

08.05.10 MS
10.13.14 MLS

Genotyping Protocol: **MMRRC 121**

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

Assay Type: PCR - cannot distinguish hemizygous animals from homozygous animals; can distinguish hemizygous animals from wild-type animals.

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat#69506). 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: HF 37 Sequence: 5'-AGC GCA TCG GTG AGT CAG G-3'
2) Name: HF 47 Sequence: 5'-TGT GTC GAA CTT GCT GTA GGT CTG-3'

Primer location: HF 37 = exon 1 of rat Nerve Growth Factor gene
 HF 47 = 3' UTR of Human Growth Hormone gene

Assay name: NGF Transgene PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
HF 37	IDT	25µM	0.3
HF 47	IDT	25µM	0.3
FastStart <i>Taq</i>	Roche (Cat#12032953001)	5 U/µl	0.2
sterile water			13

PCR Setup:

Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

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) 60°C 30 seconds
4) 72°C 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 7 minutes
7) 4°C hold until refrigerate product

Product Analysis:

For analysis on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004):

Alignment Marker: QX Alignment Marker 15bp/3kb (Cat# 929522)

Size Marker: QX DNA Size Marker 100bp-3kb (Cat# 929553)

Method: AH320 Injection: 20s at 2kV

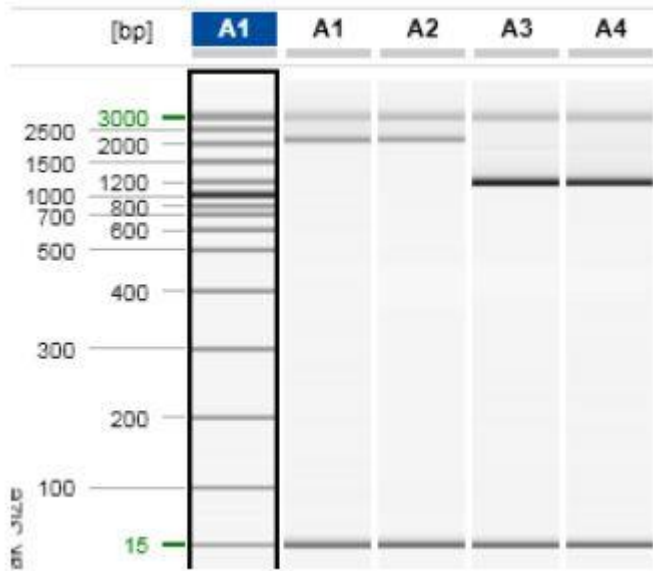
Separation: 320s at 6kV

Expected products:

Transgene positive: 1.1kb product

Transgene negative: a ~2.2kb nonspecific band may be observed in WT samples

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Lane A1 (blue): 15bp-3kb size marker
Lanes A1 (white) and A2: Transgene negative samples (displays the 2.2kb nonspecific band)
Lanes A3 and A4: Transgene positive samples (1.1kb product)

Please note: the 15bp and 3kb bands are reference markers specific to the QIAxcel method and do not represent expected products.