The Importance of Monitoring Genetic Background in Inbred Mouse Colonies

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Genetic Monitoring

The importance of genetic monitoring

The use of genetically modified mice has become an established tool to study gene function in many animal models of human diseases. However, a gene functions in the context of the genome as a whole. **The phenotype of a single gene mutation is modulated by a large number of background genes; two congenic strains carrying the same null mutation can have divergent phenotypes dependent on their genetic background.** When inbred strain colonies are separated and raised in a different environment, such as another laboratory, substantial substrain differences may occur. This implies the importance of a well-defined, homogenous genetic background for the analysis and interpretation of phenotypes associated with genetic mutations.[3]

Multiple studies have demonstrated that phenotypic differences between mutant and control mice can be the result of variance in the genetic background, and that this may lead to misinterpretation of results.[3,10,11,11–13] Variation may exist even among strains from a given repository. Several data sources are available to illustrate this point. According to information provided by the developers of 659 congenic, semicongenic, spontaneous and induced mutant strains with the C57BL/6 background deposited at the RIKEN BioResource Center, there were frequent mixed backgrounds among the C57BL/6 substrains (2%) or uncertain C57BL/6 substrains (14%).[14] Indeed, another study investigating the purity of mouse lines assigned a C57BL/6 label found that only 29% of the assessed mice had a pure genetic background consistent with the C57BL/6 designation. Based on their findings, the authors estimated that at least every second genetically modified line could lead to unexpected and non-reproducible results, irrespective of the investigated gene of interest.[15]

To avoid problems related to an insufficiently defined genetic background, a substantial body of recent research [3,8,10,11,13,14,16–22] has advocated that, for each study involving genetically modified mice, at least a detailed description of the origin and genetic background of both the wild type (WT) control and the altered strain of mice is essential.[6]

A primer on mouse strains and genetic background

Inbred strains are artificial populations of mice which are analogous, but not identical, to cloned animals of each sex; mice of the same inbred strain are said to be isogenic.[1] The phrase "inbred strains" serves as an umbrella term for derivatives such as congenic strains, recombinant inbred strains, recombinant congenic strains, and consomic strains.[1] Congenic strains are defined as two inbred strains which are genetically identical, except for the targeted gene and its flanking region.[3] **The genetic background of a given inbred strain refers to its genetic makeup (inclusive of all alleles at all loci), with the exception of the mutated gene of interest and the surrounding region, which is often derived from a different strain.** This "other material" comprises the residual heterozygosity referred to above.[4]

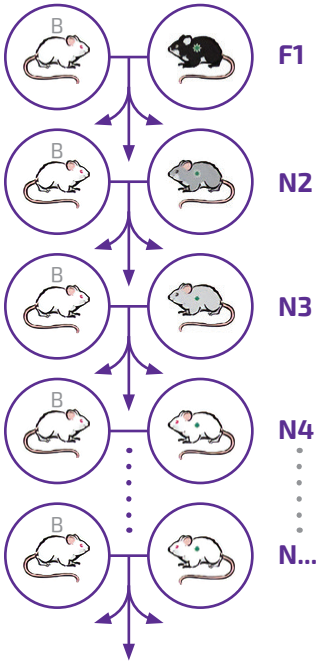

Figure 1. The successive steps in the establishment of a congenic strain via the backcrossing process. At each generation, a breeder carrying the targeted character (marked with a green dot) is back-crossed to a partner of the recipient (B) strain. Image provided courtesy of Dr. Fernando Benavides.[3]

A primer on mouse strains and genetic background (continued)

In 2011, Keane and colleagues[5] conducted an extensive analysis of genomic variation in 17 inbred strains, and catalogued an extraordinarily large number of variants, including 56.7 M SNPs, 8.8 M small indels and 0.28 M structural variants across both the classic laboratory strains and the wild type-derived lines. These analyses illustrated the potential to relate sequence variation to aspects of phenotypic variation between mouse strains, and provided an insight into the molecular and genetic basis of quantitative traits that distinguish the phenotypic characteristics of inbred strains.[6]

Strain divergence

Substrains are genetic variants of an inbred strain.[4] Genetic variation arises by accidental genetic contamination due to errors in animal management, or by genetic drift.[7] Human error is the most significant cause of variation. Genetic contamination is the accidental intercrossing of mouse strains as a result of errors in animal management. Common sources of error include mis-marking cage cards, mis-recording identification numbers, or accidentally pairing the wrong animals.[2] Inconsistent documentation of the background strain, as well as the use of inconsistent nomenclature, have also been identified as common offenders. **It has been acknowledged that within the international research community there exists a relatively free circulation of knockout mouse strains between many laboratories worldwide.**[3] As a result, what may seem a minor error within a single laboratory has the potential for far-reaching consequences.

Although inbred mouse strains are remarkably stable, genetic drift can occur through spontaneous mutations that can become fixed. Drift most commonly occurs in mouse colonies arising from the same strain which have been physically separated.[3,9] **Regardless of adherence to appropriate inbreeding protocols, mutations constantly occur during inbreeding, generating new polymorphisms.** While a proportion of these new mutant alleles are eliminated by inbreeding, another proportion may become progressively fixed in the homozygous state, replacing the original allele. Known as genetic drift, this genetic variation can lead to distinct substrains at different breeding facilities over time.[1,10]

Figure 2. While ostensibly the same, there is growing evidence of significant phenotypic differences between substrains of the C57BL/6 inbred mouse.[8]

Components of a strong genetic monitoring program

Monitoring for Phenotypic Change

The experienced eyes of the animal husbandry team are a critical component of any genetic monitoring program. Institutional guidelines for colony management are the primary means used to establish and maintain inbred mouse colonies. **They are also the principal means by which breeding errors that lead to genetic contamination are prevented.** Initial and periodic training of animal husbandry personnel should provide the foundation for any genetic monitoring program.[11]

Guidelines should clearly define the process for identifying and assessing phenotypic change.[11] Once the process is defined, it must be strictly followed. Any observed anomalies in phenotype are to be investigated, and mice exhibiting changes should promptly be removed from the breeding colony. Changes may be noted on physical examination (body size, coat color, skeletal structure) or as part of your research (unexpected behavioral responses, changes in tumor susceptibility).[2]

However, using this method alone may cause contamination to remain unidentified; not all evidence of genetic contamination is phenotypically obvious.[2] Several studies[3,10,13] provide sobering examples of such cases. Fahey and colleagues[11] strongly recommend surveillance with laboratory methodologies as a part of genetic monitoring programs.

Single Nucleotide Polymorphism (SNP) Monitoring

Even with the best trained animal care personnel and a robust genetic monitoring program, it is still appropriate and necessary to complement this surveillance with laboratory methodologies that objectively identify genetic homogeneity within inbred strains of mice.[11] **SNPs offer a robust means of differentiating mouse strains, and SNP testing has been shown to be fast, efficient, and cost-effective genotyping method for genetic monitoring programs.** SNP analysis is especially helpful:[2,11]

- When adding new breeders
- When ensuring a lack of genetic contamination is critical (i.e. at the beginning and/or end of a study)
- When used to spot check individual mice from each litter to ensure the genetic background is free from contamination
- When used to increase the speed of a backcross via marker-assisted breeding

Allele-Specific Genotyping

There are several known mutations that have resulted from genetic drift. Among these are the Nnt[10], rd8[23], and Dock2[13] mutations.[15] Routinely checking breeders for these mutations of concern via standard SNP genotyping is recommended.[2]

Best practices for strain maintenance

The University of Michigan Animal Care & Use Program advises the following strategies for maintaining inbred and mutant strains:[2]

For All Strains:

- Use brother x sister mating schemes
- **SNP analyze breeders prior to use**
- Periodically refresh inbred lines. Use pedigreed animals from a high-quality vendor or cryopreserved founders to refresh lines.

For Mutant Strains:

- Back-cross to the parent strain every 10 generations to minimize drift
- **Confirm the presence of mutant alleles with phenotyping and genetic testing**
- **Genetically test newly created lines**
- Periodically monitor transgene copy number and expression

For Newly Acquired Strains (Purchased, Donated, or Self-Created):

- Validate both the background and mutations prior to beginning experiments

Additionally, Guerts and colleagues[3] note that, even if the knockout strain is sufficiently backcrossed into an inbred line, it is important to use non-transgenic control mice derived from the same inbred line, as this will minimize strain differences due to genetic drift.

Minimizing human error

Step 1:

- Maintain consistent breeding and husbandry practices.
- Develop and adhere to a clear colony management strategy.
- Make high-quality training opportunities for the animal care and laboratory team a priority.

Step 2:

- Detailed breeding records are essential.
- Use clear cage labels. Adhere to standards for correct nomenclature.
- Clear and consistent animal identification minimizes the potential for error.

Step 3:

- Maintain separation of strains in the room or on the rack.
- Only open one cage at a time.

Step 4:

- For the sake of reproducibility, all reports concerning genetic experiments must include detailed information on the origin and the genetic background of the studied animals.

Figure 3. Steps to minimize human error in the care and maintenance of inbred mouse colonies.[1–3,11]

Conclusion

Genetic contamination can have wide-ranging, unexpected effects on research.

Undertaking strategies such as consistent breeding and husbandry practices, adherence to a clear colony management strategy, and routine genetic monitoring via SNP panels and allele-specific genotyping, are critical components of a high-quality genetic monitoring program.[1,2,11]

Researchers should consider the impact of background substrain in their experimental design and analysis. In order to improve data interpretation and protect the reproducibility of pre-clinical research findings, careful attention to the specific substrain of mice used for experiments or for backcrossing should be carefully evaluated and documented. Simply put, routine genetic monitoring is necessary to ensure valid experimental data.[3,10,11,13]

For More Information on Genetic Monitoring and SNP Genotyping Services

Ready to get started? Transnetyx offers externally validated genetic monitoring SNP panels that can be used to monitor your colonies and/or improve the efficiency of speed congenics/marker assisted breeding. To learn more, visit our web page at **www.transnetyx.com/monitoring**, or contact Transnetyx Genetic Solutions at +1 888 321 2113 or by email at help@transnetyx.com.

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