Genotyping Protocol: **GFP**

Genotyping Assay #1:

Assay Type: PCR

Assay Notes: This assay detects carriers of the GFP gene but does not distinguish homozygous from hemizygous animals

DNA Extraction: DNA from tail snips is 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: PL EGFP-F Sequence: CGC ACC ATC TTC TTC AAG GAC GAC
2) Name: PL EGFP-R Sequence: AAC TCC AGC AGG ACC ATG TGA TCG

Primer location: Internal to GFP gene

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
buffer	Roche	10X	2
dNTP	Roche	1.25mM	3.2
PL EGFP-F	IDT	20µM	1
PL EGFP-R	IDT	20µM	1
taq	Roche	5 U/µl	0.2
sterile water			11.6

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 2 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
GFP gene: ~370 bp product

Genotyping/Phenotyping Assay #2:

Assay Type: Fluorescence with UV light microscope

Assay Notes: Tail snips are collected and then visualized under UV fluorescent microscope with appropriate filter to detect GFP fluorescence. Negative samples from mice will have very weak low level to nonexistent auto-fluorescence whereas positive animals have very strong fluorescence when examining tail snips from 3 week old mice.