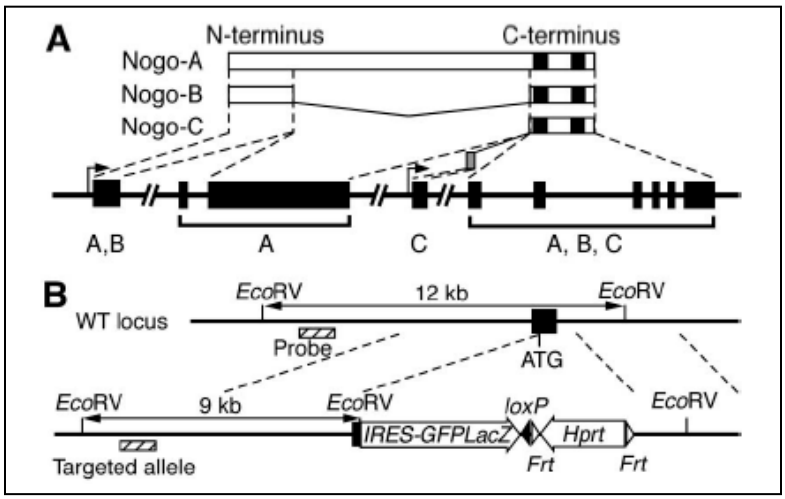
10.06.08 MS
07.30.10 HB updated

Genotyping Protocol: **MMRRC 30397**

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 200µl of AE buffer once.

Strain Description: This strain has IRES-GFPLacZ inserted into exon 1 of the reticulon 4 (*Rtn4*) gene on Chromosome 11, resulting in the deletion of exon 1 downstream of the ATG start codon. This abolishes *Nogo-A* and *Nogo-B* expression. Details can be found in Zheng et al (2003) Neuron 38:213-24.



Primer Information:

1) Name: M30399 NNGenF2 Sequence: 5'-CAG TAG CTG CAG CAT CAT CG-3'
2) Name: M30399 NNGenR3 Sequence: 5'-CTC TCC AGC ACC TCC AAT TC-3'
3) Name: M30399 IRES200R Sequence: 5'-AGA GGA ACT GCT TCC TTC AC-3'

Primer location: M30399 NNGenF2 and NNGenR3 are located in exon 1 of the *Rtn4* gene; M30399 IRES200R is located in the inserted IRES-GFPLacZ.

Assay name: Nogo Deletion (B1) PCR

Mutant 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
M30399 NNGenF2	Sigma	25µM	0.3
M30399 IRES200R	Sigma	25µM	0.3
FastStart *Taq*	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.

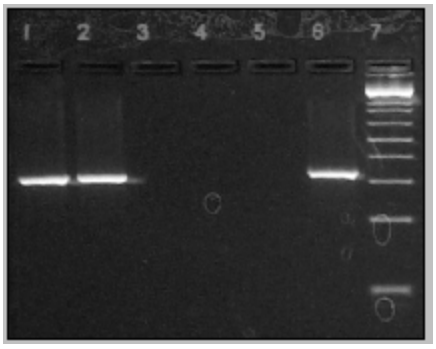
Cycle Parameters:

1)	95°C	5 minutes
2)	94°C	1 minute
3)	68°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 a 3% agarose gel with ethidium bromide staining.

Expected products: 350bp mutant band

Example gel:



<350bp

Wells 1 and 2 are positive for the mutant allele. Well 3 and 4 are blanks, and Well 5 is a wild-type control, and well 6 is a heterozygous control. Well 7 is 1Kb+ Ladder (Invitrogen Cat# 10787-018).

WT 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
M30399 NNGenF2	Sigma	25µM	0.3
M30399 NNGenR3	Sigma	25µM	0.3
FastStart *Taq*	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.

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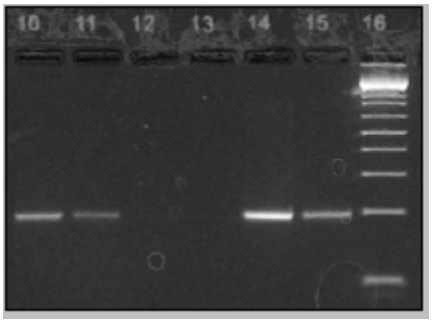
Cycle Parameters:

1)	95°C	5 minutes
2)	94°C	1 minute
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	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: 200bp wild type band

Example gel:



<200bp

Wells 10 and 11 are positive for the WT allele. Wells 12 and 13 are blanks; Well 14 is a WT control, and Well 15 is a heterozygous control. Well 16 is 1Kb+ Ladder (Invitrogen Cat# 10787-018).

Product Analysis:
Wild Type: 200bp on WT gel, no product on Mut gel
Heterozygous: 200bp on WT gel, 350bp on Mut gel
Homozygous: no product on WT gel, 350bp on Mut gel