09.03.14 MLS

Genotyping Protocol: **MMRRC 16990**

Assay Type: PCR - can not distinguish hemizygous animals from homozygous animals; Can distinguish transgene negative from transgene positive 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 in which tTA is driven by the human glial fibrillary acidic protein (*hGFAP)* promoter. Details can be found in Pascual et al.(2005) Science. 310(5745):113-6.

Primer Information:
1) Name: tTA F Sequence:5'-CCC TTG GAA TTG ACG AGT ACG GTG-3'
2) Name: MP1 R Sequence: 5'-TGG TGT ATG AGC GGC GGC GAC GGC AG-3'

Primer location: Primer tTA binds to the tetracycline transactivator and MP1 binds to the mouse Protamine 1 gene.

Assay Test Name: GFAP-tTA

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
tTA F	Sigma-Genosys	25µM	0.3
MP1 R	Sigma-Genosys	25µM	0.3
sterile water			5.1

PCR Setup:

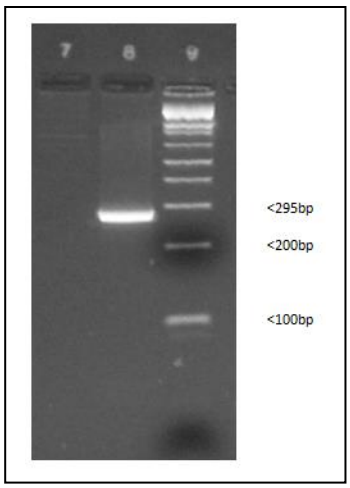
Final Reaction: 16µl master mix & 4µl DNA template (10-20 ng/µl)

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 1 minute
3) 64°C 1 minute
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 a 3% agarose gel with ethidium bromide staining
Transgene positive = 295bp product
Transgene negative = no product

Example Gel:



Lane 7 displays a transgene negative animal (no product)
Lane 8 displays a transgene positive sample (295bp product)
Lane 9 displays 1kb+ DNA Ladder (Invitrogen Cat#10787-018)