

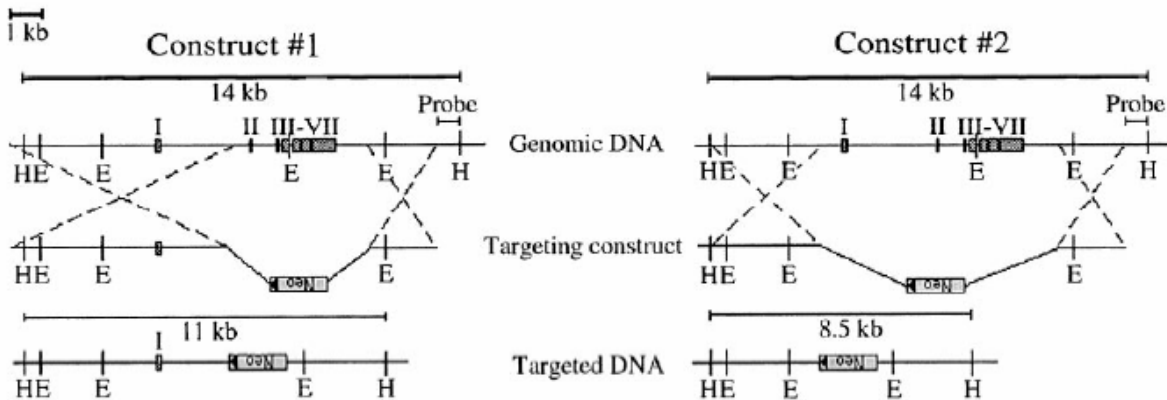
02.04.10 MS
 07.30.10 HB updated
 02.28.14 MLS

Genotyping Protocol: **MMRRC 20**

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 carries a Neomycin cassette, inserted such that all exons but exon 1 of the TNF receptor associated factor 4 gene (*Traf4*) on Chromosome 11 are eliminated. Details can be found in Shiels et al (2000) American Journal of Pathology 157:679-88.



Primer Information:

- | | |
|------------------|---|
| 1) Name: Traf4.F | Sequence: 5'-CCC TGT CTC CAA AAA CCA AA-3' |
| 2) Name: Traf4.R | Sequence: 5'-TAG GGC AGG GGA CTA CAT TG-3' |
| 3) Name: Neo F | Sequence: 5'-CAT TCG ACC ACC AAG CGA AAC ATC-3' |
| 4) Name: Neo R | Sequence: 5'-ATA TCA CGG GTA GCC AAC GCT ATG-3' |

Primer location: Traf4.F is located in intron 2 and Traf4.R is located in exon 4 of the *Traf4* gene on Chromosome 11. Neo F and R are located in the Neomycin cassette.

Assay name: Traf4 KO PCR

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
Traf4.F	Sigma	25µM	0.3
Traf4.R	Sigma	25µM	0.3
FastStart <i>Taq</i>	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 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 the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

Alignment Marker: QX Alignment Marker 15bp/1kb (Cat# 929521)

Size Marker: QX DNA Size Marker 50bp-800bp (Cat# 929556)

Method: AM320 Injection: 10s at 5KV

Separation: 320s at 6KV

Expected product: WT: 348 bp

Mut 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
Neo F	Sigma	25µM	0.3
Neo R	Sigma	25µM	0.3
FastStart <i>Taq</i>	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 3 minutes
- 2) 94°C 30 seconds
- 3) 68°C 30 seconds
- 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 the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

Alignment Marker: QX Alignment Marker 15bp/1kb (Cat# 929521)

Size Marker: QX DNA Size Marker 50bp-800bp (Cat# 929556)

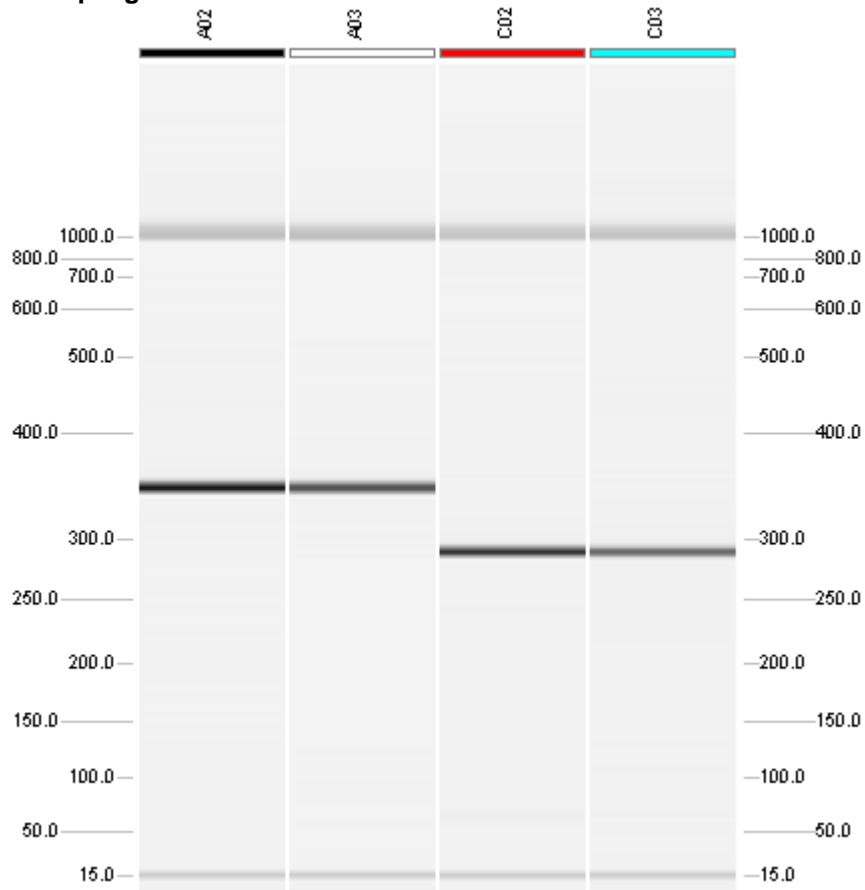
Method: AM320 Injection: 10s at 5KV

Separation: 320s at 6KV

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Expected product: Mut: 289bp

Example gel:



Lanes A02 and A03 display samples positive for the WT allele (348bp band).

Lanes C02 and C03 display samples positive for the mutant allele (289bp band).

Please note: the 15bp and 1kb bands are reference markers specific to the QIAxcel system and are not expected products.