



Genetic Monitoring

Researchers are increasingly aware of the importance of validating the genetic status of their research models. Accurate and efficient genotyping is critical for the validation and interpretation of research results. Benefits of genotyping include more efficient breeding and faster production of research models. Charles River offers a variety of DNA-based tests to facilitate the periodic genetic monitoring of laboratory rodents. Genotyping is most often performed by DNA amplification techniques (PCR and Q-PCR) or SNP analysis.

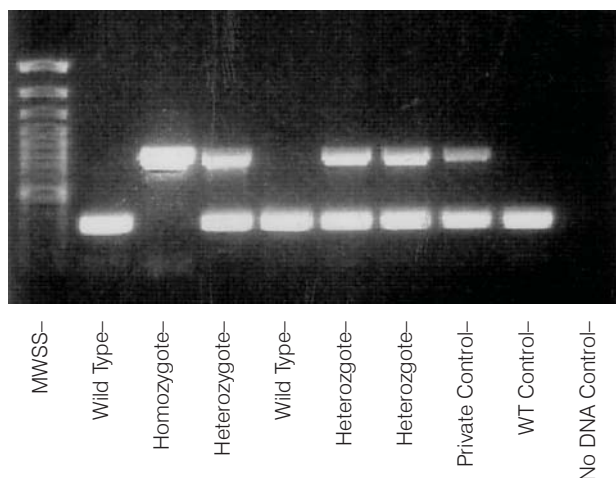
GENOTYPING VIA DNA AMPLIFICATION

Polymerase Chain Reaction (PCR)

Charles River can determine the genotype or carrier status of animals within your colony by utilizing a biopsy sample for target DNA and primers specific for the detection of the mutation of interest. The resulting amplification of the target DNA between the primer-binding sites allows us to determine the genetic nature of the individual with respect to gene insertion or deletion and therefore its genotype.

In a typical PCR, primers are designed to bind in opposite directions to complementary strands of target DNA. The target is amplified exponentially with each PCR cycle. The resulting amplified product(s) are separated by size, yielding banding patterns which allow for the interpretation of each individual's genotype. Please note that zygosity information can be obtained from PCR assays for knockout and knock-in models only. Quantitative PCR is recommended for determination of transgene zygosity.

Figure 1



In Figure 1, DNA amplification was used to determine the genotype of individuals submitted from a knockout strain of mice. For this customer, Charles River was able to detect all three possible genotypes in a single reaction with three primers. Homozygous knockout individuals exhibit an 800 base pair band, wild-type individuals exhibit a 300 base pair band, and heterozygotes exhibit both bands. (The lane labeled MWSS contains a molecular weight sizing standard marker.)

Strain-Specific Genetic Verification

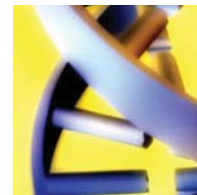
Charles River Laboratories maintains a library of strain-specific assays for common research models. Transfer and validation has already been performed on these assays, allowing samples to be genotyped efficiently and with a rapid turn-around time. Assays include:

- Mouse: NOD, SCID, RD1, PGP, PCK, OB (*Lep^{ob}*), DB (*Lep^{db}*), NU (*Foxn1^{nu}*), Nnt^{C57BL/6J}, Disc1^{del}, Hr^{hr}, and MHC Class I and Class II
- Rat: Zucker fatty (*Lep^{fa}*), Koletsky fatty (*Lep^{cp}*), Ahr, RNU (*Foxn1^{nu}*), and MHC Class I and Class II



Quantitative PCR

Quantitative PCR (Q-PCR, also called real-time PCR or Taqman® PCR) provides accurate determination of relative levels of starting nucleic acid, so it can be used to determine transgene zygosity or copy number. Unlike the endpoint reading of standard PCR, Q-PCR measures a fluorescent signal after each PCR cycle. There is no post-PCR processing, and analysis of data occurs during the logarithmic phase of the PCR reaction. For more information please refer to our Zygosity and Expression Testing and Molecular Phenotyping datasheets.



Genotyping via SNP Analysis

Point mutations, either spontaneous or induced, can be genotyped by single nucleotide polymorphism (SNP) analysis. We employ an Applied Biosystems 7900HT Real-Time PCR system to genotype point mutations using fluorescence-based TaqMan® technology.



Transfer and Validation of Protocols

Charles River can transfer and validate an existing assay, optimize an existing assay or develop a new protocol in response to your research needs. Transfer and Validation studies assess protocol efficacy and are routinely performed prior to accepting production samples.

Sample Submission and Test Results

Genetic monitoring results are reported to you within 5-14 days following receipt of samples. However, Transfer and Validation of new protocols may require additional time. Our preferred test article is a tailsnip (approximately 0.5 cm in length) immersed in 70% ethanol and refrigerated (4°C) prior to shipment. These samples should be shipped overnight on cold packs. Charles River will be happy to consult with you on the best way to ship each sample type. Please notify the laboratory prior to shipping samples and include the first page of the sample accession form.

Genetic management of rodent colonies requires the execution of a comprehensive, long-term testing regimen. Charles River provides many accurate and efficient DNA-based tests to facilitate the periodic monitoring of genetic mutant models. Please contact Charles River directly for accession forms, or other questions regarding the genetic testing services of knockout or transgenic rodents.