

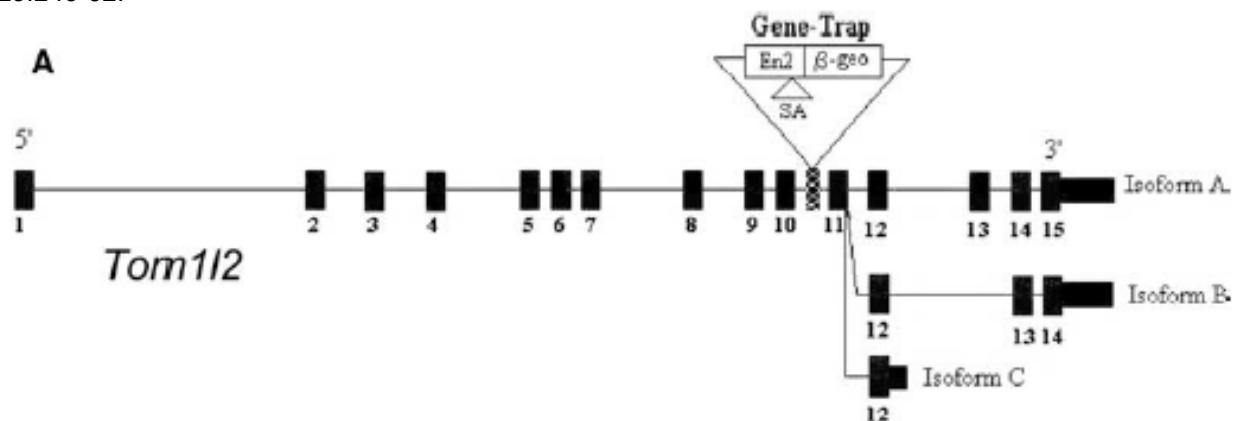
02.24.10 MS
08.02.10 HB updated
03.03.14 MLS

Genotyping Protocol: **MMRRC 30620**

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 Characteristics: This strain has the gene-trapping vector (pGT1lxf) inserted between exons 10 and 11 in the Target of Myb-1-like 2 gene (*Tom1l2*) on Chromosome 11. Details can be found in Girirajan et al (2008) Mamm Genome 19:246-62.



Primer Information:

- | | |
|--------------------------|---|
| 1) Name: M30620 Tom1l2 F | Sequence: 5'-AAA ACC ATC TTG TGG CTT GC-3' |
| 2) Name: M30620 Tom1l2 R | Sequence: 5'-TGA CGC TTT ACC TTA TGC TCA G-3' |
| 3) Name: M30620 Insert F | Sequence: 5'-GAC GTC TCG TTG CTG CAT AA-3' |
| 4) Name: M30620 Insert R | Sequence: 5'-GAC CTG ACC ATG CAG AGG AT-3' |

Primer location: M30620 Tom1l2 F and R are located between exons 11 and 12 of the *Tom1l2* gene on Chromosome 11. M30620 Insert F and R are located on the inserted genetrap.

Assay name: Tom1l2 PCR

PCR Master Mix Components:

M30620 WT:

component	manufacturer	concentration	μ l/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M30620 Tom1l2 F	Sigma	25 μ M	0.3
M30620 Tom1l2 R	Sigma	25 μ M	0.3
FastStart Taq	Roche (Cat# 12032953001)	5 U/ μ l	0.2
sterile water			13

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M30620 Mut:

component	manufacturer	concentration	μl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M30620 Insert F	Sigma	25μM	0.3
M30620 Insert R	Sigma	25μM	0.3
FastStart Tag	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:

WT PCR:

- 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

Mut PCR:

- 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:

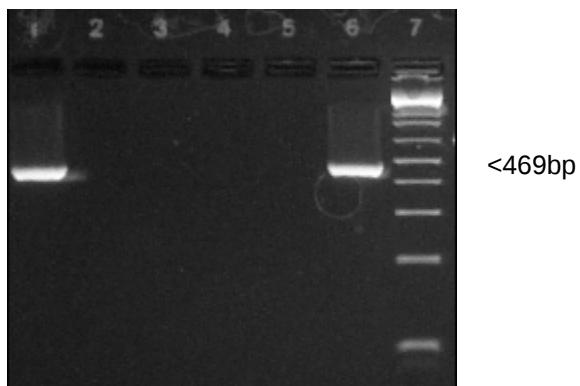
WT: 651bp
 Mutant: 469bp

<u>Genotype</u>	<u>Mut PCR</u>	<u>WT PCR</u>
WT	No product	651bp
Heterozygous	469bp	651bp
Homozygous	469bp	No product

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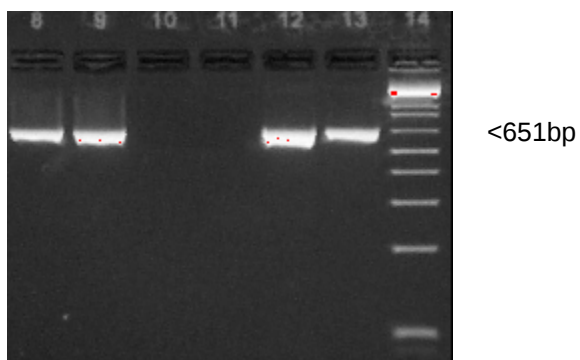
Example gels:

Mut Gel:



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

WT gel:



Wells 8 and 9 are positive for the WT allele. Wells 10 and 11 are blanks. Well 12 is a WT control and Well 13 is a heterozygous control. Well 14 is 1Kb+ Ladder (Invitrogen Cat# 10787-018).