

Genotyping Protocol: MMRRC 30747

Laboratory note: M30747 has 2 separate tests: Robo1 PCR and Robo2 PCR

Assay Type: PCR- can distinguish heterozygous animals from homozygous animals

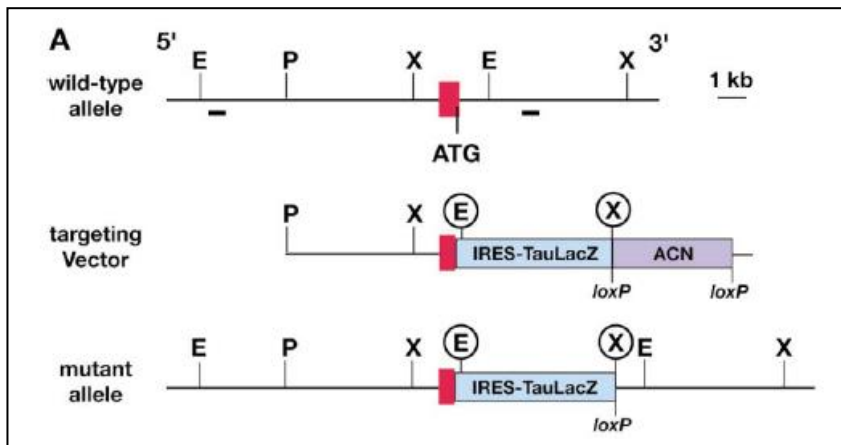
DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat# 69506). Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200ul of AE buffer once.

Strain Description: This strain carries two mutations:

Robo1: There is a TM-beta-geo-IRES-AP insertion in exon 3 of the roundabout homolog 1 gene (*Robo1*), which is on Chromosome 16. Details can be found in Long et al (2004) Neuron 42:213-23.

Robo2: There is IRES-TauLacZ-loxPNeomycin inserted into exon 1 of the roundabout homolog 2 gene (*Robo2*), which is on Chromosome 16. Details can be found in Grieshammer et al (2004) Dev Cell 6:709-17.

Robo2:



Robo1 PCR:

Primer Information:

- | | |
|-------------------------|--|
| 1) Name: M30403 robo1-1 | Sequence: 5'-TGG CAC GAA GGT ATA TGT GC-3' |
| 2) Name: M30403 robo1-2 | Sequence: 5'-GAA GGA CTG GTG GTT TTG AG-3' |
| 3) Name: M30403 robo1-3 | Sequence: 5'-CCT CCG CAA ACT CCT ATT TC-3' |

Primer Location: M30403 robo1-1 and robo1-2 are located in the *Robo1* gene on Chromosome 16. M30403 robo1-3 is located in the insertion.

Assay name: Robo1 PCR

PCR Master Mix Components:

component	manufacturer	concentration	$\mu\text{l}/\text{rxn}$
Buffer with MgCl_2 (green cap)	Roche	10X	2
dNTPs	Promega (cat # U1515)	1.25mM	3.2
M30403 robo1-1	Sigma	25 μM	0.3
M30403 robo1-2	Sigma	25 μM	0.3
M30403 robo1-3	Sigma	25 μM	0.3
FastStart <i>Taq</i>	Roche (cat # 12032953001)	5 U/ μl	0.2
sterile water			12.7

PCR Setup:

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

All reactions were performed in 200 μl 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) 66°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:

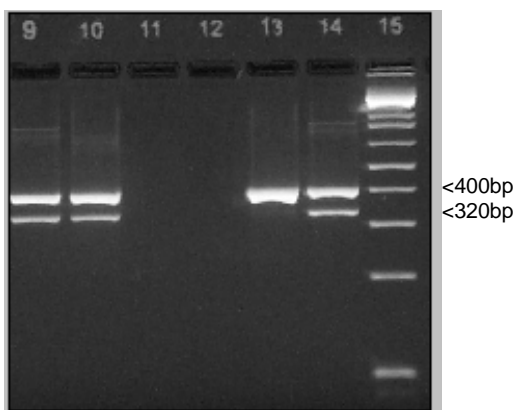
All products were analyzed on a 3% agarose gel with ethidium bromide staining.

Heterozygous: 400bp, 320bp

Homozygous mutant : 320bp

Wild Type: 400bp

Example gel:



Wells 9 and 10 are heterozygous. Wells 11 and 12 are blanks. Well 13 is a WT control, and Well 14 is a heterozygous control. Well 15 is 1Kb+ Ladder (Invitrogen Cat# 10787-018).

MS 9/09
ECB 2/22/11
02.19.14 MLS

Robo2 PCR:

Primer Information:

- | | |
|-------------------------|---|
| 1) Name: M30409 robo2 1 | Sequence: 5'-AAG TGC AAC GTC TCT GAA GTC CC-3' |
| 2) Name: M30409 robo2 2 | Sequence: 5'-GGC GGA ATT CTT AAT TAA GGC GCG-3' |
| 3) Name: M30409 robo2 3 | Sequence: 5'-TTC TTT AGA AGG CAC AAC AAT CTC AGA G-3' |

Primer Location: M30409 robo2 1 and robo2 3 are located within the *Robo2* gene on Chromosome 16. M30409 robo2 2 is located in the IRES-TauLacZ-loxpNeomycin.

Assay name: Robo2 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
M30409 robo2 1	Sigma	25µM	0.3
M30409 robo2 2	Sigma	25µM	0.3
M30409 robo2 3	Sigma	25µM	0.3
FastStart <i>Taq</i>	Roche (cat # 12032953001)	5 U/µl	0.2
sterile water			12.7

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) | 63.5°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.

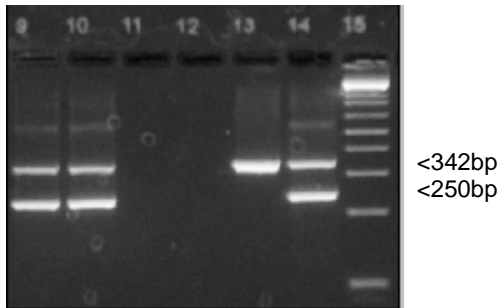
Heterozygous: 250bp, 342bp

Homozygous mutant: 250bp

Wild Type: 342bp

MS 9/09
ECB 2/22/11
02.19.14 MLS

Example gel:



Wells 9 and 10 are heterozygous. Wells 11 and 12 are blanks. Well 13 is a WT control and Well 14 is a heterozygous control. Well 15 is 1Kb+ Ladder (Invitrogen Cat# 10787-018).