03.10.16 MS

Genotyping Protocol: **MMRRC 9963**

Strain Characteristics: Random integration of the LacZ transgene into the *Mitf* gene locus.

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: LacZ F 2-6-07 Sequence: 5'-GAA TCT CTA TCG TGC GGT GGT TGA-3'
2) Name: LacZ R 2-6-07 Sequence: 5'-GCC GTG GGT TTC AAT ATT GGC TTC-3'

Primer location: Both primers bind to the LacZ gene

Assay Name: LacZ PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.6
LacZ F 2-6-07	IDT	20µM	0.2
LacZ R 2-6-07	IDT	20µM	0.2
FastStart *Taq*	Roche (Cat#12032953001)	5 U/µl	0.2
sterile water			12.8

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

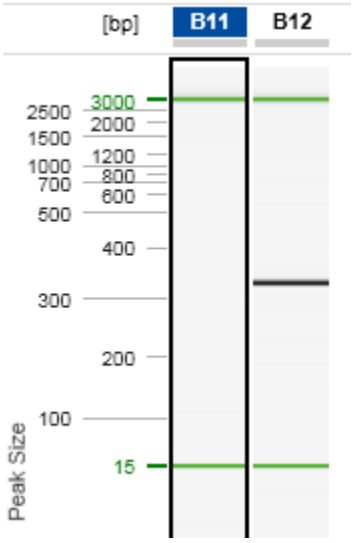
All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:
1) 94°C 5 minutes
2) 94°C 30 seconds
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 7minutes
7) 4°C hold until refrigerate product

Product Analysis:
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).
Alignment Marker: QX Alignment Marker 15bp/3Kb (Cat# 929522)
Size Marker: QX DNA Size Marker 100-3Kb (Cat# 929553)
Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

Expected Products:
Transgene positive: 341 bp
Transgene negative: no product

Example gel:



Lane B11 displays a transgene negative sample (no product)
Lane B12 displays a transgene positive sample (341bp product)

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