

Frequently Asked Questions – Genetic Monitoring

What is genetic monitoring and how can it be useful for my research?

The genetic background of genetically engineered mice can influence phenotype. Inattention to
a mutant's genetic background can substantially confound research results. Each strain of mice
has unique background alleles that may interact with and modify the expression of a mutation,
transgene, or other genetic insert.

To ensure the validity and reproducibility of a study, all animals must be genetically identical (except for the gene or modification of interest). Genetic Monitoring is a single nucleotide polymorphism (SNP)-based testing service that can be used to ensure this genetic purity by testing the integrity of inbred and congenic strains.

When should I consider using genetic monitoring for my mouse colonies?

- If you are crossing two genetically modified lines together (such as a floxed or conditional line with a Cre line), you should consider testing some of the initial offspring to determine if you have the background strain of interest.
- Consider testing the genetic integrity of your colonies at one or more of the following points, depending on your use case:
 - When introducing new breeders.
 - When ensuring a lack of genetic contamination is critical, i.e. at the beginning and/or end of your study.
 - Routinely spot-checking individuals from each of your litters to ensure the genetic background is free from contamination.
 - To increase the speed of a backcrossing program (via speed congenics/marker assisted breeding)

How long does it take to get results?

• The expected turnaround is around three weeks once an order is submitted.

What information will the results give me?

• You will receive the full results for each SNP marker run, along with a report that details the quality of the genotyping run and a calculation of the strain(s) that make up the background of the mouse.

How do I place an order and send samples for the service?

- If we have genotyped your sample within the last six months, we already have it! If not, you'll
 need to place a normal genotyping order for the samples, wait for those results, and then place
 the genetic monitoring order from the genotyping results page. Learn how to place an order by
 visiting Transnetyx Support Hub
- If you would like to order genetic monitoring on mice that do not require genotyping (for example, testing an in-house wild type colony to confirm the absence of genetic contamination), please contact Transnetyx Genetic Services. We will happily provide you with assistance in placing such an order.

My lab already uses Transnetyx for genotyping. Do I need to send additional samples to Transnetyx when ordering genetic monitoring?

• If a particular sample that is stored at Transnetyx is diluted or discarded, the user may be required to send in a new sample. Transnetyx stores each genotyping sample submitted for six months.

I want to order Genetic Monitoring services to ensure the genetic purity of my mouse lines. Do I need to send samples from all animals produced in a generation?

- The frequency of testing and the number of samples tested can vary in a genetic monitoring program. This will depend on your confidence in your source of mice, breeding history, and size of colonies. Some possible scenarios:
 - Test some or all new breeders before putting into service depending on your confidence in their background.
 - Test one mouse from every breeding pair or every other breeding pair of a colony.
 - Test study animals at the end of experiments if you are concerned with phonotypes or results.

 Consulting with Transnetyx or your animal facility can help you decide how best to utilize Genetic Monitoring services.

I want to order Speed Congenics/Marker Assisted Breeding testing to reduce the amount of time it takes to transfer my mutated lines from a 129 background to a C57BL/6 background. Do I need to send samples from all animals produced in each generation?

- Testing is necessary only for animals that carry your gene or modification of interest.
 Testing 15 -20 animals from each generation will give you the best chance of producing fully congenic mice in 5 generations of backcrossing. If fewer than 15 samples are sent per generation, the number of backcross generations required to obtain a fully congenic line will be increased.
- It is advisable to fix the sex chromosomes before testing starts. The best method to fix the Y chromosome is to cross female carriers to wildtype males of the recipient strain to produce F1. The X chromosome is then fixed by mating F1 carrier males to wildtype females of the recipient strain. The offspring of this cross and all subsequent back crosses should then be tested to select the most suitable males for the next back cross.

Do I need to know the background of my strain for Genetic Monitoring?

• While the MiniMUGA panel does not require that the constituents of the strain background be known, it provides valuable context for interpreting the results. Donor and recipient strains are needed in order to choose breeders in a Speed Congenics program.

Can the Transnetyx Genetic Monitoring service help me to identify genetic drift in my mouse lines?

 No. Genetic drift occurs through random mutations across the genome accumulating over time in a given population of mice. SNP based panel testing cannot detect mutations that occur at loci other than those targeted. However, Transnetyx offers allele-specific SNP genotyping for several mutations that are the result of genetic drift (for example, Nnt, rd8, and Dock2). Allelespecific genotyping identifies mutant alleles within a strain and is an important component of any genetic monitoring program. You can order genotyping of these loci via our standard genotyping ordering process.

What quality control measures are in place to ensure the accuracy of the Genetic Monitoring service at Transnetyx?

 Every sample that is run is qualified based on its performance in our genotyping system. If sufficient sample was obtained via the original genotyping order, the sample will be run through the genetic monitoring system. The MiniMUGA panel was developed by the lab of Fernando Pardo-Manuel de Villena (UNC, Chapel Hill). The samples are run on an Illumina BeadChip array by Neogen Genomics

What is the density of your Genetic Monitoring service?

• The MiniMUGA array contains over 9700 SNP markers across the autosomes and X and Y chromosomes that are informative in 241 inbred strains.

What is the cost of testing per sample?

• \$40

Where can I access more information about the MiniMUGA panel and its contents?

- <u>Consensus Genotypes</u>
- Report Summary and FAQ
- Inbred Strains and Diagnostic counts
- Content and Performance
- <u>MiniMUGA Poster (UNC)</u>