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Overview

Mouse Nomenclature and Maintenance of Genetically Engineered Mice

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Modern genetic engineering technologies enable us to manipulate the mouse genome in increasingly complex ways to model human biology and disease. As a result, the number of mouse strains carrying transgenes or induced mutations has increased markedly. Thorough understanding of strain and gene nomenclature is essential to ensure that investigators know what kind of mouse they have, and what to expect in terms of phenotype. Genetically engineered mice alter gene function by over-expressing, eliminating, or modifying a gene product. The resulting phenotype is often unexpected and not completely understood, necessitating special care and potentially complex breeding and husbandry strategies. Animal care technicians responsible for routine maintenance of the colony, facility managers, veterinarians, and research personnel working with mice should be well informed about the nature of the mutation, distinguishing characteristics, and necessary precautions in handling the mice. Personnel working with mice also must be aware of the multitude of factors intrinsic to the mouse and present in the environment that can influence reproductive performance. Finally, diligent adherence to the maintenance of genetic quality in conjunction with cryopreservation of germplasm is the best insurance against loss of a colony.

Mouse nomenclature

Strain and gene nomenclature can be confusing and overwhelming to the uninitiated mouse user. A standardized nomenclature system is nonetheless crucial to establish the identity or non-identity of strains available from various sources. Cross-species standardization of gene names and symbols facilitates access, comparison, and interpretation of the vast amount of data being generated by various genome projects. Even with a standardized system in place, not everyone is compliant. This creates difficulty understanding what gene or strain is being discussed, especially for investigators outside a particular area of expertise. The amount of gene expression data currently being generated is likely to create unsuspected bridges between research areas. Understanding and use of standardized nomenclature will greatly improve retrieval and analysis of information. The essential tenets of mouse nomenclature will be addressed in this paper. The complete rules set forth by the International Committee on Standardized Genetic Nomenclature for Mice are available on the Web through the Mouse Genome Database (1).

As early as 1921, the mouse research community recognized that a set of rules for naming and referring to strains was necessary to effectively communicate results from mouse-based experimental research (2). The first formal nomenclature committee convened in 1939 (3). Since its inception, the International Committee on Standardized Genetic Nomenclature for Mice has developed and revised nomenclature conventions so mouse strain names could be informative unique identifiers. For strains carrying engineered mutations, a name may also convey the technology used to generate the strain (i.e., transgenic versus targeted mutation). Proper nomenclature provides information about the specific substrain used and its producer. This assists investigators by ensuring that they will obtain the correct mouse for their experiments. Widespread use of standardized nomenclature provides advantages to all mouse users. Unfortunately, strain nomenclature is frequently long and awkward, and does not convey all the necessary information about a strain. Although it is acceptable to use appropriate abbreviations when referring to a strain, full strain nomenclature should be used in publications, ordering, and breeding and facility records.

Inbred strain nomenclature. Inbred strains are defined as those derived from 20 or more consecutive brother × sister matings, and are designated by the letter F followed by the number of generations of filial breeding (F20). Residual heterozygosity will essentially be eliminated following F60. Most commonly used inbred strains have been inbred for more than 200 generations. Inbred strains offer several advantages over outbred or random-bred mice. In addition to their genetic and phenotypic uniformity, commonly used inbred strains are well characterized (4, 5). These advantages outweigh the lack of robustness, often-overlooked strain specific characteristics like retinal degeneration, low fecundity, and high cost of some inbred strains.

Inbred strain nomenclature is a combination of parent strain and substrain designations. A parent strain is designated by a brief symbol made up of uppercase letters or numbers or combination of letters and numbers. Inbred strain names may be rooted in their coat color, origin, or a defining characteristic. For example, Clarence Cook Little's first inbred strain DBA, originally called dba, is named for its coat color genetics (D for dilute, B for brown, and A for nonagouti). The CBL and C57BR

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Table 1. Strain 129 origin of commonly used embryonic stem (ES) cell lines

129 Strain of origin	ES cell line
129P2/OlaHsd	E14TG2a
	HM-1(*Hprt*$^{b\text{-}m1}$)
129P3/JEms	mEMS32
129X1/SvJ (JAX Stock No. 000691)	RW-4
	PJ1-5
129X1/SvJ × 129S1/Sv-+^{p} +$^{Tyr\text{-}c}$ *Kitl*$^{Sl\text{-}J}$/+[a] (JAX Stock No. 000691 & 000090)	R1 (+$^{Kitl\text{-}Sl\text{J}}$)
129S1/Sv-+^{p} +$^{Tyr\text{-}c}$ *Kitl*$^{Sl\text{-}J}$/+[a] (JAX Stock No. 000090)	W9.5 (+$^{Kitl\text{-}Sl\text{J}}$)
	CJ7 (+$^{Kitl\text{-}Sl\text{J}}$)
129S2/SvPas	D3
129S4/SvJae	J1
129S4/SvJaeSor	AK7
129S6/SvEv	EK.CCE
	CP-1
129S6/SvEvTac	TC1
129S7/SvEvBrd-*Hprt*$^{b\text{-}m2}$	AB1 (+$^{Hprt\text{-}bm2}$)
	AB2.1 (*Hprt*$^{b\text{-}m2}$)

[a]Origin of 129S1/SvImJ (Jackson Laboratory [JAX] Stock No. 002448)

Table 2. Revised 129 strain names

Abbreviation	Full Name	Former Name	JAX Stock No.
129P1	129P1/ReJ	129/ReJ	001137
129P2	129P2/OlaHsd	129/OlaHsd	NA
129P3	129P3/J	129/J	000690
129X1	129X1/SvJ	129/SvJ	000691
129S1	129S1/Sv-+^{p} +$^{Tyr\text{-}c}$ *Kitl*$^{Sl\text{-}J}$/+	129/Sv-+^{p} +$^{Tyr\text{-}c}$ *Mgf*$^{Sl\text{-}J}$/+	000090
129S1	129S1/SvImJ	129S3/SvImJ (formerly 129/SvImJ)	002448
		129/Sv-+^{p} +$^{Tyr\text{-}c}$ +$^{Mgf\text{-}Sl\text{J}}$/J	
129S2	129S2/SvPas	129/SvPas	NA
129S4	129S4/SvJae	129/SvJae	NA
129S6	129S6/SvEvTac	129/SvEvTac	NA
129T2	129T2/SvEmsJ	129/SvEms-+^{Ter}?/J	002065

NA = not applicable

parent strains derived from inbreeding black (BL) and brown (BR) progeny from a mating of female 57 to male 52 in Little's line C (6). Likewise, New Zealand White (NZW) and New Zealand Black (NZB) provide information on origin and coat color of the mice. Inbred strains also may be named for more application-based phenotypes like the non-obese diabetic (NOD) and nude (NU) inbred strains.

Substrains are strains of mice that have diverged from their parent strain for 20 or more generations (10 generations each from a common ancestor), have demonstrated residual heterozygosity left over from the time of separation, or carry new mutations not found in the parent strain. Substrain designations are appended to the parent strain nomenclature following a forward slash (e.g., C57BL/6J). Substrain designations are a combination of numbers and a laboratory registration code ("lab code"). A lab code usually consists of three to four letters (first letter is uppercase) and identifies the particular institute, laboratory, or investigator that produced and/or maintains a mouse strain (e.g., J for The Jackson Laboratory, Mcw for Medical College of Wisconsin, Crl for Charles River Laboratories). The Institute for Laboratory Animal Research (ILAR, http://dels.nas.edu/ilar/index.asp) assigns lab codes and maintains a master registry.

Inbred strain names may change as more information is obtained about their genetic makeup and relationship to other strains. The nomenclature for 129 strains provides a prime example. L. C. Dunn originally produced the 129 inbred strain at Columbia University. The Jackson Laboratory obtained the mice in 1945, and again in 1948 following the 1947 Bar Harbor Fire. L. C. Stevens used 129 mice as a background strain for research on testicular teratomas and several different 129 substrains were subsequently distributed to outside investigators over the next 50 years. The 129 strains can be classified into three lineages: parental, steel, and teratoma. Investigators have bred out coat color mutations (e.g., pink-eyed dilution and albino) and bred in selectable markers (e.g., *Hprt*, *Gpi1*) (7). Work with teratocarcinomas and early gene transfer experiments in the late 1970s and early 1980s led to the eventual development of embryonic stem (ES) cells lines derived from several different 129 strains (Table 1). Extensive analysis of the numerous 129 strains and their ES cell lines reveals more genetic variability than can be explained solely by genetic drift (7, 8). This variation has considerable impact on experimental strategies centered on targeted mutagenesis technologies. Homologous recombination efficiency can be increased by matching the DNA library used for obtaining the targeting construct to the ES cell line. Additionally, by matching the ES cell line to the appropriate 129 strain, it is possible to create a pure strain that would differ from its inbred strain control only by the targeted gene. Given the importance of understanding the origin of 129 strains, their nomenclature was modified to create separate parent strains (9, 10). These include strains derived from the original 129 parental strains (129P), strains derived from lines carrying the steel-J mutation (129S), strains derived from lines carrying the teratoma mutation (129T), and 129X (the X denotes the documented genetic contamination of the 129X1/SvJ strain) (Table 2). Despite the genetic contamination in its history, 129X1/SvJ mice currently are completely inbred (7, 8, 11).

How important are genetic differences among the 129 strains? Will they affect experimental results? Is one better than another? Such questions arise in the minds of researchers informed of the detected differences in simple-sequence length polymorphic (SSLP) DNA markers. There are several documented functional differences among 129 strains. Although they all have the same major histocompatibility complex haplotype (*H2*^{b}), there are apparently enough differences in minor histocompability genes to cause fairly rapid tail skin graft rejection across the parental, steel, and teratoma lineages (7). Also, the 129P and 129X strains carry recessive mutations in the tyrosinase (*Tyr*) and pink-eyed dilution (*p*) genes that impair vision and may affect results of spatial learning and memory tests. In addition, there are documented differences in reproductive performance (4) and in responses to behavior testing (12, 13).

Unfortunately, most 129 strain nomenclature in the literature is outdated, incomplete, and/or misleading. Many ES cell lines are derived from strains within the 129S lineage; yet, the strain origin is generally referred to as simply "129/Sv" in the Materials and Methods portion of references, leading researchers to conclude erroneously that 129/SvJ (now 129X1/SvJ) mice are the best match. In fact, although there are ES cell lines derived from 129X1/SvJ, it is the most genetically distinct and is not a good match to 129S-derived ES cell lines. A thorough investigation of the origin of the 129 strain must be conducted prior to use.

Gene nomenclature. Mouse gene symbols are italicized, and the first letter is capitalized. Human gene symbols are designated in italics, with all capital letters. Mouse and human protein symbols are designated by all capital letters and are not

italicized, making them indistinguishable. Spontaneous mutations are alleles of initially unknown genes and are given allele names and symbols on the basis of phenotype (e.g., diabetes, *db*). Recessive mutations (i.e., requiring two copies of the mutated allele to manifest the phenotype) are represented by all lowercase letters, whereas dominant (i.e., one or two copies of the mutated allele produce the phenotype) and semidominant (i.e., one mutant allele produces an intermediate phenotype) spontaneous mutations are represented by an uppercase first letter, followed by lowercase letters. Once the gene responsible for the mutant phenotype has been identified, the allele symbol is superscripted to an approved gene symbol (e.g., the diabetes mutation is a point mutation in the leptin receptor gene, $Lepr^{db}$). The Mouse Genomic Nomenclature Committee (1; email: nomen @informatics.jax.org) approves and assigns gene names and symbols. Gene names and symbols may change as the function of a gene is better understood or to better correspond with gene symbols of other species (primarily human).

The International Committee for Standardized Mouse Nomenclature distinguishes clearly between transgenes (insertion of exogenous DNA, usually leading to an overexpression of the transgene and a semidominant phenotype) and targeted mutations that "knockout" or alter an endogenous gene product. Transgenes are designated by Tg, followed by a designation for the DNA insert in parentheses (preferably the gene symbol), then a number indicating the founder line, and finally, a lab code. Italics are not used for transgenes. For example, Tg(CD8)1Jwg is a transgene containing the human *CD8* gene, the first transgenic line using this construct, described by the laboratory of Jon W. Gordon (Jwg). The promoter also may be designated within the parentheses to clarify the transgene expression pattern; Tg(Zp3-cre)3Mrt designates the *Cre* transgene with a *Zp3* promoter, the third transgenic line from the laboratory of Gail Martin (Mrt).

The correct gene symbol followed by tm (for targeted mutation), an allele number, and the lab code (all superscripted, in italics) designate targeted alleles of genes. For example, $Apoa1^{tm1Unc}$ represents the first targeted mutation in the apolipoprotein AI (*Apoa1*) gene made in the laboratory of Nobuyo Maeda at the University of North Carolina at Chapel Hill (Unc)(14).

The *Cre-lox* technology has simplified the process of creating targeted alleles to replace an endogenous gene with another gene creating a "knockin." The $En1^{tm1(Otx2)Wrst}$ designation denotes a knockin of the orthodenticle homolog 2 (*Otx2*) gene into the endogenous engrailed 1 (*En1*) locus made in the laboratory of Wolfgang Wurst (Wrst). The *En1* promoter drives expression of a bicistronic *Otx2* and IRES-LacZ reporter mRNA. A *loxP*-flanked neomycin cassette was removed in vivo by *Cre*-mediated recombination (15).

The genetic background of mice with induced mutations is given prior to the gene, transgene designation, or allele symbol. Many strains are maintained on a mixture of C57BL/6 and 129 genetic backgrounds (e.g., B6;129- $Trp53^{tm1Tyj}$) because 129-derived ES cell lines are commonly used in gene targeting and chimeric mice are mated to C57BL/6 to determine germline transmission. Mutations transferred from a mixed to an inbred background by repeated backcrossing are designated by use of congenic nomenclature. For example, B6.129S2-$Trp53^{tm1Tyj}$ indicates that the $Trp53^{tm1Tyj}$ mutation originated in the 129S2 parent strain (via the D3 ES cell line) and was subsequently backcrossed to the C57BL/6J inbred strain for at least five generations. A strain is considered congenic to its inbred strain partner following 10 backcross generations (N10); however, the guidelines permit use of congenic nomenclature for incipient congenics (backcrossing ≥ N5 to the host strain) (1, 16). Although generation numbers (e.g., N10F21 for B6.129S2-$Trp53^{tm1Tyj}$) are not part of a strain name, the backcross generation should be obtained and considered prior to use of a congenic strain. Care must be taken in deciphering symbols used in strain nomenclature; the semicolon used to denote a mixed background versus a period used to denote a congenic background is a subtle but critical distinction.

The use of genetically defined inbred mice has increased markedly with the ability to genetically manipulate the mouse genome. The strain nomenclature used by many investigators and mouse suppliers in publications and in product literature is improper or incomplete. Scientists need complete information to make informed decisions about selecting and using appropriate mouse strains. Just as critical is the use of proper nomenclature in the mouse room on cage cards and in breeding records. Lack of attention to this type of detail may compromise entire research endeavors.

Maintenance of genetically engineered mice

With the sequencing of its genome, the mouse has become the premier platform for modeling human disease in biomedical research (17-19). Thousands of genetically engineered transgenic and targeted mutant mouse models have been produced in the last 20 years, and production is accelerating. There is need for additional resources to produce, house, and distribute these models. An understanding of the special problems in housing, care, and breeding of genetically engineered mice is equally important.

Genetically engineered mice may be generated in a researcher's own laboratory or, increasingly, by an institutional or regional core facility. Commercial production facilities producing custom transgenic and targeted mutant mice also are an option. Mouse models are frequently distributed from individual laboratories to the scientific community. Caution should be taken when accepting these models as there may be little or no standardization in genetic quality and/or health. Repositories like the Induced Mutant Resource (IMR) at The Jackson Laboratory and the Mutant Mouse Regional Resource Centers (MMRRC) were created to protect the health and genetic purity of transgenic strains and mice carrying induced mutations. The National Institutes of Health's National Center for Research Resources (NCRR) provides most of the funding. The NCRR is committed to ensuring that mice are distributed with as few restrictions as possible to maximize the opportunities for major advances in understanding and treating human disease. Repositories also alleviate the burden on individual investigators to distribute strains.

General handling. Genetically engineered mice alter gene function by over-expressing, eliminating, or modifying a gene product. The resulting phenotype is often unexpected and not completely understood, necessitating special care and potentially complex breeding and husbandry strategies. The mice thus require specialized care, particularly given the considerable cost involved in generating such mice. Every effort should be made to ensure the establishment and health of the colony. Animal care technicians responsible for routine colony mainte-

Table 3. Breeding schemes for maintaining genetic engineered mice

Type of mating	Genotype[a]	Genotypes produced	Conditions of use	Controls depend on genetic background
Homozygote × Homozygote	-/- × -/- or Tg/Tg × Tg/Tg	100% -/- or 100% Tg/Tg	Brother × sister matings Both sexes viable and fertile Genotype periodically to confirm homozygosity	Inbred/Congenic: matching inbred strain Mixed: problematic (B6129F2 approximate for some strains)
Heterozygote × Heterozygote	+/- × +/-	25% -/- 50% +/- 25% +/+	Brother × sister matings One or both sexes not viable or fertile as homozygote Genotype every generation	Inbred/Congenic: wild-type, siblings or from the colony; matching inbred strain Mixed: wild-type, siblings or from the colony; B6129F2 (approximate for some strains)
Heterozygote × Homozygote	+/- × -/-	50% +/- 50% -/-	Brother × sister matings One sex not viable or fertile as homozygote Genotype every generation	Inbred/Congenic: heterozygotes, siblings or from the colony; matching inbred strain Mixed: heterozygotes, siblings or from the colony; B6129F2 (approximate for some strains)
Heterozygote × Wildtype	+/- × +/+	50% +/- 50% +/+	Brother × sister matings Used for dominant mutations Used to propagate colony when recessive mutants not required (commonly used for embryonic or perinatal lethals) Genotype every generation	Recessive: not applicable Dominant: wildtype, siblings or from the colony; matching inbred strain
F1 Hybrid (or Inbred) × Hemizygote	F1 × Tg/0 or Inbred × Tg/0	50% Tg/0 50% +/+	Avoid lethality of homozygous state Avoid phenotype due to insertional mutations Genotype every generation	Inbred/Congenic: wild-type, siblings or from the colony; matching inbred strain Hybrid: wild-type, siblings or from the colony

[a]Tg = transgene, - = targeted mutation, + = wild-type, 0 = no transgene.

nance, facility managers, veterinarians, and research personnel working with mice should be well informed about the nature of the mutation, distinguishing characteristics, and any necessary precautions. All personnel handling such mice should be trained to observe and record any deviations in phenotype, behavior, and/or reproductive performance.

Breeding strategies. The breeding of mice carrying transgenes or induced mutations presents several challenges not found in the care and maintenance of standard inbred strains. Prior to setting up a breeding colony, these three questions should be asked: what do you have; what do you want; and what can you identify, either phenotypically or genotypically? The most economical and efficient way to maintain a strain is by homozygous sibling matings on an inbred strain background, though alternate breeding schemes may be required to propagate certain transgenes or mutations. Table 3 provides a list of possible breeding schemes, expected genotypes from these matings, conditions for use, and appropriate experimental controls.

Transgenes or genetic mutations may adversely affect the breeding performance of a strain, creating special husbandry problems. For example, the transgenic strain over-expressing the promoter region and exon 1 of the human Huntington's disease gene, B6CBA-TgN(HDexon1)62Gpb/J, causes signs of disease beginning at nine to 11 weeks of age (20). Hemizygous females are not fertile, and hemizygous males have only a three- to four-week breeding window during which only about 50% will breed. Depending on the assisted reproduction techniques available to the researcher, various breeding strategies may be used. This strain can be successfully maintained by transplanting ovaries from hemizygous females into histocompatible females and mating them to B6CBAF1 males. If ovarian transplantation is not feasible, trio matings can be set up with two B6CBAF1 females and one hemizygous TgN(HDexon1)62 male.

Maintenance of strains created for conditional mutagenesis requires more complicated breeding schemes and poses particular husbandry challenges. The *Cre-lox* mice combine transgenic and targeted mutation technologies to create tissue-specific knockouts (21, 22). Mice carrying a transgene with a tissue-specific promoter and a *Cre* reporter and mice carrying a targeted mutation flanked with *loxP* sites are developed independently, then intercrossed. Panels of multiple *Cre* mouse strains with diverse, expression-specific promoters can be generated and crossed with a single *loxP* strain. This strategy optimizes research systems and circumvents the laborious traditional procedures of developing individual mutant constructs, some of which may prove lethal to the animal. Inducible systems like the tetracycline-expression systems (i.e., Tet-Off and Tet-On) are binary transgenic systems in which expression from a target transgene is dependent on the activity of an inducible transcriptional activator (23). Expression of the transcriptional activator is regulated reversibly and quantitatively by exposing the transgenic animals to variable concentrations of tetracycline derivatives such as doxycycline. The generation of conditional mutant strains, using either *Cre-lox* or tetracycline-inducible systems, requires mating transgenic mice to test strains to verify appropriate expression patterns. Though a detailed explanation of the care and maintenance of conditional mutant mice is beyond the scope of this article, their use requires propagation of multiple independent strains, periodic intercrossing, genotyping, complex record keeping, and specialized care including compound treatment.

Record keeping. Breeding and maintaining genetically engineered mice necessitates accurate and detailed record keeping. Transgenic and gene-targeting core facilities, as well as institutions housing multiple strains for numerous researchers, need to be able to monitor the multiple activities necessary to maintain and monitor the colony efficiently. Colony management software now exists for animal care and use committee (IACUC), facility (census and billing), and breeding (including pedigree and genotyping records) management. Common commercial packages include products by Locus Technologies (www.locustechnology.com), Topaz (www.topaztracks.com), and

Progeny (www.progeny2000.com). Facilities also may develop customized databases appropriate for their needs, using Microsoft Access or Filemaker Pro. Although use of computer-based tracking systems is increasing, these should not replace some type of paper ledger and pedigree chart on each specific strain in the mouse room for use by the animal caretakers and researchers.

Housing. Special housing considerations may be important, depending on the nature of the mutation. Immunocompromised mice need to be maintained under strict specific-pathogen-free conditions, preferably in a barrier facility. The immune status of a strain is not always apparent or as straightforward as in the *Rag1* targeted mutant mouse, a model that lacks functional B and T cells (24). Mice carrying transgenes or induced mutations may exhibit variable degrees of immune function and, in some instances, compromised immunity may be an unexpected trait. The severity of the phenotype also may depend on the microbiological environment, as with the *Il2*- and *Il10*-targeted mutations (25, 26). An investigator may not always have multiple housing options, but it is highly recommended that the immune status of mutant mice be determined and taken into consideration when establishing a colony.

Troubleshooting. Unexpected breeding problems may arise despite the best efforts to establish appropriate and strain-specific breeding and husbandry conditions. Diagnosing the problem is difficult, as it probably results from a combination of factors. Possibilities include strain-specific effects, acute or chronic illness, mutation/transgene effects, and environmental factors. Begin troubleshooting by analyzing the whole mouse and its environment. Table 4 provides a summary of various intrinsic and extrinsic factors that may affect reproductive performance of the mouse (4, 27-30).

Frequently, the genetic background of choice for maintaining genetically engineered mice is C57BL/6. Nonetheless, it may be necessary or even preferable to maintain a transgene or an induced mutation on another inbred or hybrid background to alleviate breeding problems or cope with specific strain characteristics. Inbred strain characteristics, references, and information on reproductive performance are available through Michael Festing's online version of Inbred Strain Characteristics (4) as well as in the *Handbook on Genetically Standardized JAX Mice* (30).

Environmental enrichments. Confinement of laboratory animals may lead to boredom and undue stress. Mice are communal animals, and individual housing should be avoided whenever possible. The addition of environmental enrichments, such as nestlets (Ancare, Bellmore, N.Y.; VWR 10279-140), may improve reproductive performance, especially if the bedding type used is not suited to nest building. It is important to obtain approval from the researcher prior to altering the cage environment.

Safeguarding and rescue. Clearly, maintenance of genetically engineered mice is a complex and costly process. The best insurance against loss of a colony is diligent adherence to genetic quality maintenance in conjunction with cryopreservation of germplasm to protect against accidental loss.

Genetic monitoring to ensure the quality of genetically engineered strains consists of verifying the presence of a transgene or mutated allele. The mating schemes used for many induced mutant strains result in litters containing a combination of wild-type, heterozygous, and/or homozygous mutant mice (Table 3). The genotype of mice from such matings must be determined prior to experimental use, breeding, or distribution. In individual research laboratories, Southern or dot blot analysis frequently has been used for this purpose. Southern blotting is too slow and expensive when large numbers of mice are being distributed. Use of polymerase chain reaction analysis for allele-specific genotyping is ideal because it is rapid, standardized, does not require the use of radioisotopes, and is adaptable to automation.

Table 4. Intrinsic and extrinsic factors that influence reproductive performance

Intrinsic (mouse)	
Age	Mice will breed until ~9 months of age, depending on strain and mutant gene. Mice first mated when older than 3 months of age probably will not breed.
Obesity	Obese males lose interest in breeding. Obese females are less likely to get pregnant.
Illness	Acute illness may cause loss of pups during gestation (e.g., parvovirus) or at weaning (e.g., MHV). Chronic illness (e.g., pneumonia or colitis) also may adversely affect breeding performance. *Consult with Veterinary Services if you have questions about the health of your mice or their suitability for breeding.*
Strain type	Random-bred mice have higher fecundity than inbred mice. Reproductive performance varies among inbred strains. Some inbred strains display behaviors that affect breeding ability (e.g., SJL male aggression).
Extrinsic (environment)	
Light	Breeding/production is better with a 14/10-h vs. 12/12-h light/dark. Disruption of light cycles may have long-term effects.
Diet	Fat content too high or too low. Some strains do well on a breeder chow (9-11% fat) while others become obese and exhibit decreased reproductive performance.
Noise	Avoid high traffic areas and sudden noises.
Vibration	May cause cannibalism of litters, resorption of fetuses, or failure to thrive.
Temperature	Colder better than too warm (recommended 18-24°C).
Humidity	Acceptable range 40-70% (ideal, 45-55%).
Handling	Change in handlers. Change of smell of familiar handler. Type of handling (forceps vs gloved hand, rough vs calm).
Seasonal	Although isolated from outside stimuli, mice still show seasonal changes in reproductive changes performance (strain dependent).

It is also important to verify strain background as a safeguard against accidental genetic contamination. Monitoring for genetic contamination can be accomplished using a panel of biochemical and immunological markers or informative DNA markers, such as SSLP or single-nucleotide polymorphisms (SNP) that span the genome (31).

To avoid recurrences, it is important to diagnose sources of genetic variability at the time of discovery. The most likely causes are due to errors in breeding (e.g., human error in setting up the mating, mating between an offspring and its parent, or mating by an escapee) or genotyping (e.g., misidentification of a mouse or DNA sample, error in protocol or analysis, error in recording). There also may be some genetic variability resulting from incomplete inbreeding or in colonies of mixed genetic background.

Many errors can be avoided by: housing strains of different

coat color next to one another (this may not be possible when maintaining numerous colonies of genetically engineered mice on a black C57BL/6 genetic background); using different-colored cage cards; maintaining proper breeding records; capturing and euthanizing all escapees; and maintaining foundation stocks (30). Animal care technicians need to be properly trained in basic genetics, nomenclature, record keeping and animal identification, basic strain characteristics, and deviant recognition. Personnel also need to be trained in tissue collection procedures for genotyping. Retro-orbital or tail vein blood collection, or tail snipping are common collection methods. Cryopreservation of germplasm is the best safeguard against accidental loss of a colony due to disease, sudden reproductive failure, genetic contamination, or other catastrophic events. Cryopreservation provides a cost-effective storage method when there is not an immediate demand for a strain. Techniques are now available for preserving embryos (32), spermatozoa (33, 34), and ovaries (35, 36). As a quality-control measure, mice providing the gametes or embryos for cryopreservation, especially those carrying mutant alleles, should be genotyped prior to their use.

Conclusions

The mouse has emerged as the principal experimental organism to facilitate our understanding of human biology and for the development of treatment for diseases (37-39). The phenotypic characteristics and pathophysiology of mice carrying spontaneous or genetically engineered mutations are usually attributed solely to alterations in the modified gene. The genetic background and surrounding environment are repeatedly overlooked parameters that can significantly affect the phenotype. Thorough understanding of strain- and gene-specific genetics, nomenclature, characteristics, and maintenance requirements for mutant mice is crucial. This requires proper training, cooperation, universal adherence to nomenclature guidelines, and communication among all personnel working with mice. Numerous training opportunities are available through the American Association for Laboratory Animal Science (www.aalas.org), The Jackson Laboratory (www.jax.org/courses), and Charles River Laboratories (www.criver.com).

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