08.10.10 MS

Genotyping Protocol: **MMRRC 251**

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) Name: M251 (Penk1) F Sequence: 5'- AAC CTT GTC AGA GAC AGA ACG GGT-3'
   Genomic location:Chromosome 4
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 Line 251 PCR

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
M251 (Penk1) 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

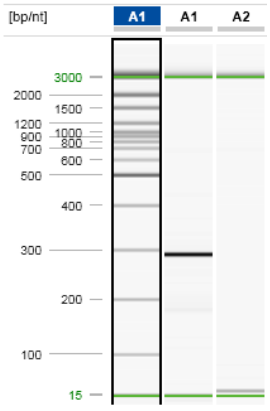
PCR Setup:
Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

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 1 minute
3) 63°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: 282 bp
Transgene + = 282 bp product
Transgene negative = no product

Example gel:



Lane A1 (highlighted in blue) displays a 15bp-3kb size marker
Lane A1 (not highlighted) displays a transgene positive sample (282bp product)
Lane A2 displays a transgene negative product (no product)

Please note: The 15bp and 3kb bands are reference markers specific to the Qiaxcel method and do not represent expected products.