08.24.10 MS
06.03.14 MLS

Genotyping Protocol: **MMRRC 315**

Assay Type: PCR (cannot distinguish hemizygous animals from homozygous animals. Can distinguish transgene positive animals from transgene negative animals.)

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit(Cat# 69506). Kit directions for animal tissues were performed with a minor modification as follows: elute in 200µl of AE buffer once.

Primer Information:

1) Name: M315 (Gfap) F#2 Sequence: 5'-CAA CCC GTT CCT CCA TAA AGG C -3'
Genomic location: Chromosome 11, immediately upstream of *Gfap* start codon

2) Name: Gensat GFP Rev Sequence: 5'- TAG CGG CTG AAG CAC TGC A -3'
Binds to the GFP transgene

Assay Name: MMRRC 315 PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25 mM	3.2
M315 (Gfap) F#2	IDT	20 µM	0.3
Gensat GFP Rev	IDT	20 µM	0.3
FastStart *Taq*	Roche (Cat#12032953001)	5 U/µl	0.2
sterile water			13

PCR Setup:
Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Applied Biosystems 2700 thermocycler.

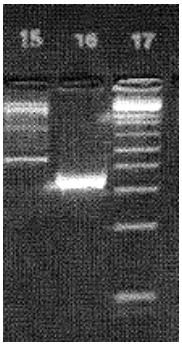
Cycle Parameters:

1) 95°C 5 minutes
2) 94°C 1 minute
3) 60°C 1 minute
4) 72°C 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 7 minutes
7) 4°C hold until refrigerate product

Product Analysis:
All products were analyzed on a 3% agarose gel with ethidium bromide staining.

Expected product: 310 bp

Example gel:



Lane 15 displays a transgene negative sample (no product. This gel displays some non-specific bands).
Lane 16 displays a transgene positive sample (310bp product).
Lane 17 displays 1 Kb+ Ladder (Invitrogen Cat# 10787-018).