

MU/H Genotyping Protocol: **MMRRC lines carrying GFP**

Strain Characteristics: Random integration of the Green Fluorescent Protein gene.

Assay Type: PCR - can not distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy kit. Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

Primer Information:

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

Primer location: GFP F = GFP transgene
 GFP R = GFP transgene

PCR Master Mix Components:

Master Mix:

component	manufacturer	concentration	µl/rxn
buffer	Roche	10X	2
dNTP	Roche	1.25mM	3.2
GFP F	IDT	25µM	0.3
GFP R	IDT	25µM	0.3
FastStart taq	Roche	5 U/µl	0.2
sterile water			13

PCR Setup:

Final Reaction: 19µl master mix & 1µl 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 5 minutes
2) 94°C 30 seconds
3) 61°C 30 seconds
4) 72°C 45 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