08.05.10 MS

Genotyping Protocol: **MMRRC 249**

Assay Type: PCR (cannot distinguish heterozygous 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) M249 (Cck) F — Sequence: 5'- TAG GAA CTT CGC TTG GCT ACG G -3'
Genomic location: Chromosome 9, upstream of *Cck* start codon

2) Gensat GFP Rev — Sequence: 5'- TAG CGG CTG AAG CAC TGC A -3'
Within GFP transgene

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25 mM	3.2
M249 (Cck) 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)

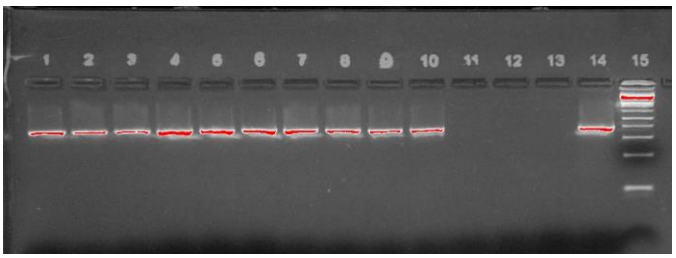
All reactions were performed in 200µl thin walled PCR tubes and were run in Applied Biosystems 2700 thermocycler.

Cycle Parameters:

1)	95°C	5 minutes
2)	94°C	30 seconds
3)	65°C	30 seconds
4)	72°C	30 seconds
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 at 100 volts for 30 minutes on a 3% agarose gel with ethidium bromide staining
Expected product: 374 bp

Example of Gel:



Lanes 1-10 are positive (374 bp band)
Lanes 11 and 12 are extraction and PCR blanks, respectively.
Lane 13 is a WT control.
Lane 14 is a positive control
Lane 15 is 1Kb+ Ladder (Invitrogen Cat# 10787-018)