A simple and reliable method for testing transgene zygosity using quantitative real-time PCR

S.K. Morton,[1,2] B.K. Baillie,[2] C.E. Hill[2] and K.I. Matthaei,[1] [1]Department of Translational Bioscience, The John Curtin School of Medical Research, The Australian National University, Canberra, ACT 0200, Australia and [2]Department of Neuroscience, The John Curtin School of Medical Research, The Australian National University, Canberra, ACT 0200, Australia.

Introduction. The upkeep of transgenic animal models is costly, so the most efficient and effective breeding strategy is ideal. Having to genotype animals is not only time consuming but costly. Usually breeding cages are set up using one heterozygous parent that is mated with a wildtype parent. This ensures that all positive transgenic offspring are heterozygous however under traditional Mendelian genetic laws, only half of the offspring will be transgenic. To improve this ratio to 75%, it is possible to mate two parents heterozygous for the transgene. However, one third of these animals will theoretically be homozygous for the transgene with the possibility of unwanted phenomena relating to gene dosage and position effect, with the potential creation of a spurious knock out (Matthaei, 2007). A better solution is to identify animals that are homozygous for the transgene and then use these for breeding with a wildtype partner, thereby ensuring that all offspring are heterozygous.

A previously published method of assessing transgene zygosity used quantitative real time PCR (qPCR) and a $2^{-\Delta\Delta Ct}$ calculation with efficiency adjustments for each real time analysis (Livak & Schmittgen, 2001). We have simplified this method by using a known single copy gene (transferrin) to normalize results from different runs.

Methods. DNA was isolated from tail biopsies under approved animal ethics protocols of the Australian National University. We have used the Corbett Research Rotor-Gene RG-3000 Real Time Analyser for qPCR to determine zygosity differences in mice transgenic for the *Lac*I repressor gene (Cronin, Gluba & Scrable. 2001), using primers for transferrin and for the *Lac*I transgene. A standard curve was created for both the transferrin and *Lac*I genes using dilutions of mouse genomic DNA as the target. The copy numbers of both transferrin and *Lac*I were then determined in every run using samples of known DNA concentration from offspring of intercrosses of either *Lac*I heterozygotes with wildtype mice, or two *Lac*I heterozygote, transgenic parents. A normalized ratio of *Lac*I to transferrin was then calculated. Potentially homozygous animals were validated by test breeding with wildtype partners.

Results. The qPCR analysis method demonstrated that DNA samples from offspring of LacI crosses varied in normalized ratios of *Lac*I to transferrin from 0.5 to 2.6. Scatter plot analysis showed that the data were clustered into 2 populations with peaks around 0.9 and 1.8, potentially heterozygous or homozygous for the *Lac*I transgene, respectively. When a cut off of 1.5 was assigned for homozygosity, ratios of wildtype:heterozygote:homozygote equated to those predicted by Mendelian inheritance. Test breeding of mice, with ratios greater than 1.5, to wildtype mice confirmed homozygosity, since all offspring were positive for the *Lac*I transgene with ratios around 1.

Conclusion. We conclude that our modified qPCR method, with internal control and cut off ratio >1.5, can be used as a rapid and reliable method to identify transgene homozygosity.

Cronin, C.A., W. Gluba & H. Scrable. (2001) The lac operator-repressor system is functional in the mouse. *Genes & Development* **15**: 1506-1517.

Livak, K.J. & T.D. Schmittgen (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25**: 402-408.

Matthaei, K.I. (2007) Genetically manipulated mice: a powerful tool with unsuspected caveats.*Journal of Physiology - London* **582**: 481-488.