

MOLECULAR PHENOTYPING FOR TRANSGENIC AND KNOCKOUT ANIMALS

Molecular phenotyping allows us to look beyond carrier status in evaluating transgenic rodents. Charles River Laboratories' Molecular Phenotyping Services provide comprehensive nucleic acid quantitation of a transgenic or knockout model. Our services are customized to provide clients with the model information they require.

Transgenic animals incorporate many copies of the transgene into their genomes. Both the number of copies inserted and the location of the transgene in the genomic DNA are essentially random. Molecular phenotyping can be used to compare and evaluate individual founder animals with the same transgene. It provides assurance that the gene of interest is being transcribed, and quantitates the level of mRNA expressed. It can also be used to determine the number of integrated copies of a transgene in an animal and to determine transgene zygosity.

Tissue-specific gene expression can be determined by assaying several tissues from the same animal. Common tissue types include heart, lung, spleen, brain, liver, or blood. Other tissue types will be considered on an individual project basis. Our Molecular Phenotyping Services combined with in-life analysis and pathology provides a comprehensive characterization program to analyze the phenotype of research models.

mRNA Quantitation for RNAi Studies

Over the past several years, the ability of an RNA molecule to modulate expression of a complementary gene has been recognized. Antisense molecules inhibit translation of corresponding mRNAs, and the RNA interference (RNAi) pathway is mediated by short double-stranded RNAs (which may be derived from sense-antisense duplexes). The phenomenon of RNAi has found wide application as a method of transient gene expression knockdown. Charles River offers a number of gene expression assays, custom-designed for the gene of interest of each individual client.

Knockdown quantitation correlates observed phenotypes to precisely measured reductions in the level of mRNA expression of the target gene.

Screening of target sequence is available. Levels of silencing can vary widely for different target sequences, even within the same transcript. It is difficult to determine *a priori* which sequences will be the most effective. We can screen numerous candidate siRNAs in a cost-effective and expeditious manner.

Additional Phenotyping Screens

In-life Analysis

Clinical and behavioral observation, growth curve, reproductive performance, DEXA scan, radiography, high resolution ultrasound imaging, grip strength, hot plate, activity level, motor coordination and gait analysis, cognitive analysis, blood pressure, and respiratory function

Pathology

Necropsy, clinical and specialty pathology, and histopathology

Visit www.criver.com/info/pheno for details on our portfolio of therapeutic phenotyping panels.

Visit www.criver.com/info/quotes for project estimates.



RNAi dosage studies via Charles River's Molecular Phenotyping Services allow precise measurement of the levels of gene knockdown corresponding to a particular RNAi dose. SiRNAs and shRNAs have been shown to activate an immune response, causing undesirable expression of interferons and cytotoxicity. Thus the lowest efficacious dose of RNAi is desired for *in vivo* studies.

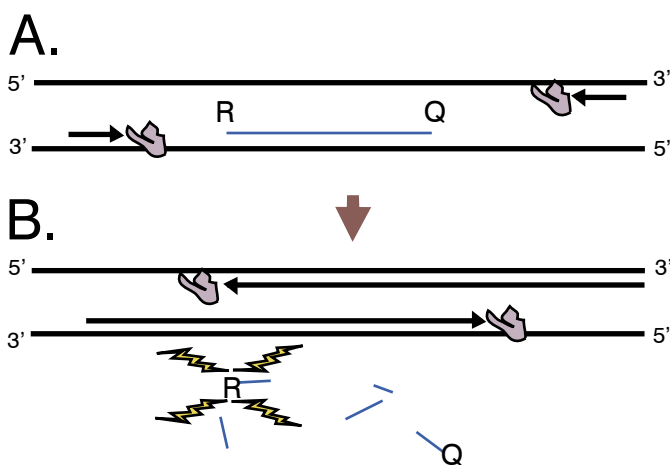
Differential effects determination is available for any tissue of interest. We can provide precise quantification of the level of transcript reduction, assessment of the effect in different tissues, and time course studies to determine the metabolic half-life of the siRNA.

Q-PCR Technology for Molecular Phenotyping

Sensitive, accurate Q-PCR is used to determine transgene copy number. This system combines the sensitivity of PCR with the specificity of probe hybridization. An oligonucleotide probe, labeled with both a reporter and quencher dye, anneals specifically to template DNA between the forward and reverse PCR primers. During the extension phase of the PCR cycle, the annealed probe is separated from the quencher dye, generating a sequence-specific signal. The probe and primer used in the reaction are custom-designed for each client's gene of interest.

The reporter dye generates a fluorescent signal when cleaved from the quencher dye, and this signal is directly proportional to the initial amount of target sequence present in the sample. Because the PCR product is analyzed at each PCR cycle, rather than a single, static measurement at the end of the PCR program (real-time vs. endpoint reading), relative differences in the quantity of starting material can be accurately determined. By comparing biopsy samples to a dilution series of control DNA, the number of gene copies in the biopsied animal can be determined.

Expression testing uses the same technique, but with an additional step. RNA is isolated from the tissue biopsy, then reverse-transcribed into DNA. The sample then undergoes a Q-PCR assay as above.



Principle of Quantitative PCR

A. Primers and a probe are designed to be complementary to the target sequence. The probe has a reporter fluorophore at one end, and a quencher molecule at the other. When reporter and quencher are in close proximity, no light is emitted.

B. At each extension cycle of the PCR reaction, the exonuclease component of Taq polymerase degrades the probe, releasing the reporter dye from the vicinity of the quencher dye. A fluorescent signal is produced, which is proportional to the number of target sequences present in the original sample.