RESEARCH MODELS

How to Refresh Your Mutant or Transgenic Mouse Strains

Author:
Peter Kelmenson,
Technical Information Scientist,
The Jackson Laboratory

Maintaining experimental mouse colonies requires a great deal of coordination. It is necessary to constantly evaluate the breeding performance, adjust the colony size to match required production, genotype the mice, and then plan and execute the experiments. With all this, it is easy to have concerns about genetic drift far down on the list of priorities. However, for mutant or transgenic mouse strains on inbred backgrounds, dealing with potential genetic drift needs to be a priority.

Why Do Mouse Models Need to Be Refreshed?

Spontaneous mutations will continually arise in any colony of mice and, through random chance, some of them will spread and become homozygous in all of the mice. If any of those mutations alter the phenotype of the strain, problems will arise that can impact research significantly. Smaller colonies of mice, like a small research colony, will be more affected by genetic drift than large colonies (Figure 1).

There are many examples of how genetic changes can lead to phenotypic changes for mutant and transgenic mouse models. The way to mitigate the impact of genetic drift on mutant and transgenic mouse strains is to refresh the genetic background of your strains every 5-10 generations by backcrossing to the inbred control strain.

Figure 1

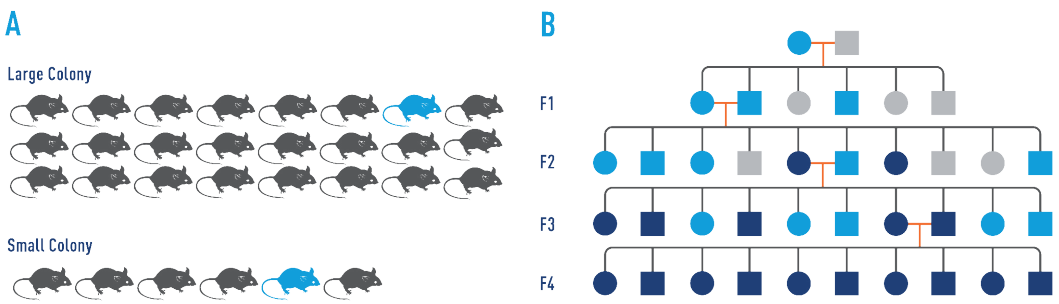

Figure 1. The risk of propagating a spontaneous mutation is higher in small colonies versus large colonies. A) The probability of using a mouse that carries any given mutation (light blue) for breeding is higher in a small colony than a large colony. B) In each round of breeding, there is a 25% chance that a new mutation will become more established in the population. For example, Mendelian inheritance predicts that the F1 generation will be composed of 50% wildtype (grey) and 50% heterozygous for the mutation (light blue). If by chance, two heterozygotes are used as breeders, the F2 generation will be composed of 25% wildtype, 50% heterozygotes, and 25% homozygotes (dark blue). This can continue until the entire colony is fixed homozygous for the mutation (F3, F4). However, the genome can drift in either direction depending on the genotypes of the mice used for breeding – the probability that the mutation becomes fixed is equivalent to the probability it will be lost entirely from the colony.

8 Steps to Genetically Refresh Your Colonies (Figure 2)

Below are detailed steps for refreshing mutant and transgenic mouse strains. C57BL/6J is used as an example, but any appropriate inbred strain can be substituted.

1. Cross females from your mutant or transgenic strain to C57BL/6J males.
2. The male progeny from that cross will have a "refreshed" Y chromosome.
3. Cross those males that carry your mutation or transgene to C57BL/6J females.
4. The male progeny from the second backcross will have a "refreshed" X chromosome, Y chromosome, and mitochondrial genome.
5. Cross those males from the second backcross that carry your mutation or transgene to C57BL/6J females.
6. Cross the males and females from this backcross together to get homozygotes (if homozygotes are needed).
7. Inbreed to maintain/expand refreshed colony.
8. Phase refreshed mice into the existing colony as older breeders are retired (see Figure 2).

If your colony has only been inbred five generations since the strain was originally obtained/created, two backcrosses should be sufficient (and step #5 can be skipped), but if your colony is at 10 or more inbreeding generations, then three backcrosses is the best approach. By regularly refreshing the genetic background of your strains, you will keep them as genetically similar to your control strain as possible, thereby ensuring the reproducibility and validity of your studies.

Genetic drift is inexorable and will impact the phenotype of every live mouse colony if not properly maintained. While it cannot be stopped, genetic drift can be limited. The Jackson Laboratory has implemented a unique Genetic Stability Program (GSP) to limit cumulative genetic drift in its most widely used mouse strains, such as C57BL/6J, by rebuilding its foundation stocks from cryopreserved, pedigreed embryos every five generations. If the strains you are refreshing are a GSP strain, then your mice will be as genetically similar today as they will be 5 or 10 years from now.

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Figure 2