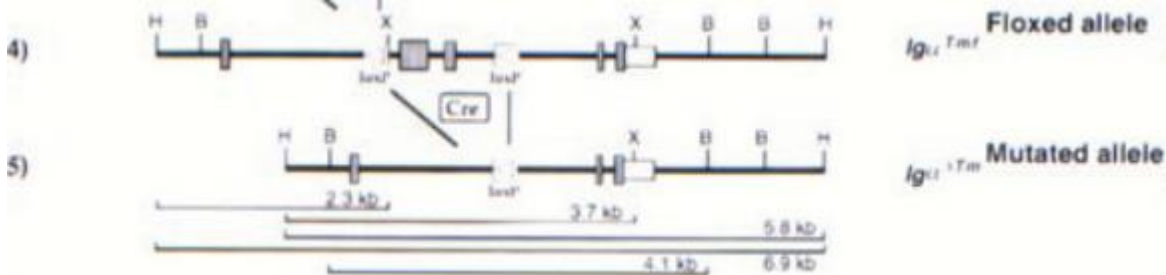


Genotyping Protocol: **MMRRC 29898**

Assay Type: PCR (can distinguish heterozygous animals from homozygous animals)

DNA Extraction: DNA from tail snips was extracted using **Qiagen's DNeasy kit (Cat#69506)**. Kit directions for animal tissues were performed with a minor modification as follows: elute in 200µl of AE buffer once.

Strain Description: Exons 2 and 3 of the mouse immunoglobulin alpha (*Iga*) gene were excised via Cre-mediated deletion. Details can be found in Kraus et al (2004) Cell 117(6):787-800.

**Primer Information:**

- 1) Name: DTMB-FOR Sequence: 5'-CCA TTG GTA CGG CTC CAC TC-3'
 2) Name: A-NEO-DEL-R Sequence: 5'-GGG GTG CAC AAC TGT AAT CC-3'

Primer Location: DTMB-FOR is located before Exon 2 of mouse *Iga*, and A-NEO-DEL-R is located after Exon 3 of mouse *Iga*.

Assay Name: Ig alpha DTM PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
DTMB-FOR	Sigma	20 µM	0.3
A-NEO-DEL-R	Sigma	20 µM	0.3
sterile water			5.4

PCR Setup:

Final Reaction: 16µl master mix & 4µl DNA template (10-20 ng/µl)

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

Cycle Parameters:

- 1) 94°C 3 minutes
- 2) 94°C 30 seconds
- 3) 65°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 a 3% agarose gel with ethidium bromide staining

Expected products:

Mutant (KO): ~195 bp
 Wild Type: 1280 bp

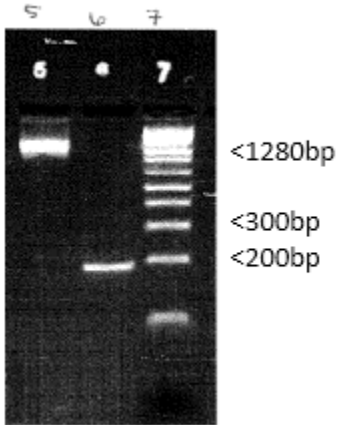
Genotypes:

Wild type: 1280bp product only

Heterozygous: 1280bp and ~195bp products

Homozygous: ~195bp product only

Example gel:



Lane 5 displays a wild type sample (1280bp product)
Lane 6 displays a homozygous mutant sample (~195bp product)
Lane 7 displays 1Kb+ Ladder (Invitrogen Cat# 10787-018)