

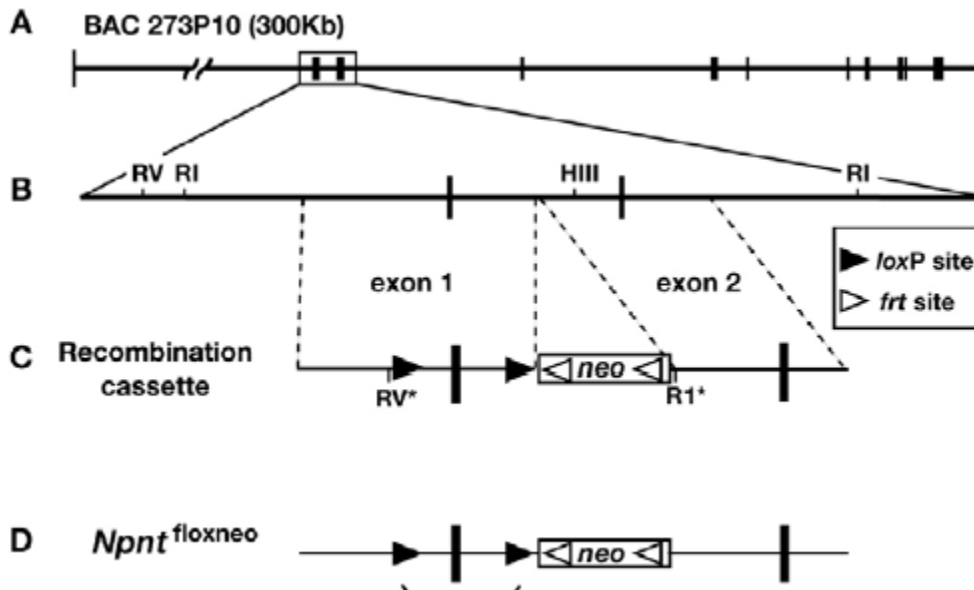
03.02.10 MS
 07.30.10 HB updated
 01.27.11 ECB

Genotyping Protocol: **MMRRC 30111**

Assay Type: PCR- can 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 200ul of AE buffer once.

Strain Description: This strain carries a floxed allele of the nephronectin (*Npnt*) gene. Exon 1 of the *Npnt* gene is surrounded by *loxP* sites, and there is a Neo cassette inserted into the intron between exons 1 and 2. Details can be found in Linton et al (2007) Development 134:2501-9.



Primer Information:

- 1) Name: M30111 NN1-A Sequence: 5'-CAG TCC ATC CTG ATC ACT ACT GGC TGT A-3'
 2) Name: M30111 NN1-C Sequence: 5'-GCA ACC TTC AGC GTC CC-3'

Primer location: M30111 NN1-A is located in the intron between exons 1 and 2, and M30111 NN1-C is located in exon 1 of the *Npnt* gene on Chromosome 3.

Assay Name: *Npnt* floxed PCR

PCR Master Mix Components:

component	manufacturer	concentration	μl/rxn
10X buffer (with MgCl ₂)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M30111 NN1-A	Sigma	25μM	0.3
M30111 NN1-C	Sigma	25μM	0.3
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/μl	0.2
sterile water			13

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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 30 seconds
- 3) 61°C 30 seconds
- 4) 72°C 30 seconds
- 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:

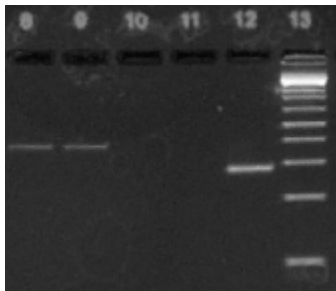
All products were analyzed on a 3% agarose gel with ethidium bromide staining.

Expected products:

WT: 279bp

Mutant: 350bp

Example gel:



Lanes 8 and 9 display homozygous samples (350bp band only).
Lanes 10 and 11 are blanks.
Lane 12 is a WT control (279bp band only).
Lane 13 is 1 Kb+ Ladder (Invitrogen Cat# 10787-018).