

Breeding Strategies for Maintaining Colonies of Laboratory Mice

A Jackson Laboratory Resource Manual



This manual describes breeding strategies and techniques for maintaining colonies of laboratory mice. These techniques have been developed and used by The Jackson Laboratory for nearly 80 years. They are safe, reliable, economical, efficient, and ensure that the mouse strains produced are genetically well-defined.

Cover Photos

Front cover: JAX[®] Mice strain B6SJLTg(SOD1*G93A)1Gur/J (002726) with red plastic enrichment toy. This strain is a model of amyotrophic lateral sclerosis (ALS), or Lou Gehrig's disease. (left), JAX[®] Mice strain C57BL/6J (000664), our most popular strain, with litter of nine-day old pups.(middle), A technician at The Jackson Laboratory—West working with mice in one of our production rooms. (right).

Table of Contents

Introduction	1
Fundamentals of Mouse Reproduction	2
Mouse Breeding Performance	4
Breeding Performance Factors	4
Optimizing Breeding Performance.....	5
Breeding Schemes.....	7
Managing Small Colonies	7
Simple Breeding Schemes	7
Complex Breeding Schemes	9
Genetic Quality.....	11
Maintaining Genetic Quality.....	11
The Jackson Laboratory Genetic Quality and Stability Programs.....	11
Costs of Maintaining Mouse Colonies	12
Reducing Costs.....	12
Cryopreservation.....	13
Advantages of Cryopreservation.....	13
Considerations for Cryopreserving a Strain.....	13
JAX® Services for Breeding and Colony Management	14
JAX® Breeding Services.....	14
JAX® Special Diets	14
JAX® Aging Service	14
Dedicated Supply of JAX® Mice.....	14
JAX® Rederivation	14
JAX® Speed Expansion.....	14
JAX® Speed Congenic Development.....	15
Strain Rescue.....	15
Microinjection & Novel Strain Creation.....	15
JAX® Sperm Cryo Kit.....	15

Table of Contents (continued)

JAX® Sperm Cryopreservation & Recovery	15
JAX® Speed (Embryo) Cryopreservation & Recovery	15
JAX® Custom (Embryo) Cryopreservation & Recovery.....	15
Genome Scanning	16
Mouse Diversity Genotyping Array.....	16
JAX® Resources for Mouse Colony Management.....	17
The Jackson Laboratory's Colony Management System	17
Course: Colony Management, Principles and Practices	17
Jackson Laboratory Online Mouse Resources.....	18
Technical Information Services.....	18
Sizing Mouse Colonies.....	19
Glossary	21
Appendix	22
Selected References	28



Introduction

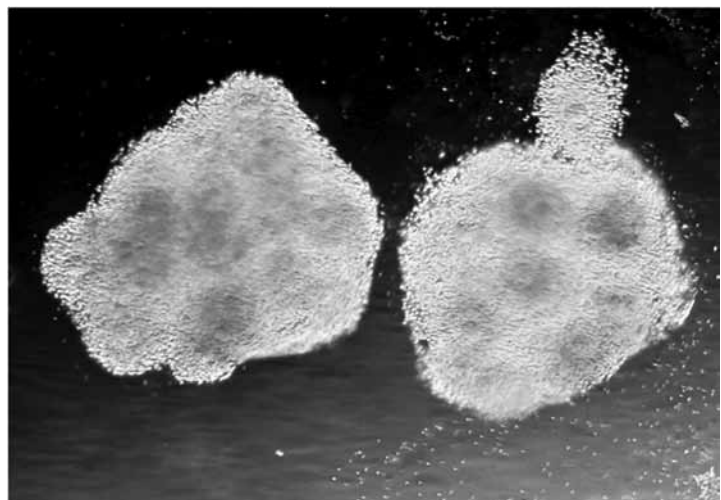
The laboratory mouse is playing an increasingly important role in biomedical research. Due to mutagenesis, transgenic, and gene-targeting technologies, the number of available mouse models is escalating. So are the costs, mouse room space, and related resources needed to accommodate these mice. Due to all of these factors, the art and science of managing mouse colonies efficiently is more important than ever. Colony managers often consult us for advice – and rightly so, for our mouse husbandry experts have been using and refining mouse colony management techniques for over 80 years. These techniques are safe, reliable, economical, efficient, and ensure that the mouse strains produced are genetically well-defined. This Manual provides valuable insights into these techniques for those who maintain their own research colonies. Its contents are guidelines: if you have questions or need more specific information, contact our Technical Information Scientists (www.jax.org/jaxmice/micetech).

If you do not have the time, facilities, or other resources to maintain or manage your own mouse colonies, JAX® Services can help. For information about our Breeding and Colony Management Services, please see page 14.

Fundamentals of Mouse Reproduction

To properly manage a mouse colony, an understanding of the fundamentals of mouse reproductive biology is essential. Some of these fundamentals are briefly reviewed below.

- **Sexual maturity.** Generally, laboratory mice become sexually mature between five and eight weeks of age. Males of most strains usually mature sexually by six weeks of age. DBA/2J (000671) and C3H/HeJ (000659) mice are precocious: females can conceive when they are as young as 23 days old. However, mice bred that early generally produce small litters. Therefore, we usually mate mice when they are six-to-eight weeks old.
- **Reproductive life span.** Typically, laboratory mice can breed for about seven to eight months, producing four or more litters (Table 1). However, some strains produce only one or two litters, usually because strain-specific characteristics or mutant phenotype affect their fertility. AKR/J mice (000648) develop leukemia, and breeders must generally be replaced when they are about six months old. C3H/HeJ mice may stop breeding early because they have a high frequency of ovarian cysts and tumors. NOD/ShiLtJ (001976) females may develop diabetes when they are 12 weeks old, but their reproductive lives can be extended with foot pad injections of Freund's Adjuvant. Reproductive life spans for each strain are average values. If a pair is breeding well beyond its expected reproductive life span, retain it until the female is not pregnant within 60 days of her previous litter's birth.
- **Fertility.** Fertility of inbred strains varies. For example, whereas nearly all breeding pairs of C3HeB/FeJ (000658) mice are fertile, less than half of C57L/J (000668) breeder pairs are fertile.
- **Gestation.** The gestation period for laboratory mice is generally consistent within a strain but varies among strains from 18-21 days. For example, it is 18.5 days in C57BL/6J (000664) mice, 20 days in BALB/cJ (000651) mice, and 21 days in A/J (000646) mice.
- **Generation time.** Generation time in laboratory mice is about 12 weeks: ~three weeks gestation, three to four weeks suckling, and two to three weeks until sexual maturity.
- **Litter size.** Litter size varies among strains (Table 1), ranging from about two to three pups/litter in some poorly breeding 129 substrains to 12 or more pups per litter in the FVB/NJ (001800) and NOD/ShiLtJ (001976) strains.
- **Weaning age.** In a mouse husbandry context, weaning refers to removing a pup from its home pen (rather than to the time a pup stops nursing and starts eating solid food). Generally, laboratory mice are weaned between 18 and 28 days of age (Table 1). Weaning age depends on weaning size and maturity. Although most strains are weaned when they are 21 days old, some benefit from being weaned when 28 days old.



Mouse oocytes

Fundamentals of Mouse Reproduction

Table 1. Reproductive information for the most widely used JAX® Mice strains, readily available in large quantities.

Strain	Mean weaning age (wks)	Rotation Length† (wks)	Mean litter size (weaned)	Mean number of litters (born)	Wean:born ratio	Percent females (weaned)
BALB/cJ (000651)	3	30	5.4	4.1	0.99	50%
BALB/cByJ (001026)	3	30	5.2	3.8	0.96	56%
B6.129P2-Apoe ^{tm1Unc} /J (002052)	4	26	4.5	3.9	0.83	44%
C3H/HeJ (000659)	3	22	5.0	3.5	0.92	44%
C57BL/6J (000664)	4	30	5.6	5.4	0.92	47%
CBA/J (000656)	3	26	4.0	5.4	0.93	48%
DBA/2J (000671)	3	26	4.7	5.3	0.93	48%
FVB/NJ (001800)	3	26	7.3	4.9	0.98	51%
NOD/ShiLtJ (001976)	3	22	7.7	3.5	0.92	49%
NOD.CB17-Prkdc ^{scid} /J (001303)	3	26	5.8	4.1	0.94	49%
NOD.Cg-Prkdc ^{scid} Il2rg ^{tm1Wjl} /SzJ (005557)	3	30	6.4	3.6	0.97	51%
129S1/SvImJ (002448)	3	30	4.9	4.6	0.89	49%

Information in the second and third columns is based on JAX® Mice and Services procedures.

Data in last four columns averaged from ~50 mated JAX® Mice females per strain (production colonies; collected between 2005 and 2007).

† Rotation length (weeks): based on our experience, the average length of time a breeding unit reliably delivers progeny (also called the optimum reproductive life span).

Mouse Breeding Performance

Breeding Performance Factors

Breeding performance of laboratory mice can be affected by many factors, including the following:

- **Birth defects in the pups.** C57BL/6J (000664) mice tend to have more pups with hydrocephaly than do other strains. A/J (000646) mice tend to have relatively more pups with cleft palates, the incidence of which can be influenced by the uterine environment.
- **Hybrid vigor.** Hybrid mice tend to have more, larger, and healthier litters than inbred strains.
- **Strain-specific behaviors.** The aggressive behaviors of some strains and the poor mothering instincts of others affect breeding performance and pup survival. For example, SJL/J (000686) males are aggressive and attack their mates and offspring; NZB/BINJ (000684) females are poor mothers.
- **Mutations and transgene effects.** Some mutations are embryonic lethal; some cause infertility or reduced fertility; some affect mammary gland function. For example the Tg(SOD1*G93A)1Gur transgene (also found in several strains) induces neurodegeneration. The severity of such effects depends on strain background.
- **Temperature and humidity.** Laboratory mice breed best when the temperature is between 65-75°F (~18-23°F) and the humidity is between 40-60%. If the temperature and humidity are uncomfortable to humans, they are probably uncomfortable to mice.
- **Light intensity and light cycle.** Because mice generally breed at night, breeding performance is best when a consistent and uninterrupted light-dark cycle is maintained. We use a 14-hour lights on/10-hour lights off cycle, but 12-hour lights on/12-hours dark works well too.
- **Noise and vibrations.** Disturbances such as changes in noise and vibration levels can decrease breeding performance and may induce the mothers to cannibalize their pups. Construction-related noises and vibration may be particularly problematic.
- **Barometric pressure.** Falling barometric pressure can make some strains hyperactive and decrease their breeding performance.
- **Odors.** Noxious fumes, perfumes, and other strong odors can decrease breeding performance.
- **Handling.** Laboratory mice respond best to calm and consistent handling. Pregnant mice, mice giving birth, wild-derived strains and mice with new litters should be handled as little as possible.
- **Nutrition.** Nutrition affects breeding performance. For example, some strains breed better when fed a diet containing 11% fat, DBA/2J (000671) mice and wild-derived strains, such as CAST/EiJ (000928), breed better when fed a lower fat diet containing 4% fat.
- **Feed.** Some strains of mice have bad teeth, no teeth, or other phenotypes that affect their ability to eat grain pellets. These mice need special foods, such as ground or dampened grain.
- **Feed placement.** Obese mice are so heavy that they cannot lift themselves up to where food hoppers are normally placed. If they do manage to hoist themselves up that high, they sometimes fall over on their backs and cannot right themselves. Therefore, their food should be placed in a relatively low position in the cage or on the cage floor (consult your institution's Animal Care & Use Committee (ACUC) for regulations about feed placement).
- **Health.** Laboratory mice may stop breeding if they are unhealthy.
- **Enrichment.** Neslets, kimwipes, or other soft fibrous material provide security and nesting materials; these may alleviate stress and improve breeding.

Mouse Breeding Performance

Optimizing Breeding Performance

Factors to consider

To optimize the breeding performance of laboratory mice, observe the following practices:

1. **Replace breeders before their reproductive performance declines.** Maintain breeder pairs of various ages by replacing a percentage of them monthly or weekly. A colony of mixed-aged breeders produces a more consistent number of pups than does a colony of even-aged breeders.
2. **Replace non-productive breeders.** The following signs indicate that breeders are non-productive:
 - a. They produce no litter within 60 days of mating (longer may be acceptable if delayed breeding is a strain characteristic).
 - b. They produce no litter within 60 days of their last litter and are not visibly pregnant.
 - c. They produce litters but do not wean pups for two to three litters.
3. **Mate mice early.** Mate mice when they are six-to-eight weeks old. Younger mice generally breed better than do older ones.
4. **Use experienced males.** Pairing young females with older males often improves breeding performance.
5. **Keep meticulous and accurate breeding records.** To evaluate the breeding performance of a mouse colony, meticulously maintain accurate records and examine them regularly. The sooner a problem is detected, the sooner it can be corrected. Cultivate habits such as the following:
 - a. Investigate deviations in breeding performance and phenotype immediately.
 - b. Compare your colony's breeding performance to that characterized by your supplier. If mutant strain breeding data are not available, use data for the inbred strain background. All mouse facilities are different: strains that breed well in one facility may not breed well in another.
 - c. Keep a colony's environmental conditions suitable and stable.
 - d. Verify the genotypes of pedigreed breeders for colonies of induced mutants (including those with a visible phenotype) with molecular or other diagnostic assays.

Note: Miscellaneous precautions

A mutation's genetic background may affect phenotype, including breeding performance. If you transfer a mutation to a different background, maintain several generations of each background until you are sure that the second background does not affect phenotype, especially reproductive performance and survival.

Try to avoid inadvertently placing selective pressure on your mouse colony. For example, if you pick only offspring from the best breeding females to perpetuate your colony, you may select for genes that change your strain's characteristics and inadvertently develop a substrain.

Litter fostering

Females of some strains are poor mothers (e.g., NZB/BLN, 000684) or cannot nurse, and a few mutations, such as toxic milk (*Atp7b^{ts}*) and lethal milk (*Slc30a4^{lm}*), render the mother's milk harmful to her pups. In such cases, litters may need foster care to survive. Fostering mice is relatively simple. The foster mother must have a healthy and well-fed litter of her own that is within one or two days of age of the fostered pups. It is very helpful if her pups are of a different coat color than that of the fosterlings. The foster litter should be no larger than the natural litter. Additionally, if the foster litter is larger than six pups, divide it between two foster mothers. Remove the proposed foster mother and place her in a holding pen. Place the fosterlings in the foster mother's home pen and cover them with some nest material or bedding so they acquire her scent and the scent of her pups. To be sure that the foster mother is feeding the pups, observe her and the pups carefully for a day or two. If litter survival is crucial, divide the litter among several foster mothers.

Mouse Breeding Performance

Optimizing Breeding Performance *(continued)*

Mating numerous females simultaneously

To induce numerous females to produce same-age pups, take advantage of the Whitten Effect (The Jackson Laboratory 1976). House the females together as densely as permitted by your institution's Animal Care and Use Committee (ACUC) guidelines. The dense co-housing suppresses the females' individualized estrous cycles. Then, induce them to resume their cycles simultaneously by exposing them to male androgen or shavings from a male cage. Mice have a four- to five-day estrous cycle and ovulate on the third day. Placing the females with a male on the third day of their cycle will result in the maximum number of pregnancies. For best results, house stud males individually for one to two weeks and then add females to the males cage.

Determining pregnancy

Frequently, you may need to know exactly when a mouse conceived. Although there are no early pregnancy tests for mice, you can tell that a female has mated in the last eight to 30 hours if her vagina contains a copulatory plug (a white or cream-colored plug of solidified ejaculate). Because mice usually mate four to six hours into the dark cycle, look for a plug as early into the light cycle as possible. Otherwise, the plug may be dislodged or dissolved. The nature and location of the vaginal plug can be a strain characteristic: it is superficially evident in some strains but deep in the vagina in others. If it is deep, it can usually be seen by opening the vagina gently with a blunt flat tooth pick or blunt metal probe. The presence of a plug indicates only that the female has mated, not that she has conceived. Pregnancy may be verified by palpation on the eleventh day of gestation (day zero is the day a plug is found).

Breeding Schemes

The heart of any colony management program is an appropriate breeding scheme. Often, a strain's phenotype may limit your choice of schemes to only one or two. To choose the best scheme, you'll need to consider several factors, including the desired genotypes, genotyping methods, necessary control mice, and your institution's ACUC guidelines.

Managing Small Colonies

Below are basic principles for managing small mouse colonies:

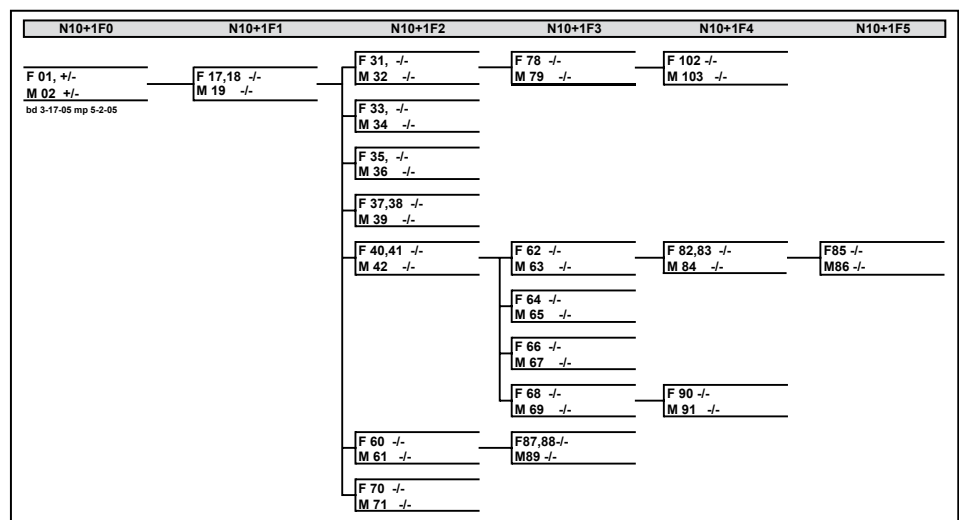
- **Maintain a minimum of six breeding pairs** representing different generations in your colony
- **Retain two generations of a strain**, and do not eliminate one until the next one is producing (for a while, you will therefore be maintaining three generations)
- **Keep the age range** of your breeders between two and eight months old; older mice may not breed reliably
- **Monitor breeding performance closely**; if performance declines, promptly take corrective measures
- **Consider backcrossing your strain** approximately every 10 generations to prevent substrain divergence
- **Contemplate cryopreserving a strain** in case breeding performance either declines, ceases, or a catastrophic event (such as a fire or flood) threatens your colony

Simple Breeding Schemes

A strain is defined as inbred if it was produced by sibling matings for more than 20 generations, after which all mice are considered isogenic or genetically identical. To remain inbred, a strain must be maintained by sibling matings or, if necessary, by parent-offspring matings. The main pedigree line should be derived from a single sister-brother breeding pair at each generation.

To produce sufficient mice for experiments, multiple sister-brother breeding pairs or trios (two females and one male) can be established. Most strains produce more progeny per cage if mated as trios because all adult cage mates generally help care for the young. Occasionally, strains that have small litters or are poor parents may be bred in harems (several females with one male). For pedigreed matings all members of the harem should be siblings, and pregnant females should be separated and housed individually. However, as mating normally occurs shortly after birth, females that are not continually housed with a male bear fewer litters. Because a male may kill the pups, we recommend not returning him to a cage with a female and her pups until she has weaned them. Recombinant inbred, congenic, chromosome substitution (consomic), and recombinant congenic strains are all specialized inbred strains and should be maintained as such.

To the right are examples of typical schemes for breeding genetically defined mutants. Actual allele symbols are used to represent spontaneous and induced mutations; "+" symbols represent wild-type alleles. The first mouse in each scheme is conventionally the female. The first three schemes apply to strains with recessive mutations, and to strains with dominant and semi-dominant mutations that are homozygous viable.



Computer-generated pedigree

Breeding Schemes

Simple Breeding Schemes (continued)

Homozygous mutant (-/-) x homozygous mutant (-/-)

This breeding scheme is used when homozygous mutants of both sexes are viable and fertile. Although all offspring produced are homozygous mutants, breeder genotypes should be verified. To be maintained on a stable inbred background, mutants should be backcrossed to the parental inbred strain about every 10 generations (for example, backcross a mutant on a C57BL/6J background to the standard C57BL/6J strain). Otherwise, an inbred substrain background will be produced. To maintain mutants on mixed or segregating genetic backgrounds (e.g., B6;129), choose breeders randomly from within a colony and backcross the offspring to F1 hybrids between the two strains that comprise the mixed background (in this case, C57BL/6 and 129 F1 hybrids or B6129F1s) about every 10 generations. Otherwise, repeated brother-sister matings will result in recombinant inbred lines.

Controls. If a mutant's genetic background is an inbred strain, that inbred strain is a suitable control. If the mutant's genetic background is mixed (i.e., B6;129), F2 hybrids between the two parental strains are acceptable controls. However, they will be only approximate controls because it is unlikely that an F2 hybrid mouse will have the same genetic mix as the mutant. It will more likely have a uniquely random mix of background genes from the two parental strains.

Heterozygous mutant (-/+) x homozygous mutant (-/-)

This breeding scheme is used when only one gender of a mutant is a viable and fertile homozygote (the other gender may be infertile or have reduced fertility, embryonic lethal, or die before reaching sexual maturity). Unless they can be recognized by a visible phenotype, all mutant mice must be genotyped or progeny tested (see below) to differentiate homozygotes and heterozygotes. If the mutants are on a mixed genetic background, backcross the offspring to appropriate F1 hybrids about every 10 generations. If the mutants are on an inbred background, prevent genetic drift by backcrossing them to the appropriate inbred strain every 10 generations.

Controls. If a mutant's genetic background is inbred, either the inbred strain or heterozygous siblings with normal phenotypes are suitable controls. If the mutant's genetic background is mixed, heterozygous littermates are suitable controls: though their backgrounds are not genetically identical, since some background alleles are segregating.

Heterozygous mutant (-/+) x heterozygous mutant (-/+)

This breeding scheme is used when homozygous mutant mice are severely impaired, infertile, embryonic lethal, or die before reaching sexual maturity. If the mutant homozygotes and heterozygotes cannot be visually distinguished, individuals must be genotyped or progeny tested (see below). If the mutants are inbred, prevent genetic drift by backcrossing them to the appropriate parental inbred strain every 10 generations. If the mutants are on a mixed genetic background, backcross the offspring to appropriate F1 hybrids about every 10 generations.

Controls. If the mutant's genetic background is inbred, the inbred strain and either wild-type or heterozygous siblings are suitable controls; if the mutant's genetic background is mixed, either wild-type or heterozygous siblings are suitable controls.

Progeny testing

When a breeding scheme produces offspring of multiple genotypes (i.e. wild-type, heterozygotes, and homozygotes), the genotypes of each offspring must sometimes be determined. If they cannot be determined visually or by molecular or other diagnostic assays, they must be determined by progeny testing. Most commonly, progeny testing involves mating the mice of unknown genotypes to a parent or a related mouse of known genotype and comparing the observed and expected phenotypes of the offspring produced. For example, the recessive beige coat color mutation is maintained on the C57BL/6J-*Lyst^{bg-1}/J* (000629) strain. These mice are perpetuated by brother-sister matings. However, to avoid substrain divergence, they are periodically backcrossed to the parental C57BL/6J strain, and the heterozygous offspring are intercrossed, producing beige mice (homozygous for the recessive beige mutation) and black mice (some heterozygous and some homozygous for the dominant black color). To determine their genotypes, the black offspring are mated to a homozygous beige mouse: if the offspring are all black, the genotype of the black mouse is wild-type; if some of the offspring are beige, the genotype of the black mouse is heterozygous.

Breeding Schemes

Complex Breeding Schemes

Maintaining transgenic strains

Maintaining a colony of transgenic mice can be challenging. The expression of a transgene may affect a strain's viability and fertility. For example, B6CBA-Tg(HDexon1)62Gpb/1J (002810) mice develop tremors and seizures by nine to 11 weeks of age. Additionally, some of the males are infertile, and they have a breeding lifespan of only three to four weeks. As another example, the *Hmga2* transgene in B6.Cg-*Hmga2*^{pg-Tg40BCha}/BmJ mice (002644) is allelic with the pygmy (*Hmga2*^{ps}) allele. As a result, homozygotes for the transgene are small and infertile.

Moreover, the integration site and number of integrated copies of a transgene may affect its expression. For example, whereas the expression of the human B cell Leukemia/Lymphoma 2 (BCL2) transgene in B6.Cg-Tg(BCL2)22Wehi/J mice (002319) is restricted to the B cell lineage, its expression in B6.Cg-Tg(BCL2)25Wehi/J mice (002320) is restricted to the T cell lineage. In B6.Cg-Tg(BCL2)36Wehi/J mice (002321), it is expressed in B- and T cell lineages. The severity of paralysis due to the SOD1*G93A transgene in mouse strain B6SJL-Tg(SOD1*G93A)1Gur/J (002726) depends on the transgene copy number.

Transgenic breeding schemes can be designed to eliminate the production of undesirable phenotypes, such as embryonic lethality or infertility. Because a transgene is an introduced allele, its copy number, expression level, or integration site may result in the lethality of Tg/Tg offspring. Therefore, a transgenic strain with such a transgene should be maintained by mating a hemizygous mouse (Tg/0) to a noncarrier or wild-type (0/0 or +/-) mouse. If the transgene does not affect embryonic or neonatal survival, approximately half the offspring will be hemizygous (Tg/0), and half will be non-carriers (0/0 or +/-). To distinguish the hemizygotes and non-carriers, every mouse must be genotyped, unless the hemizygotes have an overt phenotype.

Mice homozygous for transgenes may be produced and used in breeding, in some cases. To produce homozygous offspring, a Tg/0 x Tg/0 scheme is used; half the offspring will be hemizygous (Tg/0) and one quarter will be homozygous (Tg/Tg). If hemizygotes and non-carriers are phenotypically indistinguishable from the homozygotes (Tg/Tg), each offspring must be genotyped. The offspring must be genotyped either by progeny testing (to determine transmission frequency) or by quantitative Polymerase Chain Reaction (to determine transgene copy number).

Controls. If the genetic background of a transgenic is either inbred or a congenic, either the inbred or the congenic is a suitable control; if the transgenic's background is mixed, non-carrier siblings are suitable.

Maintaining strains by ovarian transplantation

Some strains are best maintained by ovarian transplantation. Homozygous B6C3Fe *a/a-Csf1^{op}*/J (000231) females fail to lactate, and homozygotes of both genders are extremely fragile. Therefore, we transplant ovaries from a homozygous (*op/op*) female into a recipient female of a histocompatible strain. To quickly expand the colony, the donor ovaries may be quartered and each quarter ovary transplanted into a ovariectomized recipient female. We also maintain B6.V-*Lep^{ob}*/J mice (000632) by ovarian transplantation because, though the females produce functional gametes, they cannot sustain a productive pregnancy. Additionally, we maintain colonies of B6CBA-Tg(HDexon1)62Gpb/1J (002810), B6CBA-Tg(HDexon1)62Gpb/2J (004601), and B6CBA-Tg(HDexon1)62Gpb/3J (006494) by ovarian transplantation to extend the breeding lifespans of the females. Although these females produce viable oocytes for a long time, they develop a progressive neurological disease that renders them physically incapable of mating or sustaining a pregnancy.

If a recipient's ovaries are not completely removed, she may, in addition to bearing a recipient's offspring, bear some of her own. To distinguish the two types of offspring, we use a recipient of a different, dominant coat color. Any offspring with the recipient's color will have been derived from her residual ovaries.

Breeding Schemes

Complex Breeding Schemes (continued)

Maintaining hybrid strains with two or more mutant alleles

Some strains, such as WBB6F1/J-*Kit^w/Kit^{w-v}*/J (100410), must be produced by crossing mice from two strains, in this case WB/ReJ *Kit^w*/J (000692) and C57BL/6J-*Kit^{w-v}*/J (000049). Thus, three colonies must be maintained: one for each of the parent strains, and one of the desired F1 strain.

Maintaining cre and loxP strains

Some genes have vital functions during certain developmental stages. If they are “knocked out” during those stages, the mice may die. Cre-lox technology allows a gene to be targeted after a critical developmental period passes (see the JAX® Mice website, www.jax.org/jaxmice/models/cre_intro). To maintain a cre-lox strain, three strains must be maintained: the *cre* strain, the *loxP* strain, and the cre-lox strain. Unless a cre-lox strain needs a special diet to induce or suppress gene expression, it is maintained like a transgenic strain. See our website at www.jax.org/jaxmice/research/cre for more details.

Maintaining Outbred Stocks

The genomic diversity of individual outbred mice contrasts directly with the genetic identity among individual mice of an inbred strain. To maintain genetic diversity in an outbred colony, matings between related individuals should be avoided; however, some inbreeding may be inevitable over time in any relatively small, closed outbred colony.

Therefore, the following should be considered when establishing an outbred colony:

- Use numerous, genetically diverse founder mice
- Use a defined breeding scheme that is designed to minimize inbreeding: Several different outbred breeding programs have been described (see Berry & Linder, 2007)
- While random breeding — using a random number table or computer program to select breeders — can be used, random breeding will result in occasional matings between closely related individuals
- Keep the colony at a minimum size of approximately 25 breeder males per generation

Maintaining Genetic Quality

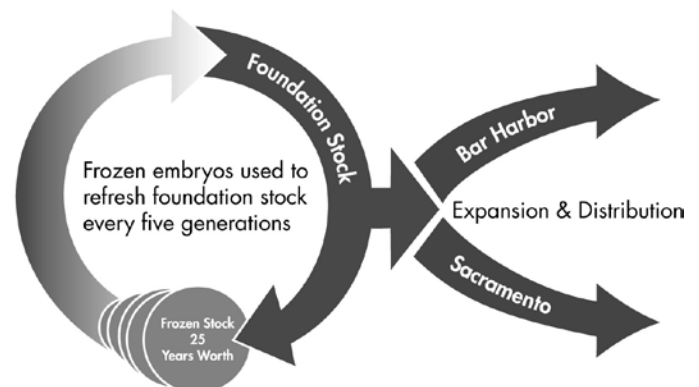
You can maintain the genetic integrity of your mouse strains, ensure the reliability of biomedical research, and help prevent genetic drift by observing the following principles:

- Obtain mice from a reliable breeding source
- Acquire new breeding stock from your supplier periodically, particularly if you maintain your own private colonies of a strain (although colonies of inbred mice expanded from our breeding stock can be maintained either by sibling or non-sibling matings, they may develop into substrains if they are expanded beyond ten generations)
- Avoid comparing results from substrains that either arose early in a strain's inbreeding regimen or that have been long-separated
- Employ proper nomenclature to describe your mouse models
- Include a detailed description of the genetic background of the mice you use in all your communications
- Use a common genetic background when possible, so that your experiments can be replicated

The Jackson Laboratory Genetic Quality and Stability Programs

As the world's leading supplier of genetically well-defined mice, The Jackson Laboratory has a rigorous Genetic Quality Control Program. This program curtails genetic contamination and genetic drift by limiting the number of generations attained in JAX® Mice colonies to less than 10 generations from the main pedigree line. Furthermore, The Jackson Laboratory's unique Genetic Stability Program nearly eliminates genetic drift by refreshing the foundation stocks of several widely used strains with cryopreserved embryos about every five generations.

For more details about these programs, visit our website at www.jax.org/jaxmice/genetichealth.



Twenty-five years from now, the mice you receive from JAX will only be a few generations away from the mice of today.

You can implement a Genetic Stability Program for your own strains using JAX® Embryo Cryopreservation and Recovery Services to cryopreserve stocks and periodically refresh your colonies with frozen embryos.

Costs of Maintaining Mouse Colonies

Although it may seem easier to maintain mouse strains “on the shelf” in your own facility, the costs of doing so should be considered. Below are two hypothetical scenarios to help you determine the cost of maintaining a certain size mouse colony for a known amount of time. (Cost estimates are for a typical academic institution and are based on our experience working with customers and breeding mice. They may vary significantly among institutions.)

Scenario 1

Overview

You need to produce 20 age-matched mice per week of both sexes for one year (1,040 mice total). This will require at least 31 breeding females on hand throughout the year, or 31 cages if paired with males. It also requires at least 6 wean/holding cages to house animals until they are used.

Cost/mouse cage

The average cost per cage should include cost of the animal care technician, cage washing, bedding, feed, and other supplies. This will vary for different institutions, but is approximately \$550/cage/year on average. This figure does not include genotyping.

Calculation of cost

$(31 \text{ breeder cages} + 6 \text{ wean/holding boxes}) \times (\$550/\text{cage/year}) = \$20,350 \text{ total}$
 $\$20,350 \div 1,040 \text{ mice} = \19.57 per mouse

Scenario 2

Overview

You need to use 40 age-matched mice every other week of both sexes for one year (20 mice per week, or 1,040 mice total). This will require at least 62 breeding females on hand throughout the year, or 62 cages paired males. It also requires at least 20 wean/holding cages to house animals until they are used every two weeks.

Cost/mouse cage

The average cost per cage should include cost of the animal care technician, cage washing, bedding, feed, and other supplies. This will vary for different institutions, but is approximately \$550/cage/year on average. This does not include genotyping.

Calculation of cost

$(62 \text{ breeder cages} + 20 \text{ wean/holding boxes}) \times (\$550/\text{cage/year}) = \$45,100 \text{ total}$
 $\$45,100 \div 1,040 \text{ mice} = \43.37 per mouse

Reducing Costs

The key to reducing the cost of maintaining a mouse colony is to maximize use of every mouse in your colony. When possible, observe the following:

- Use both sexes of mice
- Use all ages of your mice
- Use mice weekly or use an age range, such as four- to eight-week-old mice, monthly
- Replace aging breeders according to a strict schedule
- Replace non-productive breeders as soon as possible

Cryopreservation

Advantages of Cryopreservation

You may consider maintaining some strains by cryopreservation. Cryopreservation offers the following advantages:

- **Saves space**, especially when a strain is used infrequently (At The Jackson Laboratory, just 25 square feet accommodates 2,000,000 cryopreserved embryos)
- **Saves money**, although the initial cost of cryopreserving a strain may seem high, the follow-up cost of maintaining it in a cryopreserved state is only a few dollars a year, significantly lower than that for maintaining a live colony for the same amount of time. Our new and proven Sperm Cryopreservation and Recovery Service provides an economical way of maintaining a strain.
- **Insures against catastrophic loss** (fire, flood, earthquakes, disease, *etc.*)
- **Provides a method for eliminating pathogens from a mouse colony**
The techniques used during embryo transfer, the most effective way of rederiving a mouse colony to SPF conditions, are the same as to those used for recovering strains from cryopreserved embryos and sperm
- **Reduces the risk of a strain contamination due to breeding errors**
- **Prevents spontaneous loss of phenotype**
- **Slows the rate of genetic drift dramatically**
- **Diminishes the possibility that the transgene copy number will change**

New Sperm Cryopreservation and Recovery Service

The Jackson Laboratory recently developed a new cost-effective Sperm Cryo and Recovery Service for knockout and transgenic mice that results in significantly higher fertilization rates, more live births, and is feasible with more background strains than previously possible. (See page 15 for details)

Considerations for Cryopreserving a Strain

When cryopreserving a strain, the following should be considered:

- **Feasibility.** Some strains cryopreserve better than others. For example, whereas the percentage of C57BL/6J embryos that can be successfully recovered from cryopreservation is high, the percentage of A/J embryos that can be recovered is low. However, new cryopreservation techniques are continually being developed, and strain-specific protocols are improving success rates. Additionally, techniques for cryopreserving sperm, oocytes, and ovaries have been developed.
- **Recovery costs.** Recovering a strain costs money. Therefore, you may not want to manage a frequently used strain by cryorecovery alone. For some strains, our Sperm Cryopreservation and Recovery Service may be a cost-effective alternative (see page 15 for details).
- **Recovery time.** Recovering a strain from cryopreservation takes time. Plan ahead so you have the mice you need when you need them.

JAX[®] Services for Breeding & Colony Management

JAX[®] Services is a comprehensive, integrated set of mouse breeding and research services providing efficient and cost-effective solutions for mouse-based research projects. These services make The Jackson Laboratory's extensive expertise in mouse breeding, husbandry, and genetics research available to the biomedical research community. All JAX[®] Services are conducted according to the highest standards of animal health and genetic quality and are delivered to meet your schedule, your budget, and your research goals. Our colony management services are offered both in Bar Harbor, Maine, and Sacramento, California. For more information, please see the JAX[®] Services website at www.jax.org/jaxservices, call 1-800-422-6423, or email jaxservices@jax.org.

JAX[®] Breeding Services

Using investigator-supplied and/or JAX[®] Mice strains, we can produce F1, F2, and backcross progeny, congenics, coisogenics, and strains with multiple gene mutations. We can maintain or ship mice to you as needed. By combining our expertise in mouse genetics and colony management with robust *in vitro* fertilization (IVF) techniques, we can develop and implement even the most complex and challenging breeding schemes. Through the optimized use of mouse numbers and box space, we can deliver cost-effective and dependable breeding projects scaled to meet your needs.

JAX[®] Special Diets

We can produce and maintain mice on special diets to meet your specific research requirements. We work with you to select mouse strains, control diet and housing density, and to design the biospecimen and weight data collection protocol. Mice can be shipped to you or evaluated through our Efficacy Testing and Phenotyping Services.

JAX[®] Aging Service

In some mouse models, a disease condition develops only with age. JAX[®] Aging Service will maintain your research strain, or any JAX[®] Mice strain, and deliver mice to you at ages appropriate for your research projects.

The following pre-aged mice are available for purchase (learn more at www.jax.org/jaxservices/study-ready):

- Alzheimer's strain B6.Cg-Tg(APP^{swe},PSEN1^{DE9})85Dbo/J (005864)
- Study-ready Diet-Induced Obese (DIO) C57BL/6J mice aged up to 26 weeks
- Surgically altered age-onset models for Alopecia Areata

Dedicated Supply of JAX[®] Mice

We apply our unparalleled expertise in mouse husbandry to offer you a dedicated supply of JAX[®] Mice strains that are either very challenging to maintain or, because of low demand, are generally not available in large numbers. We supply you with agreed-upon quantities of these mice when you need them.

JAX[®] Rederivation

For a variety of reasons, mouse colonies may become infected with a pathogen, spread the infection to other colonies, compromise the health of the infected mice and confound your research results. Our innovative **Speed Rederivation** service uses *in vitro* fertilization (IVF) to rederive strains on common genetic backgrounds within 12-14 weeks from receipt of your mice. Typically 10 or more rederived pups are provided and the service includes sperm cryopreservation for your strain plus three years of liquid nitrogen storage. Our **Custom Rederivation** service uses either hysterectomy derivation or embryo transfer to rederive inbred or homozygously maintained colonies.

JAX[®] Speed Expansion

This service uses assisted reproductive techniques, such as IVF, to expand breeding colonies much faster than can standard colony expansion techniques.

JAX[®] Services for Breeding & Colony Management

JAX[®] Speed Congenic Development

JAX[®] Speed Congenic Development Service accelerates the creation of congenic strains through a marker-assisted breeding strategy, thereby producing a research model faster while simultaneously reducing facility, equipment and personnel costs, and the total number of mice used. This service is used to transfer genetic mutations or knockouts from one genetic background to another. Such transfers are typically made to reduce background effects, enhance the phenotype, or improve the vigor (*e.g.*, survival, breeding performance, general health, *etc.*) of mouse disease models. We use markers from our validated database of single-nucleotide polymorphism (SNP) markers to select mice carrying the gene of interest and the highest percentage of host DNA markers for each backcross mating, ultimately creating a 99.9% congenic strain in only 15-18 months. Once created, we can breed and ship mice to your specifications.

Strain Rescue

We provide a variety of options for rescuing mouse strains endangered by breeding, extreme age, health or other problems. We use assisted reproductive techniques such as *in vitro* fertilization (*IVF*) or ovary transplant to attempt to rescue your strain.

Microinjection & Novel Strain Creation

We offer both DNA pronuclear injection of embryos and ES cell microinjection of blastocysts to facilitate the creation of genetically modified mice. We inject into C57BL/6J embryos which saves you the time and money required to backcross onto this background.

JAX[®] Sperm Cryo Kit

The JAX[®] Sperm Cryo Kit enables researchers to cryopreserve novel mice strains in their own laboratories, while still enjoying the peace of mind provided by quality control (QC) testing and safe, long-term storage at The Jackson Laboratory. Our kits include a clear, easy-to-follow instruction manual and everything you need to cryopreserve three or more strains. These kits are designed for users familiar with mouse dissection techniques.

JAX[®] Sperm Cryopreservation & Recovery

This is the first truly reliable and cost-effective sperm cryopreservation and recovery service for knockout and transgenic mice. Previous mouse sperm cryopreservation methods often resulted in poor fertilization and uneconomical recovery. Our new techniques typically yield fertilization rates of over 50% and are suitable for transgenic and knockout strains maintained on C57BL/6, FVB, DBA, and C3H backgrounds, F1 hybrids of these strains, and B6129 hybrids.

This service provides inexpensive insurance against catastrophic loss of mouse strains due to disease, fire, flood, or breeding accidents, facilitates rapid production of large numbers of age-matched mice, and provides a rapid approach to generating specific pathogen-free (SPF) live mice. A minimum of 16 straws of sperm are cryopreserved for each strain and stored in liquid nitrogen at two sites for three years. Additional years of storage can be purchased. Throughout the cryopreservation process, stringent quality control checks are implemented, including sperm motility tests and an *IVF* fertilization test to two-cell embryos. Optional recovery of live-born mice (for verification of recoverability) is strongly recommended.

JAX[®] Speed (Embryo) Cryopreservation & Recovery

Using *IVF*, superovulation, and other innovations, we offer a quick, cost-effective, and reliable colony management solution for cryopreserving and rapidly recovering strains on C57BL/6, FVB/N, DBA/2, BALB/cBy, or NOD/ShiLt backgrounds. Systematic checks throughout the processes ensure successful recovery.

JAX[®] Custom (Embryo) Cryopreservation & Recovery

We can cryopreserve inbred, mutant, and genetically modified mice (whether they are homozygous, hemizygous, or heterozygous). Costs depend on factors such as strain background, fertility, and the number of mice provided to us.

JAX[®] Services for Breeding & Colony Management

Genome Scanning

This service is for investigators who wish to do their own breeding to develop congenic or consomic lines, but wish to take advantage of our SNP maker panel to type and select the offspring for each backcross. Investigators send us tail samples from the offspring (minimum of six to 10 recommended per generation), we type the tails, and tell the investigators which mice should be used for the next backcross. This service can cut in half the time needed to construct a congenic strain. In addition, this service can be used to facilitate your research in many additional ways, including the following:

- One time scan: determine the degree of congenicity of an existing congenic
- Detect cryptic unlinked segments of residual donor DNA in established congenic strains
- Detect recent inter-strain genetic contamination
- Map the location of a spontaneous or induced mutation or a non-targeted transgenic insertion

Mouse Diversity Genotyping Array

This service utilizes an innovative genotyping microarray which was designed for high-density, genome-wide profiling of single nucleotide polymorphisms (SNPs). The array was developed in the laboratories of Drs. Gary Churchill (The Jackson Laboratory) and Fernando Pardo-Manuel de Villena (University of North Carolina), both of The Center for Genome Dynamics.

Applications include the following:

- Genetic quality control
- Characterizing or comparing mouse DNA samples (e.g. from tumor tissues, cell lines, or substrains)
- High resolution mapping and genetic analysis
- Association and quantitative trait loci (QTL) studies
- Copy number variation (CNV) analysis

For more information on how these services can help you characterize the genetic background or ensure the genetic integrity of your mouse strain(s), contact our JAX[®] Services specialists at 1-800-422-6423 (from U.S.A., Canada and Puerto Rico only) or +1-207-288-5845 (from any location) or via the web at <https://secureweb.jax.org/jaxmice/servicesrequest.html>.

The Jackson Laboratory's Colony Management System

The Jackson Laboratory's Colony Management System (JCMS) is a multi-user database for managing animal colonies in a research environment. It was developed in response to increased requests within the JAX community for colony management software with an intuitive, easy-to-use interface.

Features of JCMS:

- Tracking animal status and pedigree information
- Reports on timed matings (plug dates, for example)
- Genotype logging
- Creating litter and/or mating records
- Animal pen management
- Experimental data tracking
- Cage card printing
- Export of data and creation of various reports
- Advanced database queries
- Support for handheld devices
- Tracks samples to their sources and stores their locations
- Reports on strain viability
- Timely user support via our moderated user group
- Automatically migrates your data to a MySQL backend
- Uses Microsoft Access[®]

For more information about JCMS, please visit our JCMS home page (colonymanagement.jax.org), read the JCMS discussion forum (community.jax.org/forums/9.aspx) or contact JCMS support via the web at (colonymanagement.jax.org/support).

Course: Colony Management, Principles and Practices

This newly-expanded four-day workshop was designed and is taught by The Jackson Laboratory staff. It provides invaluable training for students, scientists, animal care technicians and other personnel who manage research and production mouse colonies.

Topics include the following:

- Basic principles of mammalian genetics
- Overview of JAX[®] Mice nomenclature and uses
- Breeding strategies
- Genetic quality control
- Importation and animal health
- Resources for genetically engineered mice
- Facility design
- Considerations in tracking and storage of colony data

For further information on this and other courses taught at The Jackson Laboratory, see the Courses and Conferences website, www.jax.org/courses.

JAX[®] Resources for Mouse Colony Management

Jackson Laboratory Online Mouse Resources

The following online resources, maintained at The Jackson Laboratory, contain a great deal of useful information for managing mouse colonies.

JAX[®] Mice Database

The JAX[®] Mice database (www.jax.org/jaxmice) contains strain datasheets with detailed information for every strain of JAX[®] Mice.

Mouse Genome Informatics

The Mouse Genome Informatics website (www.informatics.jax.org) contains an immense amount of information about mouse genetics. It also has links to the following databases:

- Mouse Genome Database
- Gene Expression Database
- Mouse Genome Sequence Project Database
- Tumor Biology Database
- Gene Ontology Project Database
- Festing's Inbred Strain Characteristics Database
- International Mouse Strain Registry Database

Mouse Phenome Database

The Mouse Phenome Database (www.jax.org/phenome) is a repository for phenotypic and genotypic data on over 40 commonly used and genetically diverse inbred JAX[®] Mice strains. It is a platform for data analysis and *in silico* hypothesis testing, and enables investigators to choose optimal strains for their research, including physiological studies, drug and toxicology testing, and modeling disease processes.

Technical Information Services

Our technical information scientists are eager to help you select the appropriate JAX[®] Mice strains and controls for your research, or to suggest alternative options that will expedite your research projects.

- Visit our webpage (www.jax.org/jaxmice/support/techsupport-index) to find broad self-help guides for genotyping, mouse husbandry, and nomenclature, and helpful links to mouse strain data and resources
- See the JAX[®] Notes article featuring this dynamic group at www.jaxmice.jax.org/jaxnotes/508/508a
- Contact Technical Support at 1-800-422-6423 or via the website at www.jaxmice.jax.org/micetech



Sizing Mouse Colonies

To properly size a research mouse colony, many factors must be considered, including the following:

- Number of mice needed, utility of each sex, needs for specific genotypes and age-matched mice
- Number of strains needed (for example, a cre-lox experiment may require three breeding colonies)
- Preferred breeding scheme
- Strain productivity, genotypes and phenotypes affecting productivity, and number of unproductive matings
- Female's reproductive life span and frequency of litters
- Average number of pups per litter, average sex ratio per litter, and percent survival to weaning and adulthood
- Breeder replacement schedules
- Cage requirements, mouse room space, pair or trio breeding schemes, and allowable mouse density per cage
- ACUC regulations

Above all, colonies must be continually monitored for any environmental changes that could affect strain productivity, general behavior, litter size, pup survival, genotype frequency, phenotype, and other strain characteristics.

The example on the following page incorporates these considerations into a straightforward step-by-step algorithm. Depending on the circumstances, alternate values may be used for each step. In some situations, such as when maintaining colonies with sublethal genes or genes with variable penetrance, the algorithm may need to be modified. Additional examples are presented in the Appendix.



Mouse sperm, vas deferens and cauda epididymis

Sizing Mouse Colonies

Example:

How many breeding females are needed to produce 10 female & 10 male homozygotes per week using a homozygous female x homozygous male breeding scheme?

Strain characteristics

Breeding scheme	Homozygotes x Homozygote
Breeding lifespan	32 weeks
Number of litters produced	4 litters
Litter frequency	1 litter/8 weeks (4 litters/32 weeks)
Litter size	6 pups (3 females, 3 males)
Offspring genotypes	Homozygotes only
Percent useful offspring	100%

Number of experimental mice needed

1. Number of mice needed	20
2. Age requirements If must be same age, enter 1 If can have a two-week age range (e.g., five to six weeks old), enter 2 If can have a four-week age range (e.g., five to eight weeks old), enter 4	1
3. Frequency with which mice are needed If weekly, enter 1 If every other week, enter 2 If once a month, enter 4	1
4. Divide Line 1 by the smaller of Line 2 or Line 3 (round up to nearest whole number)	20
5. Sexes needed If both sexes needed, enter 1 If one sex needed, enter 2	1
6. Breeding scheme If homozygote x homozygote, enter 1 If heterozygote x homozygote, enter 2 If heterozygote x heterozygote, enter 4	1
7. Some surplus (insurance) mice desired If no, enter 1 If yes, enter a "fudge factor" to ensure overproduction e.g., if 10% more mice are desired, enter 1.1)	1.1
8. Number of mice to be produced weekly Multiply Lines 4 x 5 x 6 x 7 (round up to nearest whole number)	22

Colony productivity

9. Average number of pups weaned per litter	6
10. Average number of litters produced per breeder female	4
11. Average productive female's breeding lifespan (weeks)	32
12. Calculate colony productivity (number of weaned pups/female/week) Divide Line 10 by Line 11, multiply by Line 9 (round to nearest hundredth)	0.75
13. Calculate number of breeding females needed Divide Line 8 by Line 12 (round up to nearest whole number)	30

Number of breeding females needed to keep colony productive

14. Calculate number of replacement breeders needed per week Divide Line 13 by Line 11 (round up to nearest whole number)	1
15. Calculate the number of additional breeders needed to provide replacement breeders Divide Line 14 by (2 x Line 12), then multiply by Line 5 (round up to nearest whole number)	1

Total number of breeders needed

16. Add Line 13 and Line 15	31
-----------------------------	----

Number of cages needed per week

17. Breeding cages For pair breeding (one breeding female per cage): 31 cages needed (16 boxes) or trio breeding (two breeding females per cage): 16 cages needed (8 boxes)	
18. Weaning cages ~11 females & ~11 males weaned per week will require ~ 6 cages (5 animals per cage separated by sex), ~ 3 boxes	

Glossary

Allele: An alternate form of a gene or locus.

Backcross: A cross between a strain that is heterozygous for the alleles from two parental strains and one of those parental strains.

Coisogenic strain: A strain that differs from an established inbred strain by a mutation at only one locus.

Congenic strain: A strain created by backcrossing to an inbred parental strain for 10 or more generations while maintaining heterozygosity at a selected locus.

Cross: a mating of strains which are genetically different from each other at one or more loci.

Consomic (chromosome substitution strain): A strain in which one chromosome has been replaced with the homologous chromosome from another strain.

F (filial generation): A generation in a sequence of matings. The first filial generation, symbolized as “F1,” refers to the offspring of a cross between two different strains. When F1 siblings are crossed to each other, their offspring are considered to be members of the second filial generation or F2. Subsequent generations of brother-sister matings are numbered consecutively.

Genetic drift: The constant tendency of genes to evolve, even in the absence of selective forces. It is fueled by spontaneous mutations.

Genotype: The set of alleles at one or more loci. A genotype may be either heterozygous (with two different alleles), hemizygous (with only one allele), or homozygous (with two identical alleles).

Heterozygous: Possessing two distinguishable alleles at a particular locus.

Hemizygous: Possessing an unpaired allele at a particular locus.

Homozygous: Possessing two identical alleles at a particular locus.

Inbred strain: A strain that has been maintained by sibling (sister x brother) matings for 20 or more consecutive generations.

Incross: A cross between two inbred or homozygous strains.

Intercross: A cross between two heterozygous organisms.

Locus: any genomic site.

N: Describes the generation of backcrossing and the offspring that derive from it. For example, the “N2” generation describes offspring from the initial cross between an F1 hybrid and one of the parental strains. Each following backcross generation is numbered in sequence.

Outcross: A cross between genetically unrelated mice.

Phenotype: The physical manifestation of a genotype.

Recombinant inbred strain: A special type of inbred strain formed from an initial outcross between two well-characterized inbred strains followed by at least twenty generations of inbreeding.

Rotation length: Length of time before breeders are replaced (considered the optimum reproductive life span).

Segregating inbred strain: Segregating inbred strains are inbred strains in which a particular allele or mutation is maintained in the heterozygous state. They are maintained by inbreeding (usually brother x sister mating) with forced heterozygosity (selection for heterozygotes) at each generation for the locus of interest.

Substrain: A substrain has known or probable genetic differences from the parental inbred strain, or which has been separated from the parental colony for 20 or more generations.

Targeted mutant (Knockout, Knockin, etc.): A mouse or strain with a gene whose function has been altered by introduction of a recombinant construct through homologous recombination.

Transgene: A fragment of foreign DNA (DNA construct) that has been incorporated into the genome of a mouse.

Transgenic: A mouse with one or more transgenes.

Appendix

Example A1:

How many breeding females are needed to produce 10 female & 10 male homozygotes per week using a heterozygous female x heterozygous male breeding scheme?

Strain characteristics

Breeding scheme	Heterozygotes x Heterozygote
Breeding lifespan	32 weeks
Number of litters produced	4 litters
Litter frequency	1 litter/8 weeks (4 litters/32 weeks)
Litter size	6 pups (3 females, 3 males)
Offspring genotypes	25% Homozygotes, 50% Heterozygotes, 25% Wild-type
Percent experimental pups	25% Homozygotes females and males

Number of experimental mice needed

1. Number of mice needed	20
2. Age requirements If must be same age, enter 1 If can have a two-week age range (e.g., five to six weeks old), enter 2 If can have a four-week age range (e.g., five to eight weeks old), enter 4	1
3. Frequency with which mice are needed If weekly, enter 1 If every other week, enter 2 If once a month, enter 4	1
4. Divide Line 1 by the smaller of Line 2 or Line 3 (round up to nearest whole number)	20
5. Sexes needed If both sexes needed, enter 1 If one sex needed, enter 2	1
6. Breeding scheme If homozygote x homozygote, enter 1 If heterozygote x homozygote, enter 2 If heterozygote x heterozygote, enter 4	4
7. Some surplus (insurance) mice desired If no, enter 1 If yes, enter a "fudge factor" to ensure overproduction (e.g., if 10% more mice are desired, enter 1.1)	1.1
8. Number of mice to be produced weekly Multiply Lines 4 x 5 x 6 x 7 (round up to nearest whole number)	88

Colony productivity

9. Average number of pups weaned per litter	6
10. Average number of litters produced per breeder female	4
11. Average productive female's breeding lifespan (weeks)	32
12. Calculate colony productivity (number of weaned pups/female/week) Divide Line 10 by Line 11, then multiply by Line 9 (round to nearest hundredth)	0.75
13. Calculate number of breeding females needed Divide Line 8 by Line 12 (round up to nearest whole number)	118

Note: If heterozygotes are **not** needed for experiments, they can be used as replacement breeders for colony maintenance, without the need to calculate the number of additional breeding females needed per week.

Number of cages/boxes needed per week (2 cages per box)

Breeding cages	
For mating pairs (one breeding female per cage):	118 cages (54 boxes)
For mating trios (two breeding females per cage):	54 cages (27 boxes)
Weaning cages	
~44 females & ~44 males weaned/week will require ~ 18 cages (five animals per cage separated by sex), ~9-10 boxes	

Appendix

Example A2:

How many breeding females are needed to produce 20 male homozygotes per week using a heterozygous female x heterozygous male breeding scheme?

Strain characteristics

Breeding scheme	Heterozygotes x Heterozygote
Breeding lifespan	32 weeks
Number of litters produced	4 litters
Litter frequency	1 litter/8 weeks (4 litters/32 weeks)
Litter size	6 pups (3 females, 3 males)
Offspring genotypes	25% Homozygotes, 50% Heterozygotes, 25% Wild-type
Percent experimental pups	25% Homozygotes x 0.5 males = 12.5%

Number of experimental mice needed

1. Number of mice needed	20
2. Age requirements	1
If must be same age, enter 1	
If can have a two-week age range (e.g., five to six weeks old), enter 2	
If can have a four-week age range (e.g., five to eight weeks old), enter 4	
3. Frequency with which mice are needed	1
If weekly, enter 1	
If every other week, enter 2	
If once a month, enter 4	
4. Divide Line 1 by the smaller of Line 2 or Line 3 (round up to nearest whole number)	20
5. Sexes needed	2
If both sexes needed, enter 1	
If one sex needed, enter 2	
6. Breeding scheme	4
If homozygote x homozygote, enter 1	
If heterozygote x homozygote, enter 2	
If heterozygote x heterozygote, enter 4	
7. Some surplus (insurance) mice desired	1.1
If no, enter 1	
If yes, enter a "fudge factor" to ensure overproduction (e.g., if 10% more mice are desired, enter 1.1)	
8. Number of mice to be produced weekly	176
Multiply Lines 4 x 5 x 6 x 7 (round up to nearest whole number)	

Colony productivity

9. Average number of pups weaned per litter	6
10. Average number of litters produced per breeder female	4
11. Average productive female's breeding lifespan (weeks)	32
12. Calculate colony productivity (number of weaned pups/female/week)	0.75
Divide Line 10 by Line 11, then multiply by Line 9 (round to nearest hundredth)	
13. Calculate number of breeding females needed	235
Divide Line 8 by Line 12 (round up to nearest whole number)	

Note: If heterozygotes are **not** needed for experiments, they can be used as replacement breeders for colony maintenance, without the need to calculate the number of additional breeding females needed per week

Number of cages/boxes needed per week (2 cages per box)

• Breeding cages	
For mating pairs (one breeding female per cage):	235 cages (118 boxes)
For mating trios (two breeding females per cage):	118 cages (59 boxes)
• Weaning cages	
~88 females & ~88 males weaned/week will require ~ 36 cages (five animals per cage separated by sex), ~18 boxes	

Appendix

Example A3:

How many breeding females are needed to produce 40 female homozygotes (2 week age range) every two weeks using a homozygous female x homozygous male breeding scheme?

Strain characteristics

Breeding scheme	Homozygotes x Homozygote
Breeding lifespan	32 weeks
Number of litters produced	4 litters
Litter frequency	1 litter/8 weeks (4 litters/32 weeks)
Litter size	6 pups (3 females, 3 males)
Offspring genotypes	100% Homozygotes
Percent experimental pups	100% x 0.5 females = 50%

Number of experimental mice needed

1. Number of mice needed	40
2. Age requirements If must be same age, enter 1 If can have a two-week age range (e.g., five to six weeks old), enter 2 If can have a four-week age range (e.g., five to eight weeks old), enter 4	2
3. Frequency with which mice are needed If weekly, enter 1 If every other week, enter 2 If once a month, enter 4	2
4. Divide Line 1 by the smaller of Line 2 or Line 3 (round up to nearest whole number)	20
5. Sexes needed If both sexes needed, enter 1 If one sex needed, enter 2	2
6. Breeding scheme If homozygote x homozygote, enter 1 If heterozygote x homozygote, enter 2 If heterozygote x heterozygote, enter 4	1
7. Some surplus (insurance) mice desired If no, enter 1 If yes, enter a "fudge factor" to ensure overproduction (e.g., if 10% more mice are desired, enter 1.1)	1.1
8. Number of mice to be produced weekly Multiply Lines 4 x 5 x 6 x 7 (round up to nearest whole number)	44

Colony productivity

9. Average number of pups weaned per litter	6
10. Average number of litters produced per breeder female	4
11. Average productive female's breeding lifespan (weeks)	32
12. Calculate colony productivity (number of weaned pups/female/week) Divide Line 10 by Line 11, then multiply by Line 9 (round to nearest hundredth)	0.75
13. Calculate number of breeding females needed Divide Line 8 by Line 12 (round up to nearest whole number)	59

Number of breeding females needed to keep colony productive

14. Calculate number of replacement breeders needed per week Divide Line 13 by Line 11 (round up to nearest whole number)	2
15. Calculate the number of additional breeders needed to provide replacement breeders Divide Line 14 by (2 x Line 12), then multiply by Line 5 (round up to nearest whole number)	3

Total number of breeders needed

16. Add Line 13 and Line 15	62
-----------------------------	----

Number of cages/boxes needed per week (2 cages per box)

<ul style="list-style-type: none"> Breeding cages For mating pairs (one breeding female per cage): 62 cages (31 boxes) For mating trios (two breeding females per cage): 31 cages (16 boxes) Weaning cages ~22 females & ~22 males weaned/week will require ~ 10 cages (five animals per cage separated by sex), ~5 boxes
--

Appendix

Example A4:

How many breeding B6.129S2-*Trp53^{tm1Tyj}/J* females are needed to produce 40 female homozygotes per week using a heterozygous female x homozygous male breeding scheme?

Strain characteristics

Breeding scheme	Mutant Heterozygote x Mutant Homozygote
Strain lifespan (B6.129S2- <i>Trp53^{tm1Tyj}/J</i>)	Mutant Homozygotes: 3-6 months
Breeding lifespan	16 weeks
Number of litters produced	2 litters
Litter frequency	1 litter/8 weeks
Litter size	6 pups (3 females, 3 males)
Offspring genotypes	50% Heterozygotes; 50% Mutant Homozygotes
Percent experimental pups	50% Mutant Homozygotes x 0.5 females = 25%

Number of experimental mice needed

1. Number of mice needed	40
2. Age requirements	1
If must be same age, enter 1	
If can have a two-week age range (e.g., five to six weeks old), enter 2	
If can have a four-week age range (e.g., five to eight weeks old), enter 4	
3. Frequency with which mice are needed	1
If weekly, enter 1	
If every other week, enter 2	
If once a month, enter 4	
4. Divide Line 1 by the smaller of Line 2 or Line 3 (round up to nearest whole number)	40
5. Sexes needed	2
If both sexes needed, enter 1	
If one sex needed, enter 2	
6. Breeding scheme	2
If homozygote x homozygote, enter 1	
If heterozygote x homozygote, enter 2	
If heterozygote x heterozygote, enter 4	
7. Some surplus (insurance) mice desired	1.1
If no, enter 1	
If yes, enter a "fudge factor" to ensure overproduction (e.g., if 10% more mice are desired, enter 1.1)	
8. Number of mice to be produced weekly	176
Multiply Lines 4 x 5 x 6 x 7 (round up to nearest whole number)	

Colony productivity

9. Average number of pups weaned per litter	6
10. Average number of litters produced per breeder female	2
11. Average productive female's breeding lifespan (weeks)	16
12. Calculate colony productivity (number of weaned pups/female/week)	0.75
Divide Line 10 by Line 11, then multiply by Line 9 (round to nearest hundredth)	
13. Calculate number of breeding females needed	235
Divide Line 8 by Line 12 (round up to nearest whole number)	

Note: If heterozygous females and homozygous males are **not** needed for experiments, they can be used as replacement breeders for colony maintenance, without the need to calculate the number of additional breeders needed per week.

Number of cages/boxes needed per week (2 cages per box)

• Breeding cages	
For mating pairs (one breeding female per cage):	235 cages (59 boxes)
For mating trios (two breeding females per cage):	118 cages (30 boxes)
• Weaning cages	
~88 females & ~88 males weaned/week will require ~ 36 cages (five animals per cage separated by sex), ~18 boxes	

Appendix

Example A5:

How many breeding females are needed to produce 40 male homozygotes per week using a heterozygous female x heterozygous male breeding scheme with 15% non-productive breeders?

Strain characteristics

Breeding scheme	Heterozygote x Heterozygote
Breeding lifespan	32 weeks
Number of litters produced	4 litters
Litter frequency	1 litter/8 weeks
Percent non-productive breeders	15%
Litter size	6 pups (3 females, 3 males)
Offspring genotypes	25% Homozygotes, 50% Heterozygotes, 25% Wild-type
Percent experimental pups	25% Homozygotes x 0.50 males x 0.85 = ~ 10%

Number of experimental mice needed

1. Number of mice needed	40
2. Age requirements If must be same age, enter 1 If can have a two-week age range (e.g., five to six weeks old), enter 2 If can have a four-week age range (e.g., five to eight weeks old), enter 4	1
3. Frequency with which mice are needed If weekly, enter 1 If every other week, enter 2 If once a month, enter 4	1
4. Divide Line 1 by the smaller of Line 2 or Line 3 (round up to nearest whole number)	40
5. Sexes needed If both sexes needed, enter 1 If one sex needed, enter 2	2
6. Breeding scheme If homozygote x homozygote, enter 1 If heterozygote x homozygote, enter 2 If heterozygote x heterozygote, enter 4	4
7. Some surplus (insurance) mice desired If no, enter 1 If yes, enter a "fudge factor" to ensure overproduction (e.g., if 10% more mice are desired, enter 1.1)	1.1
8. Number of mice to be produced weekly Multiply Lines 4 x 5 x 6 x 7 (round up to nearest whole number)	352

Colony productivity

9. Average number of pups weaned per litter	6
10. Average number of litters produced per breeder female Because 15% of the breeders are non-productive, multiply the litters per productive female by 0.85	3.4
11. Average productive female's breeding lifespan (weeks)	32
12. Calculate colony productivity (number of weaned pups/female/week) Divide Line 10 by Line 11, then multiply by Line 9 (round to nearest hundredth)	0.64
13. Calculate number of breeding females needed Divide Line 8 by Line 12 (round up to nearest whole number)	550

Note: If heterozygotes are **not** needed for experiments, they can be used as replacement breeders for colony maintenance, without the need to calculate the number of additional breeding females needed per week

Number of cages/boxes needed per week (2 cages per box)

<ul style="list-style-type: none"> Breeding cages For mating pairs (one breeding female per cage): 550 cages (225 boxes) For mating trios (two breeding females per cage): 225 cages (113 boxes) Weaning cages ~176 females & ~176 males weaned/week will require ~ 72 cages (five animals per cage separated by sex), ~36 boxes

Appendix

Example A6:

How long will it take to expand an initial stock of five homozygous breeders to a colony producing 20 female homozygotes per week?

Strain characteristics

Breeding stock	5 females
Breeding scheme	Homozygote x Homozygote
Breeding lifespan	32 weeks
Number of litters produced	4 litters
Litter frequency	1 litter/8 weeks
Percent non-productive breeders	15%
Litter size	6 pups (3 females, 3 males)
Genotypes of offspring	100% Homozygotes
Percent experimental pups	100% x 0.5 females x 0.85 = 42.5%
Time between generations	12 weeks (8 weeks to sexual maturity; 4 weeks for mating and pregnancy)

Number of experimental mice needed

1. Number of mice needed	20
2. Age requirements	1
If must be same age, enter 1	
If can have a two-week age range (e.g., five to six weeks old), enter 2	
If can have a four-week age range (e.g., five to eight weeks old), enter 4	
3. Frequency with which mice are needed	1
If weekly, enter 1	
If every other week, enter 2	
If once a month, enter 4	
4. Divide Line 1 by the smaller of Line 2 or Line 3 (round up to nearest whole number)	20
5. Sexes needed	2
If both sexes needed, enter 1	
If one sex needed, enter 2	
6. Breeding scheme	1
If homozygote x homozygote, enter 1	
If heterozygote x homozygote, enter 2	
If heterozygote x heterozygote, enter 4	
7. Some surplus (insurance) mice desired	1.1
If no, enter 1	
If yes, enter a "fudge factor" to ensure overproduction (e.g., if 10% more mice are desired, enter 1.1)	
8. Number of mice to be produced weekly	44
Multiply Lines 4 x 5 x 6 x 7 (round up to nearest whole number)	

Colony productivity

9. Average number of pups weaned per litter	6
10. Average number of litters produced per breeder female	3.4
Because 15% of the breeders are non-productive, multiply the litters per productive female by 0.85.	
11. Average productive female's breeding lifespan (weeks)	32
12. Calculate colony productivity	0.64
Divide Line 10 by Line 11, multiply by Line 9 (round to nearest hundredth)	
13. Calculate number of breeding females needed	69
Divide Line 8 by Line 12 (round up to nearest whole number)	

Number of breeding females needed to keep colony productive

14. Calculate number of replacement breeders needed per week	3
Divide Line 13 by Line 11 (round up to nearest whole number)	
15. Calculate the number of additional breeders needed to provide replacement breeders	5
Divide Line 14 by (2 x Line 12), then multiply by Line 5 (round up to nearest whole number)	

Total number of breeders needed

16. Add Line 13 and Line 15	74
-----------------------------	----

Time to generate a colony of 74 breeders from initial stock of five breeders (generation time is ~12 weeks)

First 12 weeks: five breeding females x three females per litter x 0.85 (only 85% of the females are productive breeders) (round **down** to the nearest whole number) = **12** female breeders produced

Second 12 weeks: 17 breeding females (five original plus 12 new breeding females) x three females per litter x 0.85 (round **down** to the nearest whole number) = **43** female breeders available

Third 12 weeks: 60 breeding females (17 plus 43) x three females per litter x 0.85 = **153** females. Therefore, it will take ~36 weeks (~9 months) to produce sufficient breeding females to consistently produce 20 females per week for experiments.

Selected References

- Berry MM, Linder CC. 2007. Breeding Systems: Considerations, Genetic Fundamentals, Genetic Background and Strain Types. In: *The Mouse in Biomedical Research Volume 1 History, Wild Mice and Genetics* (Fox JG, Barthold SW, Davisson MT, Newcomer CE, Quimby FE, and Smith AL, eds.) Academic Press, pp. 53-78.
- Chia R, Achilli F, Festing MF, Fisher EM. 2005. The origins and uses of mouse outbred stocks. *Nat Genet* 37:1181-6.
- Fox RR, Witham B. 1997. The Jackson Laboratory Handbook on Genetically Standardized JAX® Mice. 5th ed. Maine: The Jackson Laboratory; 148 p.
- Lake JP, Haines D, Linder C, Davisson M. 1999. Dollars and sense: time and cost factors critical to establishing genetically engineered mouse colonies *Lab Animal* 28:24-33.
- Linder CC. 2003. Mouse nomenclature and maintenance of genetically engineered mice. *Comp Med* 53:119-25.
- National Research Council. 1996. Guide for the Care and Use of Laboratory Animals. National Academic Press. www.nap.edu/books/0309053773/html
- Silver LM. 1995. Mouse Genetics: Concepts and Applications. Oxford University Press. www.informatics.jax.org/silver.
- Standel PR, Corrow DJ. 1988. How to estimate the size and growth of an inbred mouse colony. The Jackson Laboratory internal document.
- The Jackson Laboratory. Biology of the Laboratory Mouse. New York (NY): Dover; 1976.
- Utomo AR, Nikitin AY, Lee WH. 1999. Temporal, spatial, and cell type-specific control of Cre-mediated DNA recombination in transgenic mice. *Nat Biotechnol* 17:1091-6.

Acknowledgements

Senior Editor and Technical Writer: Ray Lambert MS

Many people helped to compile, write, and lay out this manual.

Special thanks to Karen Davis, Muriel Davisson, Ph.D.,

Karen Fancher, Ph.D., Michael Greene, M.B.A.,

Stephen Linnell, M.B.A., Cathy Lutz, Ph.D.,

Linda Neleski, Steve Rockwood, B.S., Julie Soukup, B.S.,

Marge Strobel, Ph.D., Rob Taft, Ph.D., Laura Trepanier, M.S.,

Barbara Witham, B.S., Jim Yeadon, Ph.D.



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

Our mission: We discover the genetic basis for preventing, treating and curing human disease, and we enable research and education for the global biomedical community.

Phone: 1-800-422-6423
1-207-288-5845

Customer Service: orderquest@jax.org
Technical Support: micetech@jax.org

www.jax.org/jaxmice