09.02.10 MS

Genotyping Protocol: **MMRRC 10478**

Assay Type: PCR – cannot distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Sigma's Extract-N-Amp Tissue PCR Kit (Cat#XNAT2R). Kit directions for animal tissues were performed with a few minor modifications as follows: Use only 50 µl of Extraction Solution, 12.5 µl Tissue Preparation Solution and 50 µl of Neutralization Solution B.

Primer Information:
1) Name: OPTrtTA 3' Sequence: 5'-GTG GGA GAT CGA GCA GGC CCT CG-3'
2) Name: OPTrtTA 5' Sequence: 5'-CTG GGT TGC GTG TTG GAA GAT C-3'

Primer location: Primers bind to the rtTA cDNA insert.

Assay Name: OPTrtTA

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
OPTrtTA 3'	Sigma or IDT	25µM	0.3
OPTrtTA 5'	Sigma or IDT	25µM	0.3
sterile water			5.1

PCR Setup:

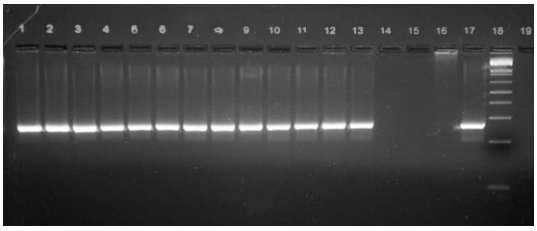
Final Reaction: 16µl master mix & 4µl DNA template (10-20 ng)

All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:
1) 94°C 3 minutes
2) 94°C 1 minute
3) 68°C 1 minute
4) 72°C 1 minute
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
Positive = 264 bp
Negative = no band

Example of Gel:



Lanes 1-13 display positive samples (264bp band).
Lanes 14 and 15 display extraction and PCR negative controls, respectively.
Lane 16 is a negative control (no band) and Lane 17 is a positive control (264bp band).
Lane 18 is 1Kb+ Ladder (Invitrogen Cat# 10787-018).