

THE Sentinel

Need to Breed Your Genetic Mutant Models?

Let Charles River determine their zygosity!

Many of you know that Charles River is committed to providing customers with state of the art infectious disease testing but did you also know that we provide genetic testing for mutant models? Here at CRL we utilize PCR technology to determine the carrier status of transgenic and knockout models. However, traditional PCR sometimes falls short when trying to determine the copy number, or zygosity of genetic mutant animals. While many traditional PCR assays are designed to determine the presence or absence of a gene, there may be no good way to determine whether the animal is a hetero/hemi or a homozygote gene carrier. Charles River offers specialized quantitative PCR testing for the determination of zygosity in your mutant models. Zygosity information is imperative for effective breeding and colony maintenance, and zygosity, in many instances, has been shown to correlate with gene expression levels.

While on a late night drive back in 1984, Kary Mullis dreamed up one of the single most important techniques of molecular biology to date, the Polymerase Chain Reaction, otherwise known as PCR. PCR has revolutionized modern science by allowing scientists to study genes that were heretofore undetectable by the average benchtop researcher. The science is based on the exponential amplification of sub-detectable levels of nucleic acid, resulting in large quantities of amplified product to analyze. While the initial application was restricted to the individual researchers, PCR applications have moved into commercial use as a diagnostic tool.

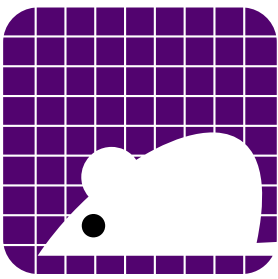
The transference of PCR technology from basic science to the diagnostic arena came with some necessary enhancements. While Kary Mullis began by manually moving samples through a series of temperature controlled water baths, PCR technology has evolved, providing laboratories with fully automated thermocyclers complete with real-time, fluorescent dye-based, product reading

capability. Please see the Fall 2000 Sentinel, ***Molecular Diagnostic Testing for Rodent Pathogens***, for a discussion of fluorogenic probe technology.

The key to applying this technology to genotyping was to make it quantitative. By allowing for periodic analysis of product production, rather than a static measurement at the end of a program (real time vs. end point reading), we are able to compare relative differences between amounts of starting material. By looking at cycle thresholds (C_t), the cycle number at which probe fluorescence is measurable above background, you can detect as little as a 2-fold difference in gene copy number.

The $\Delta\Delta C_t$ Method of Determining Zygosity

We use the Perkin Elmer ABI PRISM 7700 to perform all of our quantitative PCR assays and employ the " $\Delta\Delta C_t$ " method to determine the zygosity of a given gene. By comparing the C_t values of the unknowns to those of the control animal, we can determine if the animal carries no gene copies (wild-type), one gene copy (heter/hemizygote) or two gene copies (homozygote). In brief, the more copies of the gene, the more quickly (fewer PCR cycles) the C_t will be reached. In addition, a single copy endogenous gene is used to normalize template DNA concentration differences among samples.



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Actual data from the analysis of customer samples is shown in **Table 1**. Real-time reactions were run comparing C_t values of an animal known to be hemizygous for the transgene (X) to those of an endogenous, dual copy, gene (endo). These numbers are used to calibrate the system.

The average C_t value for the endogenous gene is 25.09 while the average C_t value for our hemizygous animal is 23.52. The difference between these two numbers, referred to as the ΔC_t , was -1.57 and this will be used later in the calculations, (**Table 1**).

Our real-time PCRs are run in triplicate to generate an average C_t value for each sample. These are run along side the endogenous gene reactions for each sample, also performed in triplicate. The difference between the two averages is calculated (ΔC_t in **Table 1**), and the calibrator ΔC_t is subtracted from each experimental ΔC_t . This value, called $\Delta\Delta C_t$, is then used to calculate a value indicative of transgene zygosity ($2^{-\Delta\Delta C_t}$).

Sample ID	Average C_t X	Average C_t endo	ΔC_t	$\Delta\Delta C_t$
Calibrator	23.52	25.09	-1.57	0
1	38.27	24.21	14.06	15.63
2	23.29	24.29	-1	0.57
3	22.15	24.37	-2.22	-0.65

Table 1. Calculation of $\Delta\Delta C_t$

In this particular mutant model set, the zygosity ranges were as follows: wild-type had a value of 0, heterozygotes had a value of X, and homozygotes were roughly 2X. Note that the absolute values are not important. In fact, the actual values will not be the same between different transgene assays, but the relative difference, (2X), between hemizygotes and homozygotes will be the same (**Table 2**).

Sample ID	$2^{-\Delta\Delta C_t}$	Genotype
1	0	WT
2	0.67	HET
3	1.57	HOM

Table 2. Zygosity Determination

While every model may have somewhat different absolute testing values, the basic trend holds true allowing Charles River Laboratories to custom design a zygosity assay for your genetic mutant model.

In addition to zygosity testing Charles River offers other services to help you genetically evaluate your animal models:

- Genetic Monitoring (PCR/Southern)
- Background Strain Characterization
- Accelerated Backcrossing/Speed Congenics

Please call us at (518) 286-0016 for details!!!


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