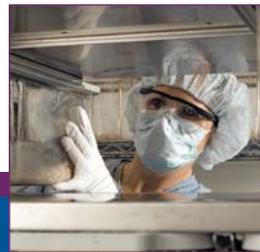


Breeding Strategies for Maintaining Colonies of Laboratory Mice

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 over 75 years. They are safe, reliable, economical, efficient, and ensure that the mouse strains produced are genetically well defined.

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Introduction

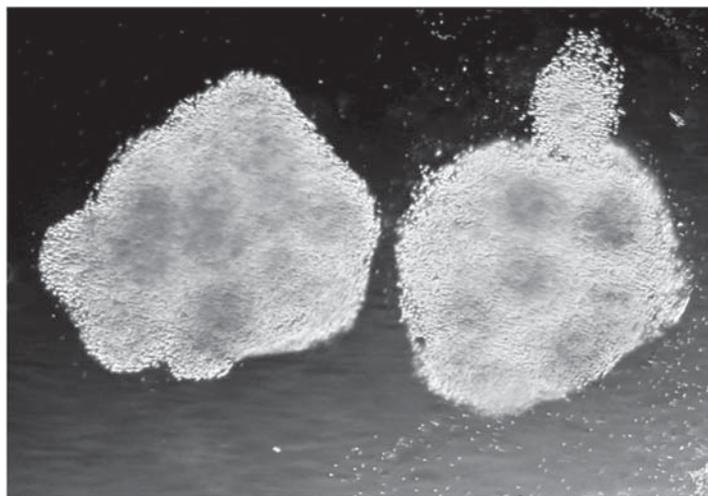
The laboratory mouse is playing an increasingly important role in biomedical research. Due to mutagenesis, transgenic, and gene-targeting technologies, the number of available mouse models is escalating. So are the costs, mouse room space, and related resources needed to accommodate these mice. Due to all 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 75 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 Support group at micetech@jax.org.

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 15

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 7-8 months, producing four or more litters (Table 1). However, some strains produce only one or two litters, usually because strain-specific characteristics 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 to five months 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.
- **Gestation.** The gestation period for laboratory mice is generally consistent within a strain but varies among strains. 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: ~3 weeks gestation, 3- 4 weeks suckling, and 5-6 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 weanling size and maturity. Although most strains are weaned when they are 21 days old, some benefit from being weaned when 28 days old. We do not wean mice before they are 17 days old without transferring them to a foster mother.



Mouse oocytes

Fundamentals of Mouse Reproduction

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

Strain (Stock Number)	Mean weaning age (wks)	Rotation Length [†] (wks)	Mean litter size (born)	Mean number of litters (born)	Wean:born ratio
BALB/cJ** (000651)	3	30	4.9	4.51	0.88
BALB/cByJ** (001026)	3	30	4.4	4.71	0.86
B6.129P2-Apoe ^{tm1Unc} /J** (002052)	4	26	3.1	5.0	0.9
B6D2F1/J** (100006)	3	38	6.5	6.4	0.9
C3H/HeJ* (000659)	3	22	4.6	5.6	0.9
C57BL/6J* (000664)	4	30	4.9	5.5	0.8
CBA/J** (00656)	3	26	4.9	4.0	0.8
DBA/2J** (000671)	3	26	4.7	5.3	0.8
FVB/NJ* (001800)	3	26	5.3	8.4	1.0
NOD.CB17-Prkdc ^{scid} /J* (001303)	3	26	4.5	7.6	0.9
NOD/ShiLtJ* (001976)	3	22	4.2	8.4	0.9

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

Data in last three columns were collected from JAX® Mice & Services Production colonies between June 2004 and May 2005.

[†] 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).

* Data from 50 pair-mated females

** Data from 100 trio-mated females

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 do inbred strains.
- **Strain-specific behaviors.** The aggressive behaviors of some strains and the poor mothering instincts of others affect breeding performance and pup survival. We list three examples: SJL/J (000686) males are aggressive and attack their mates and offspring; NZB/BINJ (000684) females are poor mothers; C57BL/6J (000664) females frequently lose their first litter.
- **Mutations and transgene effects.** Some induced mutations are embryonic lethal; some cause infertility or reduced fertility; some affect mammary gland function. For examples, the *Trp53^{tm1Tyj}* mutation (found in several strains) induces tumors that may diminish the ability of mutant females to carry litters to term, and the Tg(SOD1G93A)1Gur transgene (also found in several strains) induces neurodegeneration. The severity of such effects depends on inbred strain background.
- **Temperature and humidity.** Laboratory mice breed best when the temperature is between 16-26°C (64-79°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. Wild-derived inbred mice breed better in relatively darker conditions than do other strains.
- **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 (for example, DBA/2J, 000671) 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, and mice with new litters should be handled as little as possible. In particular, wild-derived strains benefit from as little handling as possible.
- **Nutrition.** Nutrition affects breeding performance. For example, whereas BALB/cJ (000651) mice breed better when fed a diet containing 11% fat, DBA/2J (000671) mice breed better when fed a 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.

Mouse Breeding Performance

Optimizing Breeding Performance

Factors to consider

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

- **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.
- **Replace non-productive breeders.** The following signs indicate that breeders are non-productive:
 - They produce no litter within 60 days of mating (longer may be acceptable if delayed breeding is a strain characteristic).
 - They produce no litter within 60 days of their last litter.
 - They produce litters but do not wean pups for two to three litters.
- **Mate mice early.** Mate mice when they are six to eight weeks old. Younger mice generally breed better than do older ones.
- **Use experienced males.** Pairing young females with older males often improves breeding performance.
- **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. Adopt a detective-like demeanor and cultivate habits such as the following:
 - Investigate deviations in breeding performance and phenotype immediately.
 - 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.
 - Keep a colony's environmental conditions suitable and stable.
 - Periodically verify the genotypes of pedigreed breeders for colonies of induced mutants (including those with no visible phenotype) with allele-specific molecular probes or other diagnostic assays.
- **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/BINJ, 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 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. Alternately, putting a heavy perfume scent on her nose before introducing her to the fosterlings may mask their scent. 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.

Diet Supplements

The productivity of some mice that either breed or rear their litters poorly may be improved with dietary supplements. For example, to breed successfully, BALB/cJ (000651) females need a diet of higher than average fat content.

Mouse Breeding Performance

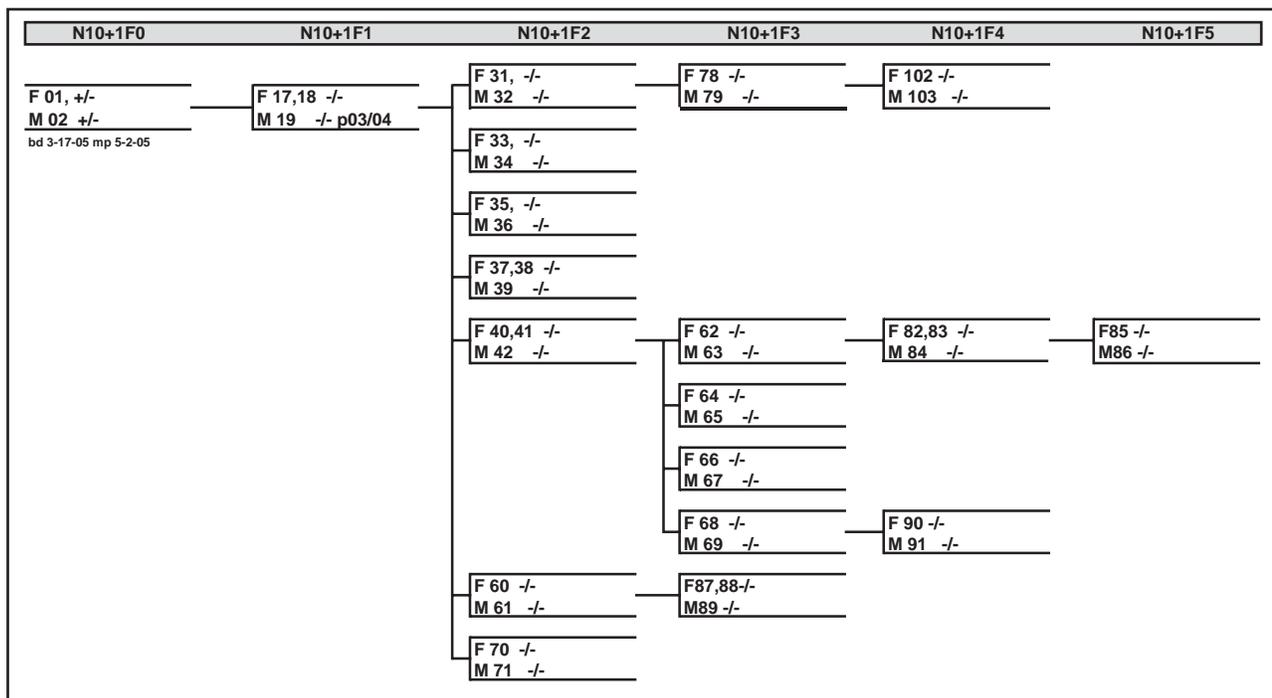
Optimizing Breeding Performance (continued)

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' estrous cycles. Then, induce them to resume their cycles simultaneously by either placing a male in their cage or exposing them to male androgen. Mice have a 4-5 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.

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 8 to 30 hours if her vagina contains a copulatory plug (a white or cream-colored plug of solidified ejaculate). Because mice usually mate 4-6 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).



Computer-generated pedigree

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, and necessary control mice.

Relatively 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). All members of the harem should be siblings. To maintain the pedigree lineage of trio or harem-mated females, the 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.

Below are examples of typical schemes for breeding genetically-defined mutants. Actual allele symbols are used to represent spontaneous and induced mutations; "+" symbols are used to 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.

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 at least every two generations. To be maintained on a stable inbred background, mutants should be backcrossed to the parental inbred strain about every 10 generations. 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, either that inbred strain or a wild-type congenic 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, die in utero, 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 next page) to differentiate homozygotes and heterozygotes. If the mutants are on a mixed genetic background, backcross the offspring to F1 hybrids about every 10 generations (to avoid producing recombinant inbred lines). If the mutants are on an inbred background, prevent genetic drift by backcrossing them to the appropriate inbred strain every 10 generations (for example, backcross a mutant on a C57BL/6J background to the standard C57BL/6J strain).

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, the same background alleles are segregating.

Breeding Schemes

Relatively Simple Breeding Schemes (continued)

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

This breeding scheme is used when homozygous mutant mice are severely impaired, infertile, embryonic lethal, die in utero, 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 (for example, backcross a mutant on a C57BL/6J background to a standard C57BL/6J inbred mouse). If the mutants are on a mixed genetic background, backcross the offspring to appropriate F1 hybrids about every 10 generations (to avoid producing recombinant inbred lines).

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, by molecular probes, or by 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 (usually recessive) 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-*Lys^{tg-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.

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 9-11 weeks of age. Additionally, only 50% of the males are fertile, and they have a breeding lifespan of only 3-4 weeks. As another example, the *Hmga2* transgene in C57BL/6J-TgN(HBBHBG)40BCha *Hmga2^{pg-TgN40BCha}*/BmJ mice (002644) is allelic with the pygmy (*Hmga2^{pg}*) 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 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 a visually distinguishable phenotype.

If transgene homozygosity is not lethal, homozygous transgenics may be produced and used in breeding. If a Tg/0 x Tg/0 scheme is used, half the offspring will be hemizygous (Tg/0). If hemizygotes and non-carriers are phenotypically indistinguishable from the homozygotes (Tg/Tg), each offspring must be genotyped. If a molecular probe for the transgene integration site is not available, the offspring must be genotyped either by progeny testing (to determine transmission frequency) or by quantitative PCR (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.

Breeding Schemes

Complex Breeding Schemes (continued)

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 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 B6SJL-Tg(SOD1G93A)1Gur/J (002726) 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.

Maintaining Strains with Two or More Mutant Alleles

Some strains, such as WBB6F1/J-*Kit^W/Kit^{W-v}* (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 Web site, www.jax.org/jaxmice/models/cre_intro). To maintain a cre-lox strain, three strains must be maintained: the cre strain, the lox 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.

Maintaining Outbred Stocks

Colonies of outbred mice are maintained by mating unrelated mice, generally randomly selected young breeders (using a random numbers table or computer program). One or more females may be mated with one or more males in appropriately sized cages. Depending on your needs and on the breeding performance of a stock, pregnant mice may be housed separately. Ideally, to keep a stock truly outbred, a colony should be maintained with at least 25 breeding pairs. Smaller colonies drift toward homozygosity because mice within them are closely related. Replacement breeders should be outcrossed every five years.

Breeding Schemes

Cryopreservation

Advantages of Cryopreservation

You may consider maintaining some strains by cryopreservation. Cryopreserving a strain has the following benefits:

- It saves space, especially when a strain is used infrequently. At The Jackson Laboratory, just 25ft² accommodates 2,000,000 cryopreserved embryos.
- It may be less troublesome, especially for strains that must be maintained by complex breeding scheme.
- It 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.
- It insures against catastrophic loss (fire, flood, earthquakes, disease, etc.).
- It provides a method for eliminating pathogens from a mouse colony. Some of the techniques used during embryo transfer, the most effective way of rederiving a mouse colony to SPF conditions, are very similar to those used for recovering strains from cryopreserved embryos and sperm.
- It reduces the risk of a strain contamination.
- It dramatically slows the rate of genetic drift.
- It reduces the risk that 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

Before 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 cryo-recovery 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.

Managing Small Colonies

Following are basic principles for managing small mouse colonies.

- Maintain a minimum of six breeding pairs representing different generations in your colony.
- Maintain 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).
- Try to keep the age range of your breeders between 2 and 8 months old: older mice may not breed reliably.
- Closely monitor breeding performance: if performance declines, promptly take corrective measures.
- Consider backcrossing your strain approximately every 10 generations to prevent substrain divergence.
- Consider cryopreserving a strain in case breeding performance either declines, ceases, or a catastrophic event (such as a fire or flood) threatens your colony.

Genetic Quality

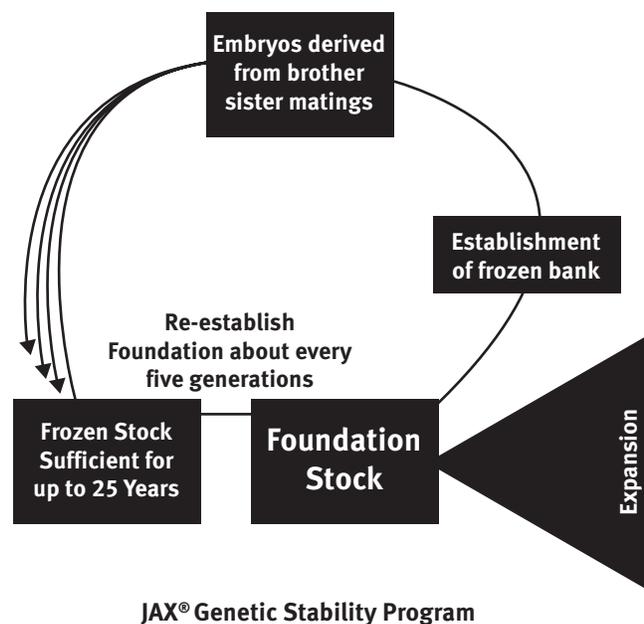
Maintaining Genetic Quality

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.
- If you maintain your own private colonies of a strain, periodically obtain new breeding stock from your supplier. 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.
- Use proper nomenclature to describe your mouse models.
- Include a detailed description of the genetic background of the mice you use in all your communications.
- When possible, use a common genetic background 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 Web site at www.jax.org/jaxmice/.



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” the costs of doing so should be considered. Following are several 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

Need. 20 mice/week for a year (1040 mice); will require having at least 30 breeding females on hand throughout the year

Cost/mouse cage. ~ \$530/year (includes cost of technician, cage washing, bedding, feed, other supplies, but not the cost of genotyping)

Total cost. 30 cages x \$530/cage = \$15,900/year = \$15.29/mouse

Scenario # 2

Need. 40 mice every two weeks for a year (1040 mice); will require having at least 115 breeding females on hand throughout the year

Cost/mouse cage. ~ \$530/year (includes cost of caretaker labor, cage washing, bedding, feed, other supplies, but not the cost of genotyping)

Total cost. 115 x \$530/cage = \$60,950/year = \$58.61/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 4- to 8-week old mice, monthly.
- Replace aging breeders according to a strict schedule.
- Replace non-productive breeders as soon as possible.

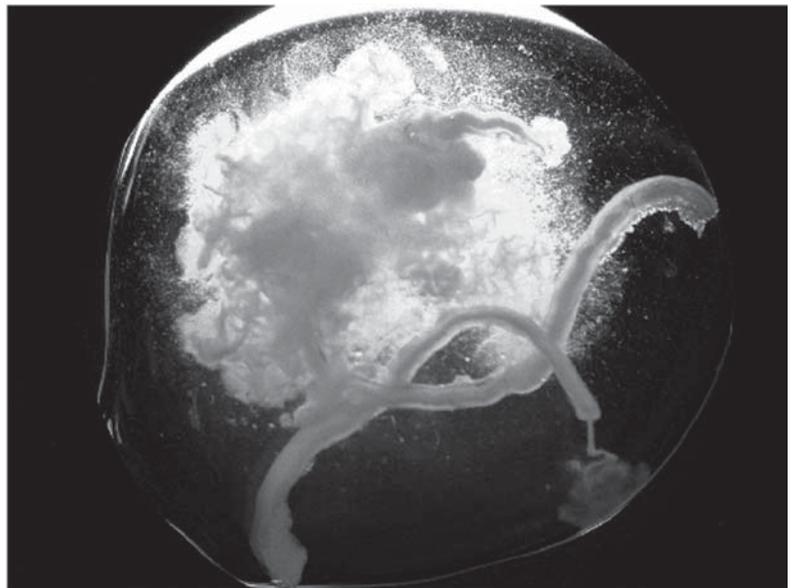
Sizing Mouse Colonies

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, average number 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 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

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 2-week age range (e.g., 5-6 weeks old), enter 2 If can have a 4-week age range (e.g., 5-8 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 3 by Line 2 (round to nearest whole number)	1
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 1 x 4 x 5 x 6 x 7 (round 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 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 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 Line 12 (round up to nearest whole number)	2

Total number of breeders needed

16.	Add Line 13 and Line 15	32
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Number of cages needed per week

17.	Breeding cages For pair breeding (one breeding female per cage): - 32 cages needed or trio breeding (two breeding females per cage): - 16 cages needed
18.	Weaning cages ~11 females & ~11 males weaned per week will require ~ 6 cages (5 animals per cage separated by sex)

JAX[®] Services for Mouse Colony Management

JAX[®] Services for Breeding and 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 Web site at www.jax.org/jaxservices, call 1-800-422-MICE (6423), or e-mail 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 and box space, we can deliver cost-effective and dependable breeding projects scaled to meet your needs.

JAX[®] Dedicated Supply

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[®] Speed Expansion

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

JAX[®] Rederivation

For a variety of reasons, a mouse colony may become infected with a pathogen, spread the infection to other colonies, compromise the health of the infected mice, and impact your research results. This service can provide you with specific pathogen free (SPF) mice rederived by hysterectomy or embryo transfer.

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[®] Sperm Cryopreservation & Recovery

This is the first truly reliable and cost-effective sperm cryopreservation and recovery service for knockout and transgenic mice. Previous sperm cryopreservation methods for these mice 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 B6;129 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 permits efficient strain distribution to other investigators as frozen embryos or 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[®] Resources for Mouse Colony Management

JAX[®] Colony Management System

The Jackson Laboratory's Colony Management System (JAX[®]-CMS) is a multi-user relational database management system for managing animal colonies in a research environment. It was developed with support from the NIH and the Howard Hughes Medical Institute in response to increased demand from our staff for a system that would manage mouse colonies with an intuitive, easy-to-use interface.

Since its initial release in 1998, JAX[®]-CMS has been widely used by The Jackson Laboratory and by several other institutions. It can perform the following tasks:

- Track animal status
- Track animal pedigrees
- Log genotypes
- Create mating records
- Create litter records
- Manage animal pens
- Track experimental data
- Print cage cards
- Create various reports
- Enter bulk data
- Perform advanced database queries

In its current form, version 2.8.1, JAX[®]-CMS runs as a Microsoft Access[®] application. It can be executed remotely from UNIX or Macintosh[®] environments using technologies such as Citrix Server. You may download this free and publicly available software from our Web site, www.jax.org/jcms. End user support is provided through our moderated listserv discussion group, which you can join when downloading the software.

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 principals 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 Web site, 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 data sheets with detailed information for every strain of JAX® Mice.

Mouse Genome Informatics

The Mouse Genome Informatics Web site (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.

Jackson Laboratory Technical Support

You can obtain expert technical advice for any JAX® Mice strain by contacting our technical support team at micetech@jax.org or 1-800-422-6423.



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

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 homozygous (with two identical alleles), heterozygous (with two different alleles), or hemizygous (with only one allele).

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 strains that are heterozygous at all loci.

Intercross: A cross between two organisms that have the same heterozygous genotype at all loci.

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.

Targeted mutant (Knockout, Knock-in, 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 Useful Offspring	25% Homozygotes

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 2-week age range (e.g., 5-6 weeks old), enter 2 If can have a 4-week age range (e.g., 5-8 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 3 by Line 2 (round to nearest whole number)	1
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 1 x 4 x 5 x 6 x 7 (round 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 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 to nearest whole number)	118

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)	4
15. Calculate the number of additional breeders needed to provide replacement breeders Divide Line 14 by Line 12 (round up to nearest whole number)	6

Total number of breeders needed

16. Add Line 13 and Line 15 Note: Approximately 44 heterozygous females and males per week not needed for experiments but useful for breeding will be produced. Therefore, breeding colony size need not be adjusted/increased to produce replacement breeders.	124
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Number of cages needed per week

17. Breeding cages For pair breeding (one breeding female per cage): – 118 cages needed or trio breeding (two breeding females per cage): – 54 cages needed	
18. Weaning cages ~11 females & ~11 males weaned per week will require ~ 6 cages (5 animals per cage separated by sex)	

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 Useful Offspring	12.5% Homozygotes

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 2-week age range (e.g., 5-6 weeks old), enter 2 If can have a 4-week age range (e.g., 5-8 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 3 by Line 2 (round to nearest whole number)	1
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 1 x 4 x 5 x 6 x 7 (round to nearest whole number)	176

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 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 to nearest whole number)	235

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)	4
15. Calculate the number of additional breeders needed to provide replacement breeders Divide Line 14 by Line 12 (round up to nearest whole number)	6

Total number of breeders needed

16. Add Line 13 and Line 15 Note: Approximately 88 heterozygous females and males per week not used for experiments but useful for breeding will be produced. Therefore, breeding colony size need not be adjusted/increased to produce replacement breeders.	246
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Number of cages needed per week

17. Breeding cages For pair breeding (one breeding female per cage): – 235 cages needed or trio breeding (two breeding females per cage): – 118 cages needed	
18. Weaning cages ~11 females & ~11 males weaned per week will require ~ 6 cages (5 animals per cage separated by sex)	

Example A3:**How Many Breeding Females are Needed to Produce 40 Female Homozygotes 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	All homozygotes
Percent Useful Offspring	50% female homozygotes

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 2-week age range (e.g., 5-6 weeks old), enter 2 If can have a 4-week age range (e.g., 5-8 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 3 by Line 2 (round to nearest whole number)	1
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 1 x 4 x 5 x 6 x 7 (round 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 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 to nearest whole number)	118

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)	4
15. Calculate the number of additional breeders needed to provide replacement breeders Divide Line 14 by Line 12 (round up to nearest whole number)	6

Total number of breeders needed

16. Add Line 13 and Line 15	124
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Number of cages needed per week

17. Breeding cages For pair breeding (one breeding female per cage): - 124 cages needed or trio breeding (two breeding females per cage): - 16 cages needed	
18. Weaning cages ~11 females & ~11 males weaned per week will require ~ 6 cages (5 animals per cage separated by sex)	

Example A4:

How Many Breeding B6.129S2-Trp53^{tm1Tyj}/J Females are Needed to Produce 40 Female & Male 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-Trp53 ^{tm1Tyj} /J)	Mutant homozygotes: 3-6 months
Breeding lifespan	12 weeks
Number of Litters Produced	2 litters
Litter frequency	1 litter/6 weeks
Litter size	6 pups (3 females, 3 males)
Offspring Genotypes	50% Heterozygotes; 50% Mutant homozygotes
Percent Useful Offspring	50% Mutant homozygotes

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 2-week age range (e.g., 5-6 weeks old), enter 2 If can have a 4-week age range (e.g., 5-8 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 3 by Line 2 (round to nearest whole number)	1
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	2
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 1 x 4 x 5 x 6 x 7 (round 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	2
11. Average productive female’s breeding lifespan (weeks)	12
12. Calculate colony productivity Divide Line 10 by Line 11, multiply by Line 9 (round to nearest hundredth)	1
13. Calculate number of breeding females needed Divide Line 8 by Line 12 (round to nearest whole number)	88

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)	8
15. Calculate the number of additional breeders needed to provide replacement breeders Divide Line 14 by Line 12 (round up to nearest whole number)	8

Total number of breeders needed

16. Add Line 13 and Line 15 Note: Approximately 44 heterozygous females and males per week not used for experiments will be produced each week, enough females to serve as replacement breeders, but likely not enough homozygous males required for breeding, especially given the reduced lifespan and fertility of mutant homozygotes. Therefore, the increased number of breeders to ensure that the colony remains productive is warranted.	96
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Number of cages needed per week

17. Breeding cages For pair breeding (one breeding female per cage): -32 cages needed or trio breeding (two breeding females per cage): -16 cages needed	
18. Weaning cages ~11 females & ~11 males weaned per week will require ~ 6 cages (5 animals per cage separated by sex)	

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 Useful Offspring	0.25 homozygotes x 0.50 males x .85 productive breeders = ~ .1

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 2-week age range (e.g., 5-6 weeks old), enter 2 If can have a 4-week age range (e.g., 5-8 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 3 by Line 2 (round to nearest whole number)	1
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 1 x 4 x 5 x 6 x 7 (round 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 Divide Line 10 by Line 11, multiply by Line 9 (round to nearest hundredth)	0.64
13. Calculate number of breeding females needed Divide Line 8 by Line 12 (round to nearest whole number)	550

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)	18
15. Calculate the number of additional breeders needed to provide replacement breeders Divide Line 14 by Line 12 (round up to nearest whole number)	28

Total number of breeders needed

16. Add Line 13 and Line 15 Note: Approximately 176 heterozygous females and males per week not used for experiments but useful for breeding will be produced. Therefore, breeding colony size need not be adjusted/increased to produce replacement breeders.	578
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Number of cages needed per week

17. Breeding cages For pair breeding (one breeding female per cage): – 32 cages needed or trio breeding (two breeding females per cage): – 16 cages needed	
18. Weaning cages ~11 females & ~11 males weaned per week will require ~ 6 cages (5 animals per cage separated by sex)	

**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 useful offspring	$0.5 \times 0.85 = .42$
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 If must be same age, enter 1 If can have a 2-week age range (e.g., 5-6 weeks old), enter 2 If can have a 4-week age range (e.g., 5-8 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 3 by Line 2 (round to nearest whole number)	2
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 1 x 4 x 5 x 6 x 7 (round 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 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 Divide Line 10 by Line 11, multiply by Line 9 (round to nearest hundredth)	0.64
13. Calculate number of breeding females needed Divide Line 8 by Line 12 (round to nearest whole number)	73

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)	3
15. Calculate the number of additional breeders needed to provide replacement breeders Divide Line 14 by Line 12 (round up to nearest whole number)	4

Total number of breeders needed

16. Add Line 13 and Line 15	77
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Time to generate a colony of 77 breeders from initial stock of 5 breeder (generation time is ~12 weeks)

First 12 weeks: 5 breeding females X 3 females per litter X 0.85 (only 85% of the females are productive breeders) = 12 female breeders produced
Second 12 weeks: 17 breeding females (5 original plus 12 new breeding females) X 3 females per litter X 0.85 = 43 females
Third 12 weeks: 60 breeding females X 3 females per litter X 0.85 = 153 females Therefore, it will take ~36 weeks (9months) to produce sufficient breeding females to consistently produce 20 females per week for experiments. Therefore, it will take ~36 weeks (9 months) to produce sufficient breeding females to consistently produce 20 females per week for experiments.

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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, Dorcas Corrow, B.S., Muriel Davisson, Ph.D., Michael Greene, B.S., M.B.A., Chip Leighton, M.B.A., Stephen Linnell, M.B.A., Cathy Lutz, Ph.D., 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.



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