09.01.10 MS
04.18.13 MLS

Genotyping Protocol: **MMRRC 396-398**

Assay Type: PCR- cannot distinguish heterozygous animals from homozygous animals.

Strain Characteristics: This strain carries the Atrophin-1 Transgene, which consists of the full-length cDNA's of atrophin-1, encoding 26 and 65 consecutive glutamines, driven by the mouse prion protein promoter. Details can be found in Schilling et al (1999) Neuron 24:275-286.

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.

Primer Information:
1) Name: PrP-SenseJ Sequence: 5'-CCT CTT TGT GAC TAT GTG GAC TGA TGT CGG-3'
2) Name: PrP-Anti-SenseJ Sequence: 5'-GTG GAT ACC CCC TCC CCC AGC CTA GAC C-3'
3) Name: AT-3818-5' Sequence: 5'-AGG TGG GGA GGT GGC GAG GAT-3'

The PrP primers will detect endogenous PrP sequences and serve as a control. The AT-3818-5' and PrP-Anti-SenseJ primers will detect the transgene.

Assay Name: Atrophin-1 PCR

PCR Master Mix Components:
Run separate reaction for Transgene and WT gene:

Master Mix for WT gene:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25 mM	3.2
PrP-SenseJ	Sigma Genosys	25 µM	0.3
PrP-Anti-SenseJ	Sigma Genosys	25 µM	0.3
FastStart *Taq*	Roche (Cat#12032953001)	5 U/µl	0.2
Sterile Water			13

Master Mix for Tg gene:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25 mM	3.2
AT-3818-5'	Sigma Genosys	25 µM	0.3
PrP-Anti-SenseJ	Sigma Genosys	25 µM	0.3
FastStart *Taq*	Roche (Cat#12032953001)	5 U/µl	0.2
Sterile Water			13

PCR Setup:
Final Reaction – for both WT and Tg reactions: 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 (for both WT and Tg reactions):

1) 94°C 3 minutes
2) 94°C 30 sec
3) 70°C 30 sec
4) 72°C 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 10minutes
7) 4°C hold until refrigerate product

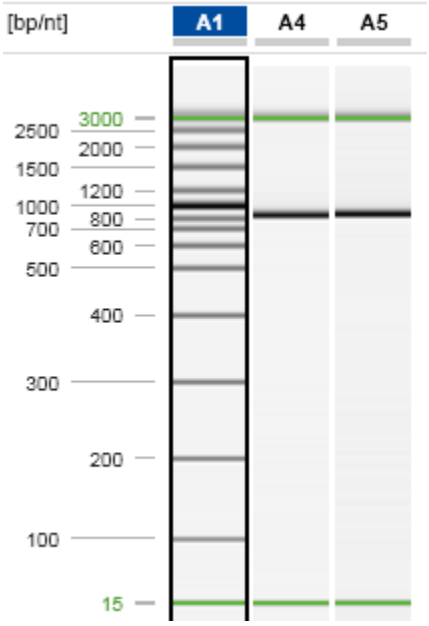
Product Analysis:
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

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

Wild type (WT) gene product: 700 bp
Transgene (Tg) gene product: 450 bp
*All samples will have a 700bp WT band. Transgene positive animals will have the 450bp Transgene positive band as well as the 700bp WT band.

Example gels:

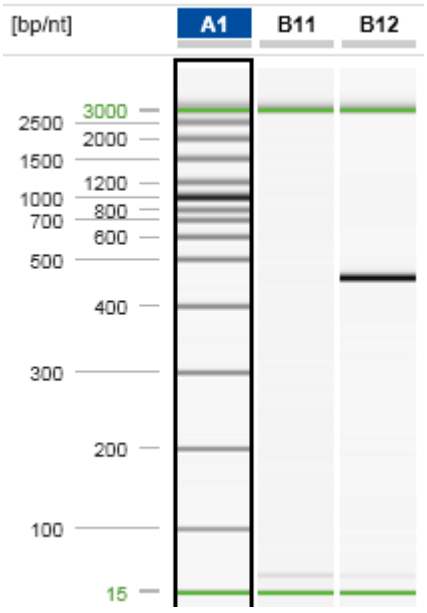
WT Gel:



Lane A1 displays a 15bp-3kb size marker. Lane A4 is a transgene negative sample and Lane A5 is a transgene positive sample. Both samples display the 700bp WT gene product.

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

Tg Gel:



Lane A1 displays a 15bp-3kb size marker. Lane B11 displays a transgene negative sample (no product). Lane B12 displays a transgene positive sample (450bp product).

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