

BACKGROUND STRAIN CHARACTERIZATION

Genetically defined rodent strains with stable, identifiable phenotypes have played a central role in the advances made in biomedical research. However, experiments designed to study any phenomenon based on complex gene interactions must take into account possible genetic variability in rodent strains, and the effect this may have on the observed phenotype. Differentially fixed modifier loci can influence phenotypes such as immunological states, susceptibility to viral and bacterial diseases, and incidence and growth of tumors. The use of congenic strains serves to decrease spurious experimental results due to genetic variability, while providing insight into the contribution of background strain on model phenotype. Developed by selection and backcrossing, these strains require periodic genetic characterization to ensure strain/line purity prior to extensive research or breeding programs. Genetic background characterization alerts the investigator to genetic variation, which may eventually arise even in closed breeding programs, as well as via the introduction of contaminating genetic backgrounds through misbreeding.

Charles River Laboratories Genetic Testing Services has microsatellite and SNP panels appropriate for the genetic monitoring of any inbred strain of mouse or rat.

Why is Background Strain Characterization recommended?

Time and money may be wasted when experiments are performed with animals that are not of the expected background strain. Misbreedings can occur undetected, and errors may occur in pedigree records. Research models are often exchanged among many institutions, making their precise breeding history difficult to track. Additionally, congenic animals may not have been fully backcrossed, or information on the number of backcross generations may not be available. Given that these events may occur in even the best-managed colonies, animals (colony or experimental) should have their background strain confirmed.

Detection of Misbreeding

Consequences of an undetected misbreeding can be severe, even to the point of invalidating research studies. For a known or suspected misbreeding event, a panel of 32 SNP markers can quickly, easily, and inexpensively identify offspring from a genetically contaminated mating event. A larger panel of microsatellite markers, scanning all chromosomes, is necessary to detect ancestral misbreedings.

Evaluation of Backcrossing

Using traditional backcrossing methods, 10 generations of backcrossing are required to establish congenic lines.

We recommend background strain characterization at the following times:

- When establishing a closed breeding colony
- When new strains are received from collaborators
- During a backcrossing (to check extent and/or for speed congenics)
- If unusual phenotypes appear (i.e., unusually large litters may indicate hybrid vigor)
- At the commencement of a series of experiments
- Annual monitoring of in-house colonies



Sometimes a desirable research model does not have the necessary documentation, the number of backcross generations has not been tracked, or the animals have been bred by both backcrossing and intercrossing. If an investigator finds that the animals are not at the desired level of preferred background strain, the strain characterization data can be used to develop a speed congenics program.

129 Substrain Characterization

In the mid 1990's, it became apparent that the 129 strain of mouse is actually several genetically distinct substrains^{1,2}. Over the course of more than 75 years of breeding, different laboratories have introduced the steel and teratoma loci, genetic contamination has occurred, and residual heterozygosity in the original colony has been differentially fixed. For generation of knockout mice, the same substrain should be used for targeting vector and ES cells; otherwise there can be a decrease in the rate of homologous recombination. For all experiments, the control animals should be of the same substrain as the genetically modified experimental animals. Because the contamination went undetected for a time, the substrain identity of 129 ES cells and/or animals may not be known to the investigator. Charles River offers microsatellite panels which can distinguish between many common 129 substrains.

Routine Genetic Monitoring

Good animal husbandry requires appropriate monitoring of inbred colonies. Closed colonies will gradually diverge from the original genotype. There is always a possibility of undetected misbreeding, error in recording of pedigrees, or other mix-ups. Early detection of such errors can avoid wasted time and effort and incorrect interpretation of experimental data.

Sample Submission

There is no one-size-fits-all answer as to the number of animals to be genotyped. Some general recommendations are available on our website, and we will be happy to discuss your individual needs. Sending samples is quick and easy. Our preferred test article is a tailsnip in 70% ethanol, kept cool but not frozen. Sample collection and shipping information can be found on the Genetic Testing Service Sample Accession Form available at www.criver.com.

1. Simpson, E.M. C.C. Lindner, E.E. Sargent, M.T. Davison, L.E. Mobraaten and J.J. Sharp (1997) Genetic variation among 129 substrains and its importance for targeted mutagenesis in mice. *Nature Genet.* 16: 19-27.

2. Threadgill, D.W., D. Yee, A. Matin, J.H. Nadeau and T. Magnuson (1997) Genealogy of the 129 inbred strains: 129/SvJ is a contaminated inbred strain. *Mamm. Genome* 8: 390-393

A Note on Outbred Strains

Outbred strains are maintained to maximize genetic diversity between the animals in a colony. Ideally, each animal should exhibit a high level of heterozygosity, and each locus has several different alleles. Thus there are no reference microsatellite panels for outbred strains. However, background strain characterization on outbred animals can serve to assess loss of heterogeneity within a breeding colony, which could help in colony breeding management.



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