

08.04.10 MS
08.03.16 MLS

Genotyping Protocol: **MMRRC 31750**

Assay Type: PCR- cannot distinguish heterozygous animals from homozygous 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.

Strain Description: This strain carries a transgene, which contains a Cre recombinase gene driven by a rat *Nestin* promoter.

Primer Information:

- 1) Name: rNes1.F Sequence: 5'-CCT TCT CTA GTG CTC CAC GTC-3'
- 2) Name: Cre.R Sequence: 5'-GCA AAC GGA CAG AAG CAT TT-3'

Primer location: rNes1.F is located in the promoter region of rat *Nestin*. Cre.R is located in the Cre gene.

Assay name: Nes-Cre PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
rNes1.F	Sigma	25µM	0.3
Cre.R	Sigma	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) 95°C 3 minutes
- 2) 94°C 1 minute
- 3) 64°C 1 minute
- 4) 72°C 1 minute
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 10 minutes
- 7) 4°C hold until refrigerate product

Product Analysis:

All products were analyzed 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 100-3Kb (Cat# 929553)

Method: AM320 Injection: 10s at 5KV

Separation: 320s at 6KV

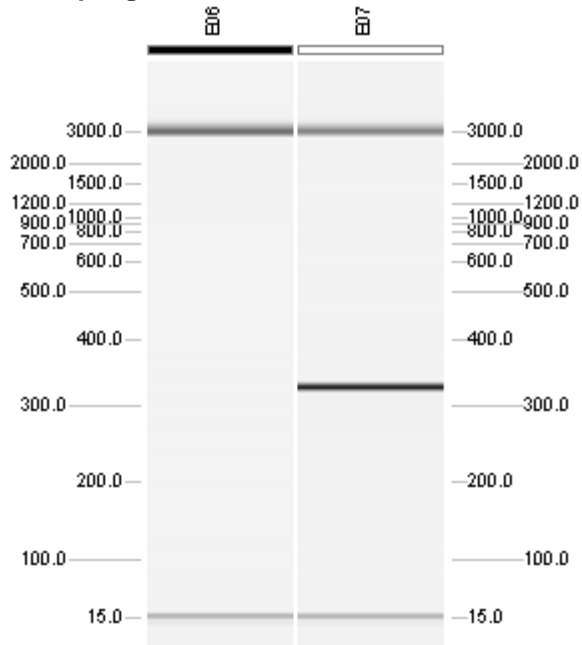
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Expected product:

Positive: 320bp

Negative: no product

Example gel:



Lane E06 displays a transgene negative sample (no product).
Lane E07 displays a transgene positive sample (320bp product).

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