08.05.10 MS
02.17.14 MLS
05.20.16 MLS

Genotyping Protocol: **MMRRC 43**

Strain Characteristics: Random integration of the Green Fluorescent Protein gene under an opsin promoter.

Assay Type: PCR - can not distinguish heterozygous animals from homozygous animals. Can distinguish hemizygous animals from WT animals.

DNA Extraction: DNA from tail snips was extracted using Sigma's Extract-N-Amp Tissue PCR Kit (Cat#XNAT2R). Kit directions for fresh or frozen tails were performed with a few minor modifications as follows: use 50 µl of Extraction Solution and 12.5 µl of Tissue Preparation Solution and 50 µl of Neutralization Solution B.

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 GFP R are both located in the GFP transgene.

Assay Name: GFP PCR

PCR Master Mix Components:

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

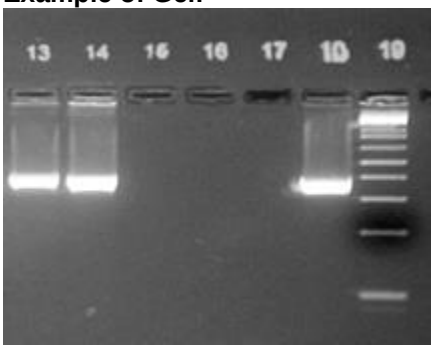
PCR Setup:
Final Reaction: 16µl master mix & 4µl DNA template

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 3 minutes
2) 94°C 30 seconds
3) 61°C 30 seconds
4) 72°C 30 seconds
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
Expected product: 375 bp

Example of Gel:



Lanes 13, 14 and 18 display positive samples.

Lanes 15, 16, 17 display negative samples.

Lane 19 is 1Kb+ Ladder (Invitrogen Cat#10787-018)