

H/M Genotyping Protocol: MMRRC Line 121

Strain Characteristics: Overexpression of Nerve Growth Factor (NGF) under MHC promoter, maintained heterozygous

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: mmrrc121F Sequence: AGC GCA TCG GTG AGT CAG G
2) Name: mmrrc121R Sequence: TGT GTC GAA CTT GCT GTA GGT CTG

Primer location: mmrrc121F = exon 1 of rat Nerve Growth Factor gene
 mmrrc121R = 3' UTR of Human Growth Hormone gene

PCR Master Mix Components:

Master Mix:

component	manufacturer	concentration	µl/rxn
buffer	Roche	10X	2
dNTP	Roche	1.25mM	3.2
mBActin 2F	IDT	25µM	0.3
mBActin 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) 62°C 1 minute
- 4) 72°C 1 minute
- 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: 1.1 Kb