08.05.10 MS
01.07.14 MLS

Genotyping Protocol: **MMRRC 244**

Assay Type: PCR to detect transgene positive animals (cannot distinguish hemizygous animals from homozygous 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 few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

Primer Information:
1) Name: M244 (Dcx) F Sequence: 5'- TTC ACAGGC AGC AGA TTG CAG C-3'
Genomic location: Chromosome X, bases 139177788 to 139177809 (-137 to -116 from the *Dcx* start codon)
2) Name: Gensat GFP Rev Sequence: 5'- TAG CGG CTG AAG CAC TGC A -3'
Binds to bases 206-224 of the GFP transgene

Assay Name: MMRRC 244 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
M244 (Dcx) F	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

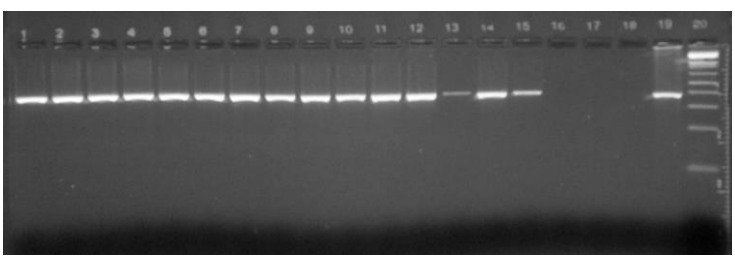
PCR Setup:
Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

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

Cycle Parameters:
1) 95°C 3 minutes
2) 94°C 30 seconds
3) 69°C 30 seconds
4) 72°C 30 seconds
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 10 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: 388 bp

Example of Gel:



Lanes 1-15 display positive samples (388bp band).
Lanes 16 and 17 are extraction and PCR blanks, respectively.
Lane 18 is a WT control (no product).
Lane 19 is a positive control (388bp band).
Lane 20 is 1Kb+ Ladder (Invitrogen Cat#10787-018).