sites with the intent to produce a phenotype based on overexpression of the gene (transgene). This method was first used in the early 1980s [4]. Transgenes can be completely "assembled" in the laboratory (Figure 1.2) by linking the expression (the promoter) and the processing and protein coding (intron, exons/cDNA, and polyadenylation signal) regions. Promoters are regions of DNA that drive and control gene expression. They can allow for ubiquitous expression of a gene in all cell types (also termed a constitutive promoter, such as the β-actin promoter), they can allow expression only within a specific cell or tissue type (liver-specific, heart-specific, neuron-specific, etc.), and can respond to factors that activate expression at a specific time. Regardless of the type of promoter, these regions in the construct allow for expression of the transgene independent of genomic location. If a transgene were to be assembled in this manner, the protein could be produced in tissues, which would not normally occupy, and at levels that are higher than normal. These types of changes often

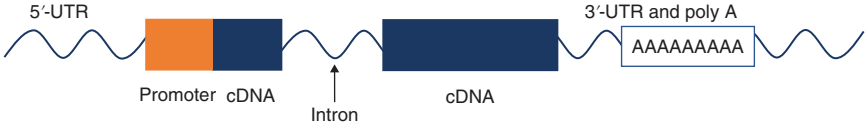


**Figure 1.2** Schematic diagram of a typical transgene construction for the production of transgenic mice.

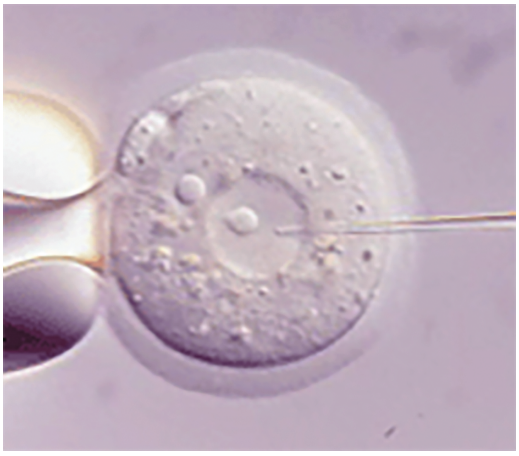

**Figure 1.3** Photo of mouse embryo being microinjected. The rounded pipette on the left is a holding pipette that stabilizes the embryo for insertion of the injection pipette on the right.

lead to phenotypes that can provide clues about the mode of action of specific proteins or serve as models of diseases.

Linearized transgenes are introduced to the mouse genome via pronuclear microinjection of mouse one-cell embryos. Embryos are collected from females early in the day following mating by flushing them from the reproductive tract into tissue culture medium. They are then placed on a microscope stage, and specialized pipettes are used to hold the embryo or inject the DNA (Figure 1.3).

Surviving embryos are then transplanted into pseudopregnant females and carried to term. Once the litter is born, pups are assayed for the presence of the transgene in their genome. This method typically results in random integration into the genome at only one site per individual (called a transgenic line founder animal), but this integration site may contain a tandem array of transgenes linked together in multiples ranging from two to several hundred. Multiple founder animals may be produced following an injection session with a given transgene and, although they contain the same transgene, it is important to note that each founder animal is unique. This is due to the fact that the site of integration and the size of the tandem array will differ for each one. Those differences often lead to different levels of expression of the transgene and, therefore, to potentially different phenotypes in offspring of the founder animal. After the founder animals are produced, the next step is to breed them with wild-type animals to assess if the transgene had been passed onto offspring. When germ line transmission of the transgene occurs, carrier animals can be used to assay its level of expression, and founder lines that produce animals with suitable levels of transgenic protein production can be used for further study.

Transgenesis performed in this manner has two main difficulties. The first is that the random integration of the transgene may result in the disruption of an endogenous gene. When the disruption is sufficient to abate or change the expression of the gene, a phenotype other than the one desired may result. In the worst-case scenario, researchers may attribute an observed change in their transgenic line to the transgene itself when it is actually due to disruption of an unrelated gene. The second difficulty is that transgene expression levels can

vary considerably from founder line to founder line for the same construct. Expression levels are greatly affected by the genomic insertion site of the transgene. One reason for using large, genomic DNA-based transgenes is to insulate the expression-controlling regions (promoter) from the surrounding genomic region.

### 1.4.3 Targeted Mutagenesis

Targeted mutagenesis is the purposeful manipulation of a specific gene target or genomic region to produce a desired effect. The two most common types of targeted mutagenesis are knockouts and knock-ins [5]. Knockouts are designed to eliminate gene function and can be either constitutive (gene function is eliminated in all tissues) or conditional (gene function is eliminated in an inducible or tissue-specific manner) [6, 7]. Knock-ins are produced by targeting a construct, for example, a transgene, to a specific location in the genome. For both knockouts and knock-ins, targeting of the gene or genomic region is carried out in embryonic stem (ES) cells using vectors that are capable of "finding" their target regions. ES cells are derived from the inner cell mass (ICM) of 4.5–5-day-old mouse embryos and are capable of becoming any tissue in the developing mouse (i.e. they are pluripotent) [8–10]. Mouse ES cells were first derived from the 129 inbred strain but have since been cultured from other inbred lines (e.g. C57BL/6) [11, 12].

The production of knockout animals begins with the selection of the target and the isolation of a portion of the genomic copy of the gene. That section becomes the basis for building a targeting vector that will be introduced into ES cells. Targeting vectors can be complicated in their design, but the basic vectors are intended to disrupt a portion of the normal coding region of a gene. They also contain a selectable marker, such as neomycin (Neo), that helps select for ES cells that have taken up the targeting vector. They may also contain a negative selection cassette, such as thymidine kinase (TK) [13, 14], to eliminate cells in which the targeting vector has integrated at a random site. Figure 1.4 is a map of a basic targeting vector containing the Neo-positive selection cassette along with a TK-negative selection cassette compared to the wild-type allele [15]. For correct targeting, the targeting vector must recombine with the wild-type locus via homologous recombination [16]. Thus, the mutated region from the targeting vector disrupted the functional gene (Figure 1.5).

Recombinant ES cells that are correctly targeted will have the Neo cassette integrated, but the TK cassette will be lost because it is outside the region of homology between the targeting vector and the endogenous locus. Subsequent selection with neomycin in culture will kill cells that do not have a neomycin cassette and enrich those cells that do. However, the targeting vector may integrate into the ES cell genome at random, and these cells will survive Neo selection in culture as well. Use of the TK vector helps reduce the total number of surviving cells, as many of the cells that have random integrations will also have an intact TK cassette. Selection with agents that kill thymidine kinase-positive cells will eliminate the cells with random integrations, further enriching correctly targeted cells. This positive–negative selection reduces the total number of ES cell clones

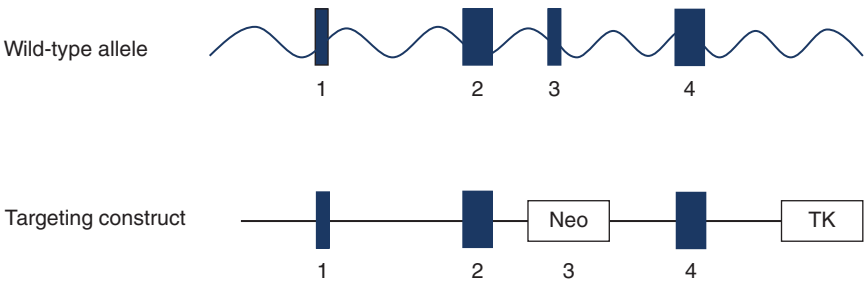


**Figure 1.4** Diagram of a basic targeting vector, used to replace a gene by homologous recombination, and the complementary wild-type allele for comparison.

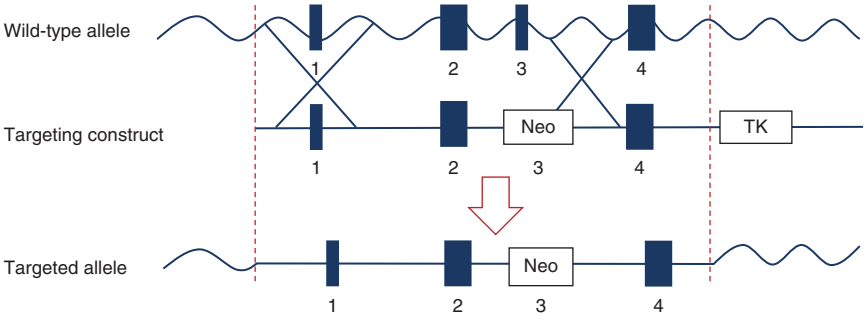


**Figure 1.5** Diagram of targeting vector replacement of an endogenous locus via homologous recombination. Homologous recombination between the targeting vector and the endogenous allele results in the introduction of the Neo cassette into the locus and the deletion of the TK cassette.

that need to be examined for correct targeting. Use of both positive and negative selection methods greatly reduces the number of clones that must be screened using more labor-intensive methods, such as Southern blotting. Once correctly targeted cells are identified, they are expanded and injected into host blastocysts (Figure 1.6).

After injection, the surviving blastocysts are transplanted into pseudopregnant recipient females. The resulting animals will be chimeric; some tissues will be derived from the host ES cells and some from the targeted ES cells. Those tissues derived from injected cells will contain the mutation, so it is essential for germinal tissue (cells that will become sperm in most cases) to be derived from the targeted ES cells if the mutation is to be passed onto the next generation. It usually takes six to eight months from targeting vector construction to F1 knockout.

If the host blastocysts were selected from a strain that has different coat color alleles than those of the ES cell origin strain, the resulting chimeras will show a mix of the two coat colors because some hair cells are derived from each strain. A high percentage of the coat color chimeras derived from the injected ES cell indicates high-percentage germ line transmission of the mutant allele into the next generation. For example, if ES cells were derived from an agouti 129 coat color strain and host blastocysts were derived from the C57BL/6 black coat color

strain, chimeras mated with C57BL/6 animals would produce either black or agouti offspring. Any agouti animal will have been produced by sperm from the manipulated ES cell and will have a 50% chance of carrying the mutation. Multiple litters of all-black offspring indicate that the manipulated ES cells did not contribute to the germ line. Figure 1.7 is a diagram of the process of producing gene-targeted mice.

### 1.4.4 Inducible Mutagenesis

The most widely used method for inducing expression of a transgene by an external stimulus is a binary transcriptional transactivation system called the "tet-on/tet-off" system [17]. The transcriptional activator gene can be regulated reversibly and quantitatively by the antibiotic tetracycline or a derivative (e.g. doxycycline, dox). dox can be delivered to the transgenic animals via drinking water or chow at a prescribed time to activate (or repress) expression of the transactivator protein. As illustrated in Figure 1.8, the tet-on system activates transcription in the presence of dox, whereas the tet-off system represses transcription in the presence of dox. The requirement for fairly high doses of antibiotic for a long time is the major drawback of these systems. Tet-off requires continuous administration of dox, and activation occurs only once dox is cleared, which can take one to seven days in adult animals. Additionally, dox is cleared more slowly in tissues, such as bone and liver. The tet-on system induces transcription rapidly, but repression depends on the clearance of dox.

### 1.4.5 *Cre–loxP* System

A null mutation is a constitutive mutation because the knockout is always on. If the gene was necessary for embryonic development, no live animals (embryonic lethal) will be produced, hampering subsequent analyses. To avoid this problem, scientists developed a system that would allow for the production of targeted alleles. Those alleles function normally until a special signal is given to create a null allele. Such models are known as conditional (or inducible) knockouts because

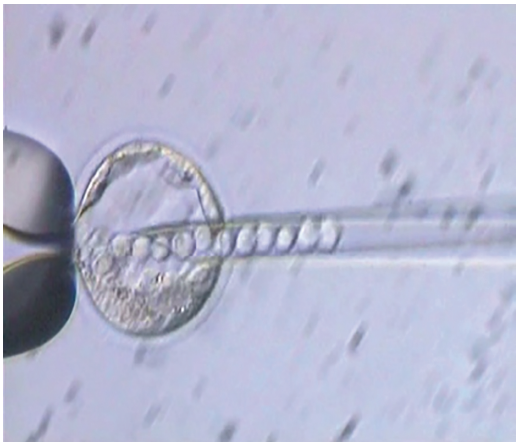

**Figure 1.6** Bright-field photo of blastocyst injection with manipulated ES cells.

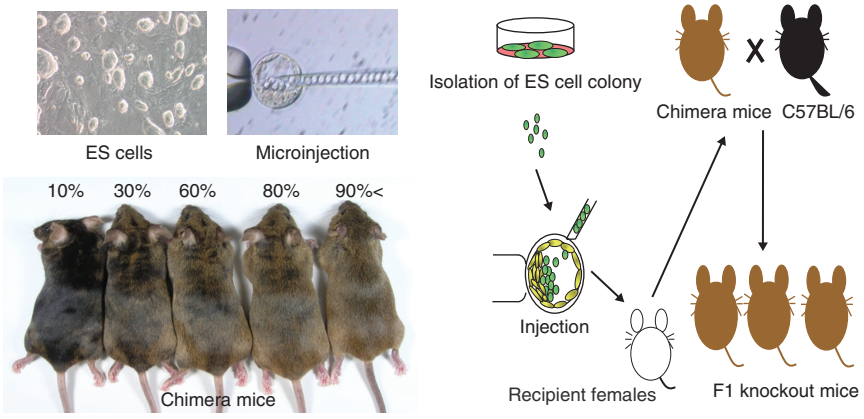


**Figure 1.7** Overview of the processes involved in targeted mouse model creation.

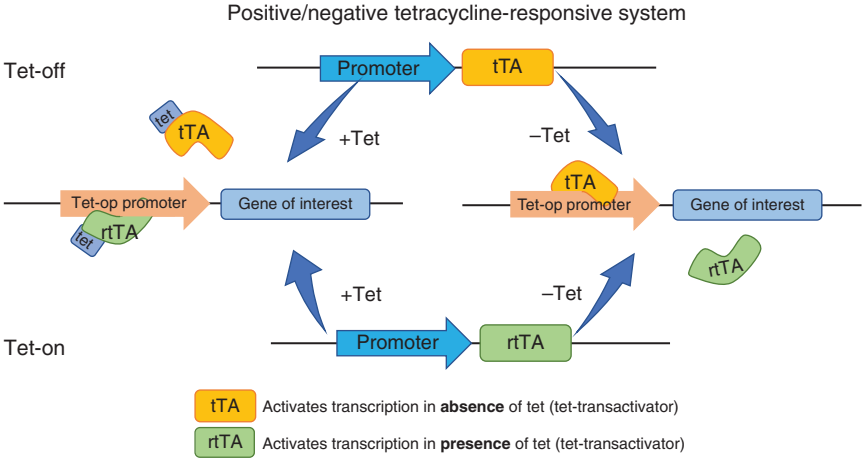


**Figure 1.8** Diagram of the function of tet-on and tet-off approaches for the regulation of transgene expression.

the gene is disrupted under only certain conditions. To manipulate conditional alleles, recombinases are used. Recombinases rearrange sections of DNA when specific recognition sequences are present in the genome. The most widely used of these for engineering targeted alleles in the mouse is the Cre recombinase from bacteriophage P1 [18]. This enzyme catalyzes site-specific recombination of DNA between sequences called *loxP* sites, resulting in the removal of the sequence between two *loxP* sites. Figure 1.9 is a schematic of the *cre–loxP* system. Because *loxP* sequences are not normally present in mammalian genomes, they can be artificially introduced into targeting vectors to flank regions that are intended to be removed later. The expression of *cre* is driven by a tissue-specific promoter so that the target locus is excised only in cells in which the promoter is active. Post-recombination, a single *loxP* site remains in the endogenous locus, whereas the region that was flanked by *loxP* sites is circularized and lost. In addition to

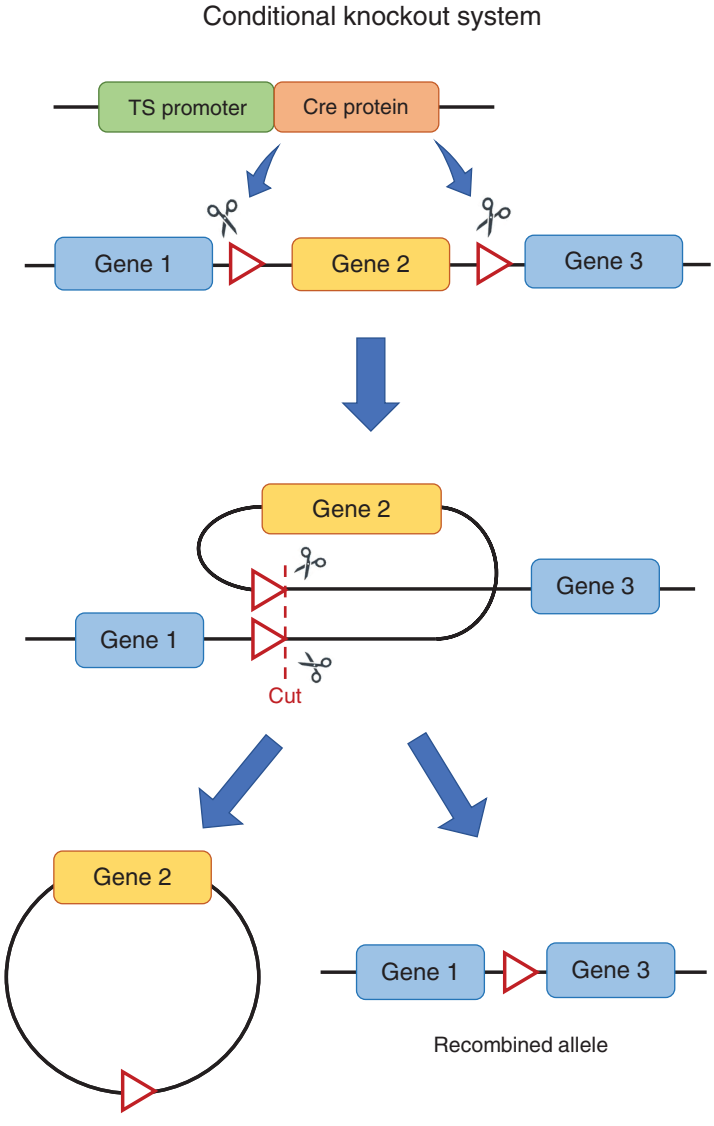


**Figure 1.9** Diagram of *cre*-mediated excision of a *loxP*-flanked region.

the *cre–loxP* system, the analogous *flp–frt* recombinase system originally isolated from yeast can also be added to target constructs [19]. The Flp recombinase finds *frt* sites and removes the intervening DNA regions.

### 1.4.6 CRISPR/Cas9 System

Recently, developed programmable editing tools, such as zinc finger nucleases (ZFN) [20] and transcription activator-like effector nucleases (TALEN) [21], enable precise modification of genomes. However, clustered regularly

interspaced short palindromic repeats (CRISPR)/Cas9 (CRISPR-associated protein 9 nuclease) technology [22] represents a considerable advance over the other next-generation genome-editing tools, reaching a new level of targeting, efficiency, and ease of use. The CRISPR/Cas9 system allows for site-specific genomic targeting in virtually any organism. Three types of CRISPR/Cas systems have been identified, of which type II is the most frequently used. This is a prokaryotic adaptive immune response system that uses noncoding RNAs to guide the Cas9 nuclease to induce site-specific DNA cleavage. This DNA damage is repaired by cellular DNA repair mechanisms, via the nonhomologous end joining either the DNA repair pathway (NHEJ) or the homology-directed repair (HDR) pathway. The CRISPR/Cas9 system has been harnessed to create a simple, RNA-programmable method for genome editing in mammalian cells and can be used to generate gene knockouts (via insertion/deletion) or knock-ins (via HDR). To create gene disruptions (Figure 1.10), a single guide RNA (sgRNA) is generated to direct the Cas9 nuclease to a specific genomic location. Cas9-induced double-strand breaks are repaired via the NHEJ DNA repair pathway. The repair is error prone, and thus insertions and deletions (INDELs) that can disrupt gene function can be introduced.

## 1.5 Resources of Laboratory Strains

A variety of mouse models have been generated, and this will likely be accelerated by the sequencing of the mouse genome. The World Wide Web offers information on the mouse strains available to the research community. The International Mouse Strain Resource (IMSR) website (www.findmice.org) is a searchable online database of international repositories of mouse strains and mutant ES cell lines, including inbred, spontaneous mutant, and genetically engineered strains. The international repositories identify, select, and import important mutant strains of mice, cryopreserve embryos, or gametes of these strains, transfer mutations onto defined genetic backgrounds when appropriate, maintain and distribute the mouse strains, and provide information on them to the scientific community.

## 1.6 Germ-Free Mice

Germ-free mice are animals that have no microorganisms living in or on them. Germ-free mice as a model of the effect of gut microbes on host gastrointestinal physiology develop an enlarged cecum (Figure 1.11). Germ-free mice are born under aseptic conditions, which may include removal from the mother by Caesarean section and immediate transfer of the newborn to an isolator where all incoming air, food, and water are sterilized [23]. Because intestinal microorganisms provide energy to the animal by breaking down dietary fiber, germ-free mice require more food and water to maintain their weight than do non-germ-free animals. Germ-free mice have immune system defects [24].

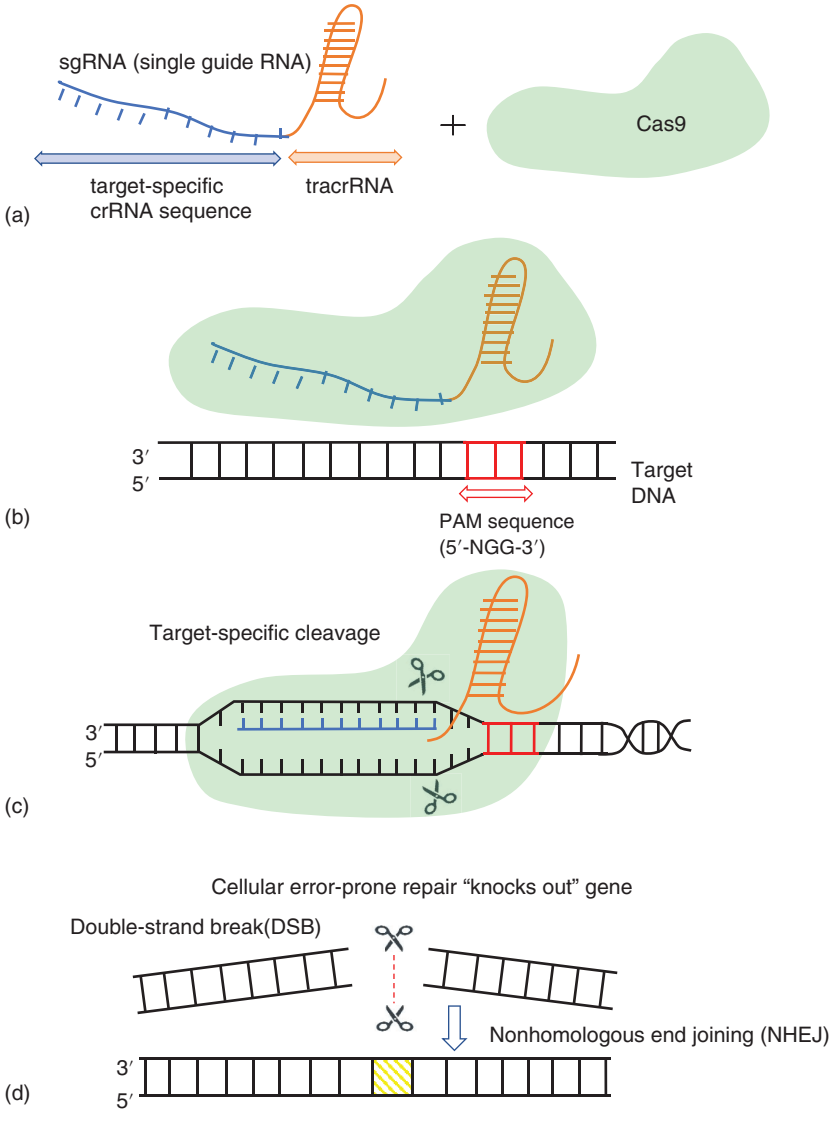


**Figure 1.10** CRISPR/Cas9-mediated gene disruption. A single guide RNA (sgRNA), consisting of a crRNA sequence specific to the DNA target and a tracrRNA sequence that interacts with Cas9 (a), binds to recombinant Cas9 with DNA endonuclease activity (b). The resulting complex specifically cleaves the target double-stranded DNA (c). The cleavage site will be repaired by the nonhomologous end joining (NHEJ) DNA repair pathway, an error-prone process that may result in insertions/deletions (INDELs) that can disrupt gene function (d).

**Figure 1.11** Representative photos of cecum. The cecum was indicated by yellow arrows.

## 1.7 Gnotobiotic Mice

Gnotobiotic mice (also gnotobiotes or gnotobionts) are animals with only certain known strains of bacteria and other microorganisms. Gnotobiotic mice are born under aseptic conditions, which may include removal from the mother by Caesarean section and immediate transfer of the newborn to an isolator where all incoming air, food, and water are sterilized. Gnotobiotic mice are normally reared in an isolator to control their exposure to viral, bacterial, or parasitic agents and are exposed only to those microorganisms that the researchers wish to have present in the animal. Gnotobiotic mice are used to study the symbiotic relationships between an animal and one or more of the microorganisms that inhabit its body. Gnotobiotic mice often have poorly developed immune systems [25].

## 1.8 Specific Pathogen-Free Mice

Specific pathogen-free (SPF) mouse strains are guaranteed to be free of particular pathogens by routine microbiological testing. The list of organisms assessed typically includes disease-causing pathogens that can affect mouse health and research outcomes, as well as opportunistic and commensal organisms that typically do not cause illness in normal, healthy mice. A list of the tested organisms from the mouse room where that particular SPF mouse strain is maintained is needed.

## 1.9 Immunocompetent and Immunodeficient Mice

Immunocompetent mouse strains produce a normal immune response following exposure to an antigen. Immunodeficient mouse strains have specific deficiencies

in major histocompatibility complex (MHC) class I, II, or both; B- or T-cell defects; or defects in both; as well as immunodeficiency because of knockdown of genes for cytokines, cytokine receptors, toll-like receptors (TLRs), and a variety of transducers and transcription factors of signaling pathways. Numerous mouse models of natural immune deficiencies and gene-deficient transgenic mouse strains are available. The use of both immunocompetent and immunodeficient mouse strains facilitates the study of the immune system under normal and disease conditions.

## 1.10 Mouse Health Monitoring

The microbiological quality of experimental mice can critically influence their welfare and the validity and reproducibility of research data. It is therefore important for breeding and experimental facilities to establish a laboratory animal health-monitoring program as an integrated part of any quality assurance system. Such a program should include pathology, serology, microbiology, parasitology, and polymerase chain reaction (PCR) infectious agent testing. Immunodeficient animals are generally more susceptible to infectious agents than are immunocompetent animals. This may allow the detection of agents that are usually eliminated by immunocompetent sentinels. A disadvantage of immunodeficient animals is that they may serve as a persistent source of infections of the other resident animals.

## 1.11 Production and Maintenance of Mouse Colony

### 1.11.1 Production Planning

Colony production and management starts with production planning; thus, it is important to understand both the purpose and the goals of the breeding colonies. For example, the purpose is to supply females for embryo or blastocyst harvest, to supply recipient females for embryo transfer, to generate novel mutant animal lines, or to perform large-scale production for specific experiments? Additionally, what are the goals for the breeding colony in terms of the number of animals needed over a specific period of time, and what characteristics are needed (e.g. sex, age, and genotype)? Finally, other factors that can influence production performance should be considered during the planning process. Factors such as genetic background, number of available breeders, life span, and health status affect the overall productivity of a breeding colony.

### 1.11.2 Breeding Systems and Mating Schemes

Breeding systems can be divided into two categories [26]. One category is permanent mating groups, including monogamous and harem mating (one male with more than one female). In permanent mating groups, the male is housed continuously with the female(s), which allows him to participate in pup care and to take advantage of the postpartum estrus. The other category is temporary

mating groups, which include polygamous and hand mating (multiple males and females). These require separation of the breeders at some point after mating.

To maximize the productivity of a single male mouse, it may be best to rotate different receptive females into his cage. This can allow for accurate staging of gestation and is commonly used for generating time-mated females for embryo harvest or, with vasectomized males, to prime the uterus of females for embryo-transfer surgeries. To maximize the productivity of female mice, they are best kept in permanently mating groups because this allows mating at the postpartum estrus. Although male-intensive, the monogamous breeding system will result in the greatest number of pups born per female over her reproductive lifespan. In contrast, harem breeding will result in the largest number of pups born per breeder cage [27]. Multiple breeder females housed in the same cage may share pup-rearing tasks. Polygamous mating is the least male-intensive breeding system, but because pregnant females are separated from the males to litter in separate cages, it leads to the fewest pups born per female, and record keeping can be difficult because male parentage is not certain.

For examples and recommendations on animal number requirements, please refer to [28]. For example, when the breeding colony is composed of five pair-mating cages, approximately 80–100 animals may be required to characterize a line from founder animals assuming that breeders exhibit no unusual adverse, life-interfering phenotypes; good productive mating frequencies; and an average reproductive life span of six to eight months.

There are many possible mating schemes for breeding genetically modified rodents, but any mating scheme should consider the genotypes of the breeders to generate offspring with the desired genotype. This is particularly important if a phenotype of interest was to be expressed only in homozygotes or is sex dependent. Therefore, having a strong foundation in genetics can be helpful when managing rodent breeding colonies. Mating two heterozygotes will produce 25% homozygote, 50% heterozygote, and 25% wild-type offspring. This scheme is useful when homozygotes show the desired phenotype but are infertile. This mating scheme is also useful if heterozygotes were to be of interest because they have a phenotype intermediate between that of wild types and homozygotes. Mating of a homozygote with a heterozygote will yield 50% homozygotes, whereas the other 50% will be heterozygote siblings. This scheme is useful when the phenotype is seen only in homozygotes and when littermate controls are requited. This mating scheme may also be chosen when one sex of homozygotes is not viable or fertile. Breeding two homozygotes will yield 100% homozygous offspring and is useful if the gene effect is to be seen only in homozygotes and if homozygotes are to be viable and fertile. Although there will be no sibling control animals, inbred animals of the same strain may be used if the mutants are to be on an inbred background. Mating of a wild type and a heterozygote yields 50% wild type and 50% heterozygous offspring. This mating scheme is useful with animals that have sex-linked mutations. When expected percentages of genotypes are given, it is probable that genotypes will appear at this ratio in offspring over time. However, it is not a guarantee that every litter will have a particular combination.

## 1.12 Mating

The mating behavior of mice generally follows a consistent pattern. There is a distinct sequence of approach by the male, acceptance by the female, mating, ejaculation, and a refractory period [29–31]. Males generally investigate females by sniffing the genitals. When females are not receptive, they avoid contact with males; when mounting is attempted, they may assume defensive postures. The copulation plug is a white plug produced (Figure 1.12) by secretions of the coagulating gland, seminal vesicles, and prostate. It fills the vagina and provides a mechanical barrier to mating another male [32]. The presence of a plug indicates successful mating and ejaculation but does not necessarily indicate pregnancy.

To avoid production problems, before setting up a pair of mice for breeding, a physical examination can rule out problems, such as a vaginal septum (Figure 1.13) or imperforate vagina. Mice with either of these conditions are unsuitable for breeding because the structure present in the vagina will interfere with both mating and parturition.

## 1.13 Gestation Period

The gestation period for mice is generally 18–21 days [33], and fetuses may be palpable by 10–12 days post-conception.

## 1.14 Parturition

Parturition occurs when the fetuses indicate their readiness. Corticosteroid hormones secreted by the placenta induce luteolysis and begin the parturition sequence. The female exhibits nesting behavior before parturition, and the nests built at that time are complex and differ from those built at other times [34]. Parturition usually occurs at night [35, 36] and takes place over a variable span of time, with a pup being delivered every few minutes [37]. The female will lick and clean the pups between each birth and remain in the nest.

## 1.15 Parental Behavior and Rearing Pups

Both males and females, whether virgin or experienced, will commit infanticide [38–43]. It is commonly seen as a male reproductive strategy, as killing a litter removes the lactational block to estrus and removes a rival's genes. Females will also kill pups when stressed or when resources are limited. The timing of copulation and remaining in contact with a female result in a change in male behavior from infanticidal to parental at about the time offspring from that mating would be born.

Retrieval behavior requires participation and feedback from the pups. Infant rodents emit high-frequency vocalizations that guide the mother to their

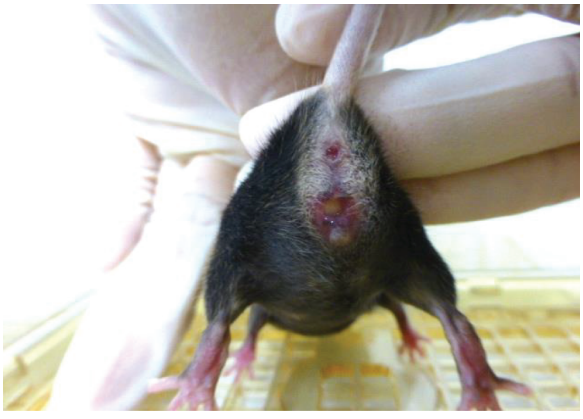

**Figure 1.12** Copulation plug in a mouse.

**Figure 1.13** A female mouse with a vaginal septum.

location and facilitate their retrieval to the nest [44]. The successful growth of pups requires adequate milk production. A certain number of suckling offspring is necessary for adequate milk production for all offspring. This number appears to be related to the suckling stimulus provided to the mother during the first day of lactation. Peak lactation in mice occurs between days 10 and 16 [45–47]. The effective nursing of pups requires synthesis of milk in the mammary glands, parental retrieval of the pups to the nest, crouching behavior by the mother, attachment of the pups to the teats, suckling behavior by the pups, and milk ejection [34, 48]. Lactation is a physiological stressor for animals that can suppress estrus or prevent implantation of blastocysts.

## 1.16 Growth of Pups

A summary of the basic reproductive parameters of pups is provided in Tables 1.3 and 1.4. Animals can be sexed at birth (Figure 1.14). Before the descent of the testes, sex is most easily determined based on the distance between the genital

**Table 1.3** Reproductive and developmental parameters of the mouse.

| | |
|---|---|
| Weight at birth | 1 g |
| Weight at weaning | 10–15 g |
| Weight at adult | 20–40 g |
| Age at weaning | 18–28 d |
| Full sexual maturity | 7–9 wk |
| Estrous cycle length | 4–5 d |
| Fertilization | 2 h after mating |
| Gestation period | 19–21 d |
| Implantation | 4–5 d |
| Fertile postpartum estrus | Yes |
| Litter size | 6–12 heads |
| First solid food intake | 11–12 d |

papilla and the anal opening (anogenital distance); this distance is about twice as long in males than in females.

## 1.17 Reproductive Lifespan

Age at sexual maturity can vary depending on the strain and environmental conditions. In general, female mice attain puberty, as defined by vaginal opening, between four and six weeks of age, and sexual maturity at six to eight weeks of age. Male mice reach puberty, as defined by sperm in the tail of the epididymis, at approximately five to seven weeks [49, 50]. Many investigators give animals an extra one or two weeks to mature to attempt to maximize pup survival. This would mean setting up breeding pairs of mice at seven to eight weeks.

The male reproductive lifespan is longer, especially when they are housed with females [51, 52], but it is usual to retire animals 8–10 months after they are placed together to breed.

## 1.18 Record Keeping and Colony Organization

The ability to keep organized breeding records is vital to the success of colony management. Breeding cages should be checked at least once per week, and basic breeding information can easily be tracked at the cage side. Collected information is often transferred into an electronic database as part of laboratory record maintenance. The database can also be used to calculate and track specific reproductive parameters, facilitate production performance evaluation, and identify problems or corrective measures during troubleshooting exercises. Failure to keep records could result in genetic contamination of the colony, trouble in production, difficulty in locating specific animals, and/or delays in experiments.

**Table 1.4** Developmental milestones of the mouse, days 0–14.

| Age | Appearance |
|---|---|
| 0–24 h | Deep red skin |
| | Milk spot visible |
| | Dark eyes (black/agouti mice) |
| Day 1 | Deep pink skin |
| | Milk spot visible |
| Day 2 | Ears appear as nubs |
| | Milk spot visible |
| | Pigment in skin begins to appear (black/agouti mice) |
| Day 3 | External ear flap begins to lift from head |
| | Milk spot visible |
| Day 4 | External ear flap fully lifted from head and |
| | Milk spot visible |
| | Skin fully pigmented (black/agouti mice) |
| Day 5 | External ear flap completely vertical |
| | Skin appears much thicker |
| | Incisors visible as white spots under gums |
| Day 6 | Milk spot gone or only faintly visible |
| | Colored fuzz appears behind ears or on dorsal neck |
| Day 7 | Colored fuzz begins to cover pup fully |
| Day 10 | External ear opens |
| | Pup fully haired |
| Days 13–14 | Eyes begin to open |

## 1.19 Animal Identification

Individual animal identification may be achieved by multiple methods. Individually numbered rodent ear tags and ear punches are commercially available from National Band and Tag (https://nationalband.com) and Kent Scientific (https://www.kentscientific.com). Example ear-clipping systems are illustrated in Figure 1.15.

## 1.20 Animal Models in Preclinical Research

Animal models have increasingly become the preferred tool in research on mechanisms of pathogenesis, drug discovery, and translational studies. In this section, we introduce the recent concept of incorporating patient-derived models into

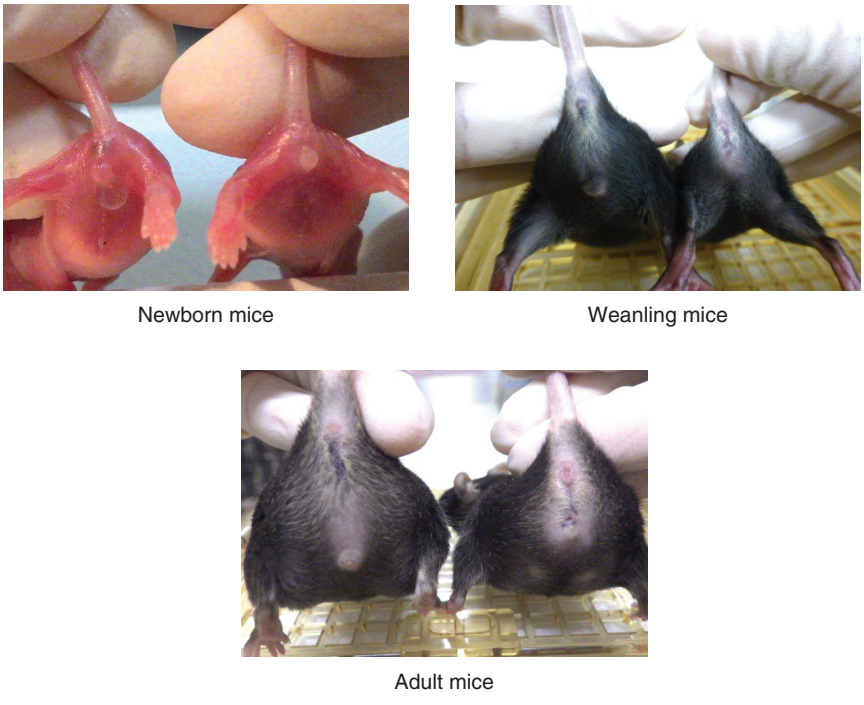


**Figure 1.14** Photographs of mice at various ages, males are on the left.

the drug discovery process, from target to clinical development. Patient-derived tumor xenograft (PDX) models facilitate oncology research as they recapitulate intratumor heterogeneity and can model the correlation of genotype with the response to particular therapies.

Cancers are among the leading causes of death worldwide and must be treated systemically by chemotherapy, hormone therapy, radiation therapy, surgery, or a combination thereof. Among these, chemotherapy is frequently applied but is associated with a low therapeutic window because of its poor specificity toward tumor cells/tissues. Development of selective therapeutic agents would overcome this limitation. Animal models are essential for testing the efficacy of anticancer agents and for mechanistic studies.

PDX models can be created by transplantation of cancerous cells or tissues from a primary tumor into immunodeficient mice (Figure 1.16). Specifically, tumors can be transplanted into sites other than those from which they originated (heterotopic transplantation) or into the corresponding site (e.g. the brain [53, 54], lung [55], liver [56], pancreas [57, 58], kidney [59], and ovary [60] [orthotopic transplantation]). PDX models are used in basic and preclinical cancer research (Figure 1.17).

Basic and preclinical cancer research using PDX models are connected, as basic research identifies therapeutic targets or strategies for preclinical testing.

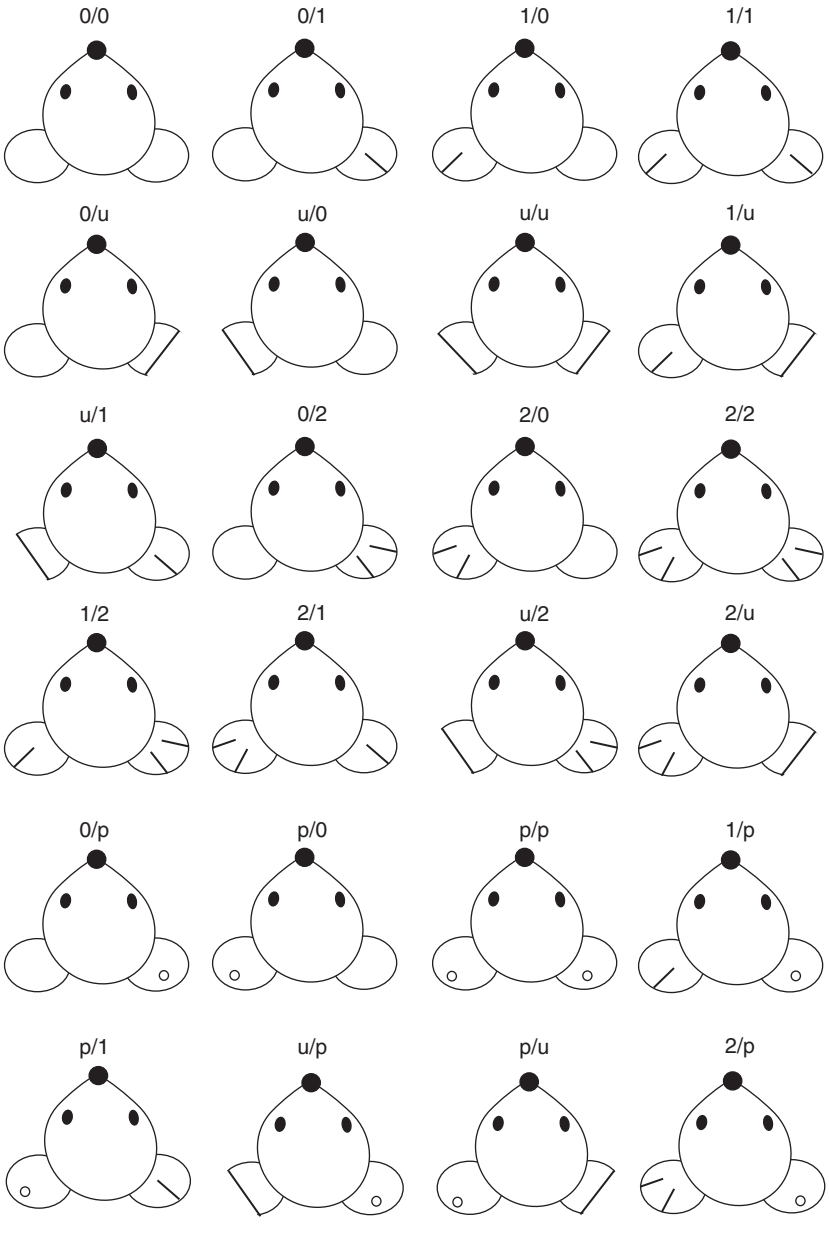


**Figure 1.15** Examples of ear clipping or punching systems for numbering mice.

In drug screening, PDX models can be used to expand small tumors for *in vitro* studies. The expanded tumor cells can be cultured and manipulated *ex vivo* and used for high-throughput screening of drugs. The identified candidate drugs and drug combinations can be further evaluated in PDX mice before use in patients

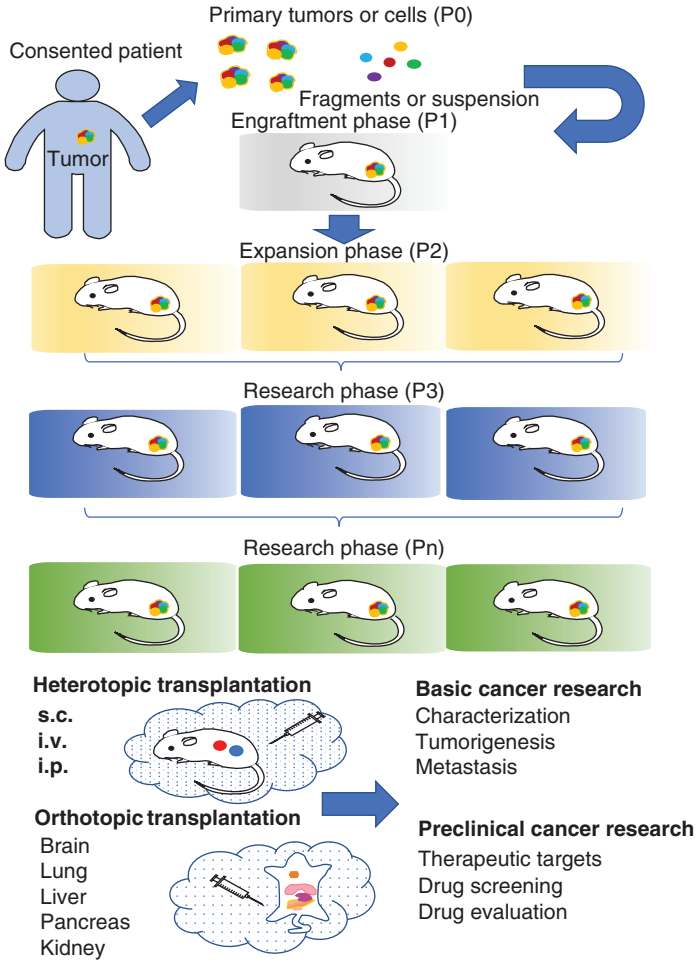


**Figure 1.16** Establishment of PDX models. Tumors from cancer patients (P0) are fragmented or digested into single-cell suspensions and transplanted into immunodeficient mice (P1) for engraftment. Next, the tumors are transplanted into secondary recipients (P2) for expansion. The expanded tumors are then cryopreserved or transplanted into P3 mice for use in cancer research. Established PDX models (Pn) used in cancer research. s.c., subcutaneous; i.v., intravascular; i.p., intraperitoneal.

or directly used in patients if the drugs have been approved. In preclinical therapeutic evaluation, PDX models can be used to determine the optimum clinical therapeutic regimens for cancer patients.

PDX models are imperfect and do not represent all cancer types and subtypes. However, the data produced by the use of PDX models enable patient stratification, identification of novel predictive biomarkers, and identification of novel biological mechanisms.

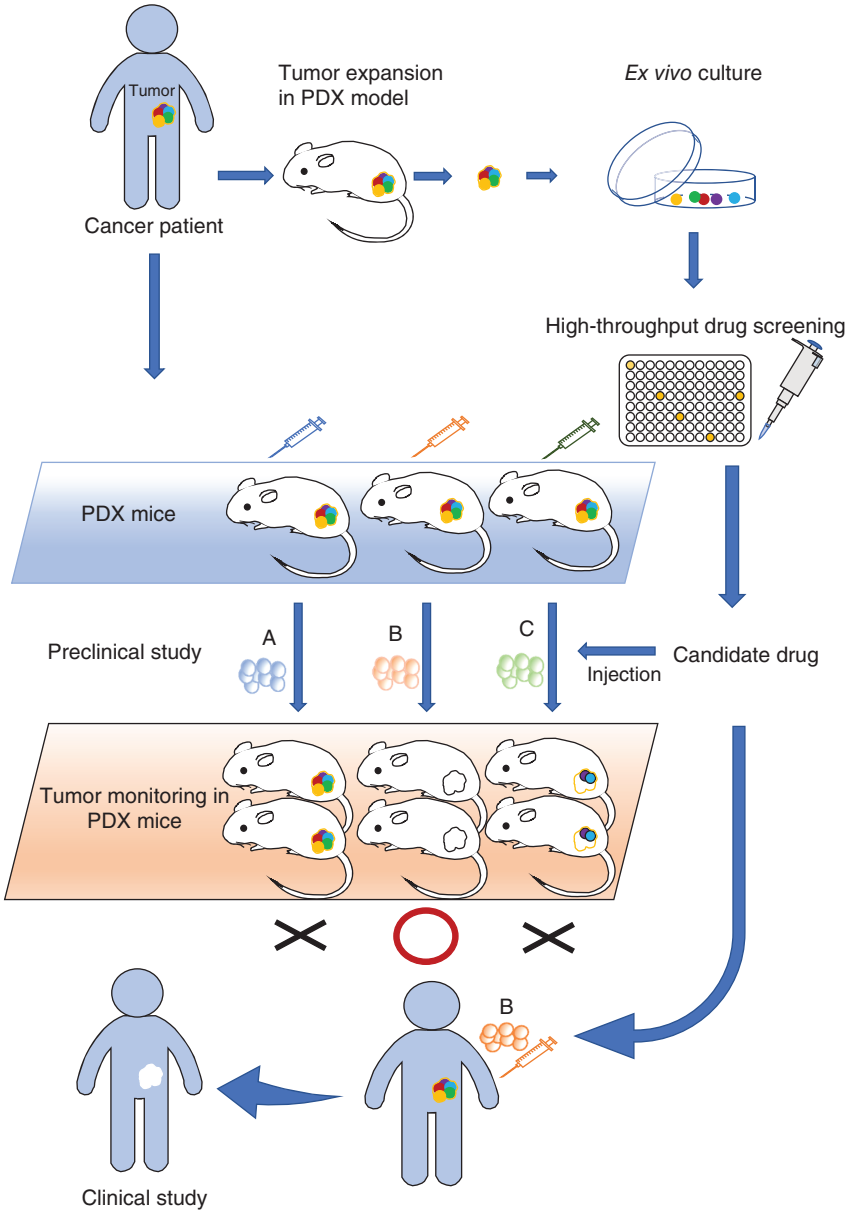


**Figure 1.17** Use of PDX models in drug screening and preclinical therapeutic evaluation. PDX mice derived from one patient are randomly assigned to receive different therapeutic regimens, followed by efficacy assessment.

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