

# TRANSGENIC OUTLOOK



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## CHARLES RIVER LABORATORIES' GENETIC TESTING SERVICES (GTS)

### Who is GTS?

GTS is the Genetic Testing Services division of Charles River Laboratories. We offer a comprehensive set of molecular-based tests for genetic monitoring of transgenic and knockout laboratory animals. Our laboratory is located in Troy, New York, part of the upstate Tech Valley region.

GTS clients include academic institutions, biotechnology, and pharmaceutical companies. In addition, GTS performs all the genetic monitoring necessary for Charles River's production facilities worldwide. The majority of our staff is employed in technical positions, and includes numerous PhD-level scientists.

### What does GTS do?

All divisions of Charles River are dedicated to accelerating the search for healthier lives. The Genetic Testing laboratory supports scientific research by providing molecular-based genotyping services. This makes genotyping available to scientists without molecular biology training or equipment. Laboratories with molecular biology experience use our genotyping services in order to free technical staff for other pursuits, or to take advantage of services otherwise not available to them.

## GENETIC MONITORING SERVICES

We offer three basic types of Genetic Monitoring Services, with several variations and options, to meet the varied needs of our clients. These services include:

- Routine Genotyping
- Strain Assessment
- Quantitation

### Routine Genotyping

The carrier status of transgenic and knockout animals can be identified by implementing and optimizing an existing protocol or through the development of a new protocol. Methods include PCR, Southern, and slot blots. Our high-throughput platform processes large numbers of samples with a rapid turn-around time. Once an assay has been transferred and validated, samples can be submitted at any time. We guarantee results 10 business days following sample receipt.



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Our highly-trained technical staff assures that assays work consistently and reliably. This allows laboratories to free their own technicians for less routine work while assuring fast, consistent results.



GTS offers background strain characterization, Max-Bax<sup>SM</sup>, and quality control microsatellite panels for rats.

### **Background Strain Assessment**

Background Strain Assessment has several distinct applications described below. GTS uses microsatellite marker panels that are polymorphic between different strains (or substrains) of inbred mice or rats. The rat genome is now available, and there have been recent advances in the development of transgenic rats. In response to this trend, we have expanded our background strain assessment services to include major inbred rat strains.

**Background strain characterization** compares an investigator's inbred animals to the expected microsatellite profile for the strain. It is important to perform background strain characterization before initiating experiments to confirm that the client's animals are from the expected strain and to save time and resources early on in the event that they are not. We commonly find instances of animal mix-ups or genetic contamination.

**Routine quality control** or rapid assessment of a suspected misbreeding event can be accomplished with a few microsatellite markers. GTS has six-marker and nine-marker panels specific for inbred mouse and rat strains.

**Max-Bax<sup>SM</sup> accelerated backcrossing** utilizes microsatellite analysis to facilitate the rapid production of congenic strains of knockout and transgenic rodents. This pioneering service allows for the directed selection of individuals with preferred genetic background, thereby reducing the total number of generations needed to product a congenic research model. This service is now available for major inbred rat strains.

### **Quantitation**

Quantitation of nucleic acids is performed by quantitative PCR (also known as Q-PCR, real-time PCR, or TaqMan PCR). As in standard PCR, two primers are used to amplify a DNA sequence of interest. In addition to these primers, a probe is designed complementary to one of the strands in the region to be amplified, adding specificity and a method of detection to the assay.

GTS offers Zygosity and Expression Testing to aid in the characterization of genetic mutant models. Zygosity testing allows us to go beyond carrier status to quantitatively determine the true transgenic nature of the client's test animal (i.e. wild type, hetero-, hemi-, or homozygote). Transgene zygosity information is imperative for effective breeding and colony maintenance. In addition, zygosity has often been shown to correlate with gene expression levels.

Expression testing measures the *in vivo* transcriptional activity of a transgene. Quantitative assessments of transgene expression will ultimately determine the relative worth of each founder within a transgenic line. Each of these tests utilizes quantitative PCR combined with state-of-the-art fluorogenic probe technology.

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The addition of a fluorogenic probe allows for real-time monitoring within the exponential phase of the PCR thereby improving assay precision and reproducibility.

## STRAIN-SPECIFIC GENETIC VERIFICATION SERVICES

Several assays have been added to our Strain-Specific Genetic Verification library. These include the *Pde6b*<sup>rd1</sup> retinal degeneration mutation found in FVB and C3H strains. This mutation leads to blindness which can result in significant changes to the animal's behavioral phenotype.



GTS has validated assays to identify the *Lepr*<sup>fa</sup> and *Lepr*<sup>op</sup> mutations.

For obesity researchers, we now offer the *Lepr*<sup>op</sup> mutation found in Koletsky rats. We continue to offer assays for the *Lepr*<sup>fa</sup> mutation. Although both mutations are found in the gene encoding the leptin receptor, they are distinct mutations and their detection requires different assays.

Other assays include the severe combined immunodeficiency (SCID) mutation and markers associated with the non-obese diabetes (NOD) phenotype.

## MOLECULAR PHENOTYPING FOR TRANSGENIC AND KNOCKOUT ANIMALS

Molecular phenotyping allows us to look beyond carrier status in evaluating transgenic rodents. GTS' 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.

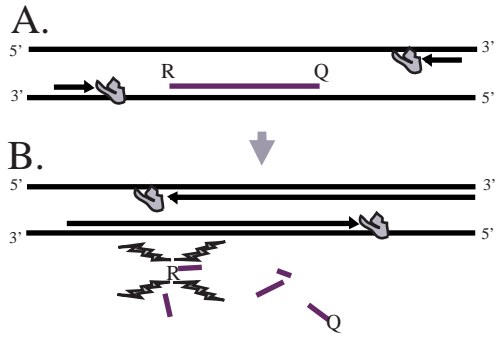
Transgenic animals incorporate many copies of the transgene into their genomes. Both the number of copies inserted and the location of the transgene in the genomic DNA are essentially random. Molecular Phenotyping can be used to compare and evaluate individual founder animals with the same transgene. 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. GTS offers a number of gene expression assays, custom-designed for the gene of interest of each individual client.

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#### 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.

**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.

**RNAi dosage studies** via GTS' Molecular Phenotyping 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.

For information on how to submit samples and pricing, please contact GTS at 518-286-0016