

Genotyping Protocol: **MMRRC line 406**

Assay Type: PCR - can distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy kit. 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: GCT TTC CCT GCT CCT GGT TCA TTT
2) Name: m406 Rwt Sequence: TTC CCA CTT CTT GAC CCT GTC GTT
2) Name: m406 Rneo Sequence: ACC TTG CTC CTG CCG AGA AAG TAT

Primer location: m406 F & Rwt: Mus musculus Delta-Sarcoglycan gene

Primer location: m406 Rneo: Synthetic construct neomycin resistance gene

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
buffer	Roche	10X	2
dNTP	Roche	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	5 U/µl	0.2
sterile water			12.7

PCR Setup:

Final Reaction: 19µl master mix & 1µl DNA template

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:

All products were analyzed on a 3% agarose gel with ethidium bromide staining

Wildtype = ~600 bp

Positive = ~800 bp

Heterozygous = ~600 bp AND ~800 bp