09.01.10 MS
09.27.13 MLS

Genotyping Protocol: **MMRRC 406**

Assay Type: PCR - can 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: m406 F Sequence: 5'-GCT TTC CCT GCT CCT GGT TCA TTT-3'
2) Name: m406 Rwt Sequence: 5'-TTC CCA CTT CTT GAC CCT GTC GTT-3'
2) Name: m406 Rneo Sequence: 5'-ACC TTG CTC CTG CCG AGA AAG TAT-3'

Primer location: m406 F & Rwt: *Mus musculus* Delta-Sarcoglycan gene
Primer location: m406 Rneo: Synthetic construct neomycin resistance gene

Assay Name: Delta-Sarcoglycan KO 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	3.2
M406 F	IDT	25µM	0.3
M406 Rwt	IDT	25µM	0.3
M406 Rneo	IDT	25µM	0.3
FastStart *Taq*	Roche (Cat#12032953001)	5 U/µl	0.2
sterile water			12.7

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 30 seconds
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:
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-3kb (Cat# 929553)
Method: AH320 Injection: 20s at 2kV
Separation: 320s at 6kV

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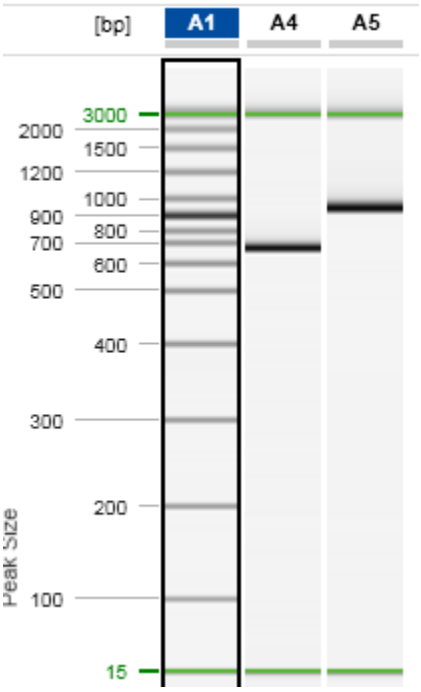
Expected Products:
Wildtype = ~600 bp
Homozygous mutant = ~800 bp
Heterozygous = ~600 bp AND ~800 bp

Example Gel:



Lane A1 displays a 15bp-3kb size marker
Lane A4 displays a wild type sample (600bp product)
Lane A5 displays a homozygous sample (800bp product)

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