

# Background Strain Characterization

*Who's that mouse?*

# THE SENTINEL

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 arise even in closed breeding programs, as well as the introduction of contaminating genes through misbreeding.

## *Scientific Significance of Background Strain Characterization*

Historically, non-DNA based tests were used to assess relatedness - the most rudimentary being coat color. Unfortunately, different modifier alleles can be present among individuals and, as a result, can confound phenotypic analysis. Skin grafting was also commonly used, and prior to DNA-based testing, the establishment of allelic profiles using biochemical and immunological markers was considered to be the gold standard. Now, genetic testing allows researchers to directly analyze DNA rather than infer relatedness from resulting protein products. In addition to being more comprehensive, DNA-based testing is less labor intensive, and thus less costly, than biochemical or skin graft testing.

Charles River offers two different methods for background strain characterization and estimating genetic variation among individuals- **microsatellite and RFLP analysis.**

## **BACKGROUND STRAIN CHARACTERIZATION METHODS**

### *Microsatellite Analysis*

Background strain characterization is performed by comparing the DNA profile of an individual animal to the established profiles of known rodent strains and lines. Nucleotide repeats (microsatellites) mapped to specific locations on each chromosome are used to evaluate genomic polymorphism. This PCR-based method scans all 20 mouse chromosomes at approximately 20 centiMorgan intervals. Results yield a defined analysis of the genome in question, and profiles are compared to detect genetic variation among individuals.

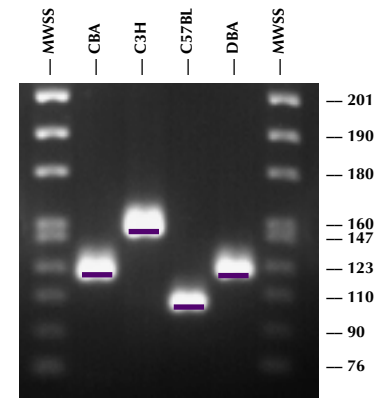
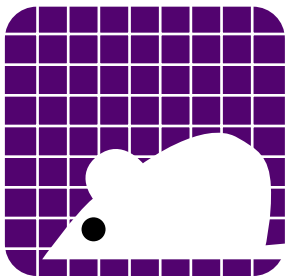


Figure 1

### *Background Strain Characterization Case Study 1*

In Figure 1, PCR is used to amplify a region of the C-reactive protein (CRP) gene as one example of a mouse strain characterization marker. Depending upon the mouse strain, this generates an amplified PCR product of either 96, 116 or 149 base pairs. In this example, mice from four different strains were examined. The C3H mouse has the 149 bp PCR product, and the C57BL has the 96 bp PCR product. As both CBA and DBA mice have the 119 bp PCR product, the CRP PCR product cannot be used to distinguish between these two strains. However, they can be distinguished using combinations of other markers (data not shown). In the

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background strain characterization assay, a set of markers is chosen so that each strain exhibits a unique banding pattern across the microsatellite data set.

### RFLP Analysis

Restriction Fragment Length Polymorphism (RFLP) analysis is used to compare DNA profiles produced by multilocus probes (minisatellites) among individuals. Restriction enzymes are used to discretely cleave DNA and the resulting banding pattern is visualized using multilocus probes. Variations occur when two individuals differ in the presence or absence of these restriction sites within their DNA, or by insertion or deletion of DNA between two sites. Variation in restriction sites occurs naturally within populations, however closely related animals (e.g., within a population of an inbred strain) will have fewer differences between DNA profiles. Strain specific marker analysis (20-80 markers) offers a broad profile of the mouse genome.

### Background Strain Characterization Case Study 2

A client requested background strain characterization of a transgenic mouse strain believed to be on a C57Bl/10 background. As can be seen in Figure 2, DNA profiles of several individuals from the transgenic line revealed:

- 1 The absence of some markers associated with the C57Bl/10 strain.
- 2 The presence of genetic markers not characteristic of C57Bl/10 individuals (believed to be 129 strain genetic markers).
- 3 Genetic variation within the transgenic strain which was alleged to be on a congenic background.

A standard Background Strain Characterization assay consists of two probe/enzyme combinations, which can analyze approximately 20-80 markers (the number of informative markers is strain dependent). However, up to 120 markers may be analyzed at an additional cost per sample.

### Test Results and Sample Submission

Results from DNA profile characterization of background strain are reported in 2 weeks by microsatellite analysis and 3-5 weeks using the RFLP methodology.

Tail snips should be collected and immersed in 70% ethanol and refrigerated (4°C) until ready for shipment. Other tissue, such as toe clips or ear punches may also be used. Samples should be shipped overnight on ice packs.

Charles River offers a variety of DNA-based tests to facilitate the periodic genetic monitoring of both transgenic and knockout strains of laboratory animals that specifically address these needs. Other DNA-based tests offered include:

- Genetic Monitoring (PCR, Southern and slot blots)
- Zygosity and Expression Testing
- Accelerated Backcrossing/MAX-BAX<sup>SM</sup>

*Please contact Charles River directly for more information on Background Strain Characterization or other questions regarding the genetic testing services for knockout or transgenic rodents.*

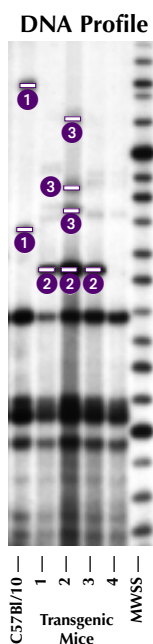


Figure 2