

## Genotyping Protocol: **MMRRC 30409**

**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 has 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.

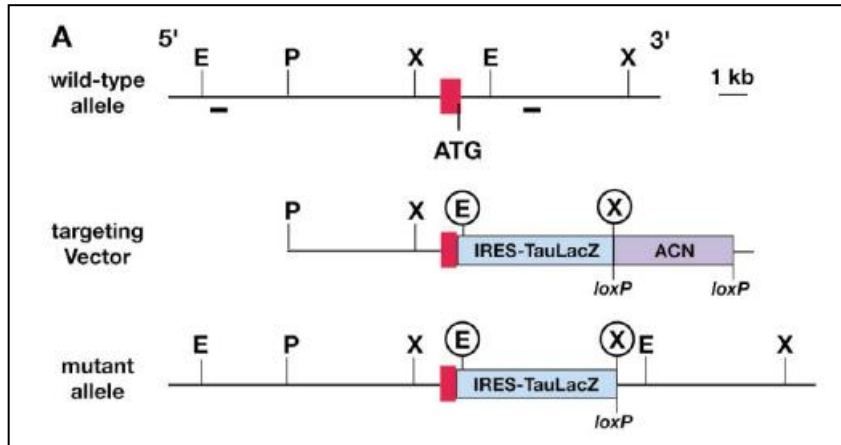


Image from Grieshammer et al (2004) Dev Cell 6:709-17.

**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 <sub>2</sub> (green cap)	Roche	10X	2
dNTP	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)

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

All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

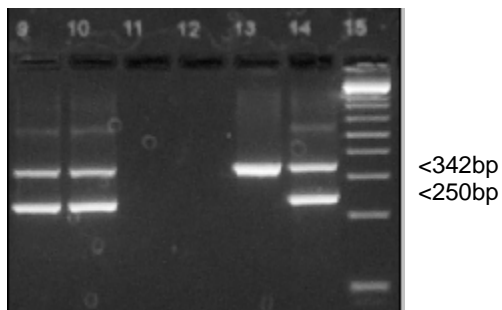
**Cycle Parameters:**

- 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: 250bp  
Wild Type: 342bp

**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).