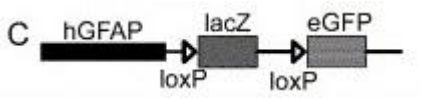
04.23.13 MLS

Genotyping Protocol: **MMRRC 16993**

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

DNA Extraction: DNA from tail snips was extracted using Sigma's Extract-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.

Strain Description: This strain carries a transgene with the human GFAP promoter, a floxed *lac*Z gene, and the Enhanced Green Fluorescent Protein. Details can be found in Casper, KB and McCarthy,KD (2006) Molecular and Cellular Neuroscience 31(4):676-84.



Primer Information:
1) Name: GFP F Sequence: 5'-CGC ACC ATC TTC TTC AAG GAC GAC-3'
2) Name: GFP R Sequence: 5'-AAC TCC AGC AGG ACC ATG TGA TCG-3'

Primer location: GFP F and R both bind to the GFP sequence in the transgene

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
GFP F	Sigma	25µM	0.3
GFP R	Sigma	25µM	0.3
sterile water			5.1

PCR Setup:

Final Reaction: 16µl master mix & 4µl 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 3 minutes
2) 94°C 30 seconds
3) 60°C 30 seconds
4) 72°C 1 minute
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

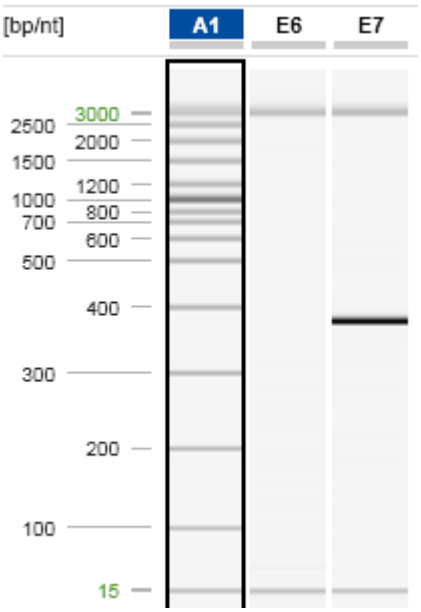
Product Analysis:

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

Transgene positive = 375 bp
Transgene negative = no band

Control DNA: positive and negative animals

Example Gel:



Lane A1 displays 15bp-3kb size marker.
Lane E6 displays a transgene negative sample (no product).
Lane E7 displays a transgene positive sample (375bp product).

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