06.21.16 MLS

Genotyping Protocol: MMRRC 37479

Assay Type: PCR; can distinguish heterozygous and 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 200ul of AE buffer once.

Strain Description: The *Amfr* gene was knocked out via the insertion of a gene trap vector into Intron 1 of the gene.

Primer Information:
1) Name: M37479 F Sequence: 5'-CAT GCC ACA AGT ACT GGC TTC G -3'
2) Name: M37479 R Sequence: 5'-ACT GCC TTC TAT GAA ACT ATG C -3'
3) Name: M37479 mut R Sequence: 5'-ATA AAC CCT CTT GCA GTT GCA TC -3'

Primer Location: M37479 F and R are located in Intron 1 of the mouse *Amfr* gene. M37479 mut R is located in the inserted gene trap vector.

Assay Name: Amfr KO PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M37479 F	Sigma	25µM	0.3
M37479 R	Sigma	25µM	0.3
M37479 mut R	Sigma	25µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			12.7

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

All reactions were performed in 200 µl 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 30 seconds
3) 62°C 30 seconds
4) 72°C 30 seconds
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:
For analysis 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 100bp-2.5kb (Cat# 929559)
Method: AH320 Injection: 20s at 2kV
Separation: 320s at 6kV

Expected products:
WT: 369bp product
Heterozygous: 212bp and 369bp products

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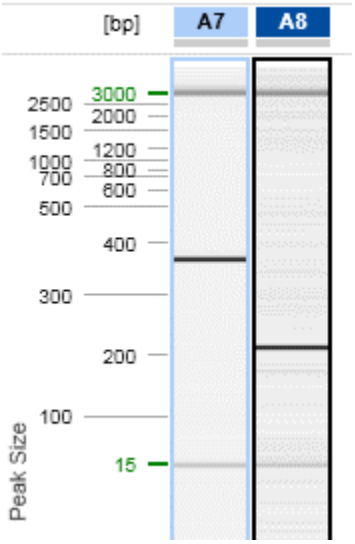
Homozygous mutant: 212bp products

Example gel:



Lane A7 displays a wild-type sample (369bp product)
Lane A8 displays a homozygous mutant sample (212bp product)

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