

## Genotyping Protocol: MMRRC Line 159

**Assay Type:** PCR - can distinguish heterozygous animals from homozygous animals. This assay only detects the mahogany allele and wild type attractin gene ( $A^{trn^{mg}}$ ,  $A^{trn}$ ). For detection of the yellow allele of the nonagouti gene ( $A^y$ ) visual inspection of the coat color is performed- animals that are  $A^y/a$  have a yellow haircoat if the animals are not homozygous for the mahogany allele and have a deep brown mahogany coat color if they are also homozygous for the mahogany allele,  $a/a$  animals are black.

**DNA Extraction:** DNA from tail snips was extracted using Sigma's RedExtract-N-Amp Tissue PCR Kit. Kit directions for animal tissues were performed with a few minor modifications as follows: Use only 50  $\mu$ l of Extraction Solution, 12.5  $\mu$ l Tissue Preparation Solution and 50  $\mu$ l of Neutralization Solution B.

### Primer Information:

- |                   |   |
|-------------------|---|
| 1) Name: Mg WT F  | Sequence: TTG GGA CCT TGA GGA TTG TTC CCT |
| 2) Name: Mg WT R  | Sequence: TCA GTT TAT GCC AGG CAA GTG CTC |
| 3) Name: Mg Mut R | Sequence: AAG CAC TGC AAT GGA AAG GCT CTG |

Primer location:	Mg WT F & R:	Intron 26 in the mouse Attractin Gene
Primer location:	Mg Mut R:	IAP element insertion in Intron 26

### PCR Master Mix Components:

component	manufacturer	concentration	$\mu$ l/rxn
REDEExtract-N-Amp PCR Reaction Mix	Sigma	2X	10
Mg WT F	IDT	25 $\mu$ M	0.3
Mg WT R	IDT	25 $\mu$ M	0.3
Mg Mut R	IDT	25 $\mu$ M	0.3
sterile water			5.1

### PCR Setup:

Final Reaction: 16 $\mu$ l master mix & 4 $\mu$ l DNA template

All reactions were performed in 200 $\mu$ l 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 20 seconds
- 3) 68°C 20 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

Mg WT allele = 450 bp

Mg Mut allele = 907 bp

Heterozygous = 450 bp AND 907 bp