08.23.10 MS
10.10.11 MS
01.06.14 MLS

Genotyping Protocol: **MMRRC 283**

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: elute in 200µl of AE buffer once.

Primer Information:
1) Name: M283 (Calb2) F Sequence: 5'- GCG CTG AGA GAG AGG CTT AAG AT -3'
Binds to Genomic location: Chromosome 8, bases 109465366 to 109465388 (+) (-40 to -18 of Calb2 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 line 283 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
M283 (Calb2) 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) 64°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 on a 3% agarose gel with ethidium bromide staining
Expected product: 264 bp

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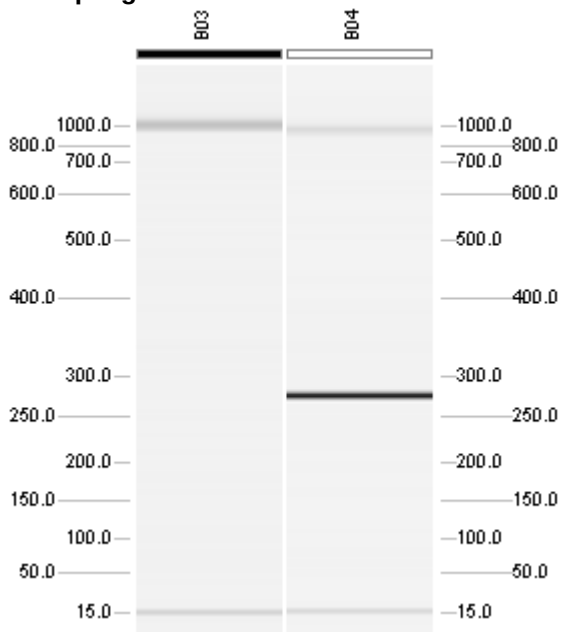
Example gel:



Lane B03 displays a negative sample (no product).
Lane B04 displays a positive sample (264bp product).

Please note: the 1000bp and 15bp bands are reference markers specific to the Qiaxcel method and do not represent expected products.