07.26.13 MLS

Genotyping Protocol: **MMRRC 29874**

Assay Type: PCR - cannot distinguish heterozygous animals from homozygous animals. Can distinguish transgene positive animals from transgene negative animals.

DNA Extraction: DNA from tail snips was extracted using Sigma's RedExtract-N-Amp Tissue PCR Kit (Cat# XNAT2R). Kit directions for animal tissues were performed with a few minor modifications as follows: Use only 50 µl of Extraction Solution, 12.5 µl Tissue Preparation Solution and 50 µl of Neutralization Solution B.

Mutation Information: This is a transgenic line carrying a dominant negative cystolic domain of synaptobrevin 2 (VAMP2) driven by a tetO promoter. Details can be found in Pascual et al (2005) Science. 310(5745):113-6.

Primer Information:
1) Name: M29874 F Sequence: 5'-TAC CAG TAA CAG GAG ACT GC-3'
2) Name: M29874 R Sequence: 5'-GAT TAT GAT CCC TCA GAG GTC-3'

Primer Location: The forward primer is located in VAMP2. The reverse primer is located in the transthyretin (*Ttr*) gene.

Assay Name: VAMP2 PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat# XNAT2R)	2X	10
M29874 F	Sigma	25µM	0.3
M29874 R	Sigma	25µM	0.3
sterile water			5.4

PCR Setup:
Final Reaction: 16µl master mix & 4µl DNA template (10-20ng DNA)

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

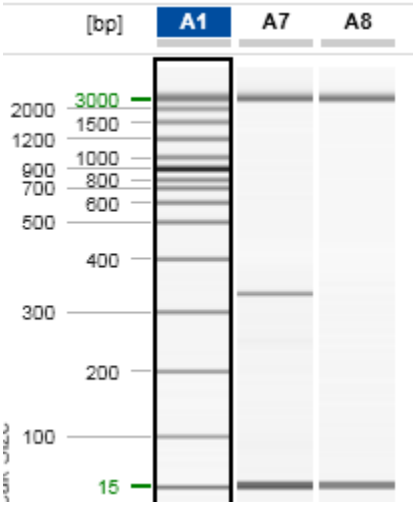
Cycle Parameters:
1) 94^{o}C 3 minutes
2) 94^{o}C 1 minute
3) 66^{o}C 1 minute
4) 72^{o}C 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72^{o}C 10 minutes
7) 4^{o}C hold until refrigerate product

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 = 300 bp
Transgene negative = no band

Control DNA: positive and negative animals

Example of Gel:



Lane A1 displays a 15bp-3kb size marker.
Lane A7 displays a transgene positive sample (300bp product).
Lane A8 displays a transgene negative sample (no product).

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