MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	336
MMRRC Strain Name	B6.Cg-*Fasl*^{gld} *Tcrb*^{tm1Mom}/Mmnc
Gene Name(s)	T-cell receptor beta chain (Tcrb) Fas ligand (TNF superfamily, member 6)/generalized lymphoproliferative disease (Fasl)
Breeding Protocol(s)	Sib-mating
Protocol Date	4/28/14

MMRRC #336 PCR Reaction

Genotyping for this strain includes TCR beta and gld (FasL mutation).

	1X
ddH_2O	13
5× Buffer	5.0
25 mM $MgCl_2$	2
10 mM dNTPs	0.5
10 µM Primer Forward	1.0
10 µM Primer Reverse	1.0
Taq	0.5
DNA	2

Taq: Apex and Chromataq 5X Buffer

TCR beta genotyping

MUTANT reaction: primers pgk OUT/FOR (341) and Tcrb REV1 (341) reaction

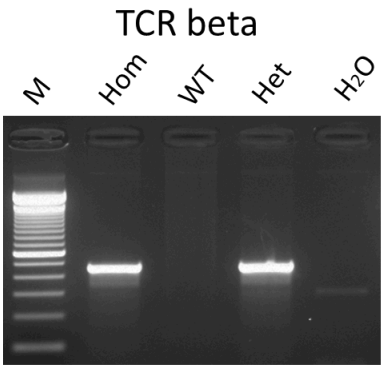
Thermal Cycler:
Step 1: 94°C for 5 min
Step 2: 94°C for 30 sec
Step 3: 61°C for 30 sec
Step 4: 72°C for 45 sec
Step 5: 35 × from step 2 to step 4
Step 6: 72°C for 7 min

The Mutant Mouse Resource and Research Center – University of North Carolina
CB# 7519, Chapel Hill, NC 27599
Phone: (919)966-4570 Fax: (919)843-1818
www.med.unc.edu/mmrrc www.mmrrc.org

Primer sequences 5' to 3': Primers are 10 µM with respect to each primer.
pgk OUT/FOR (341): TAC CGG TGG ATG TGG AAT GT
Tcrb REV1 (341): CCG TGG CAT CTA TTC TGT CA

Bands expected: targeted allele 450 bp

Run on 2% agarose gel in TAE.



Primers: pgk OUT/FOR (341) + Tcrb REV1 (341)
M: 100 bp DNA ladder (Invitrogen)

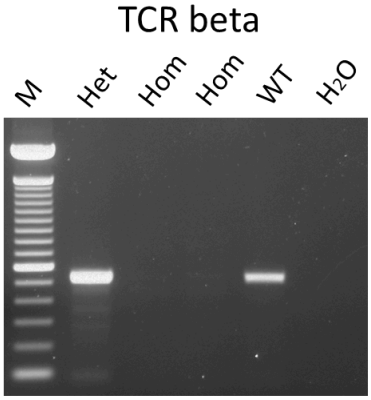
WT reaction: Primers Tcrb FOR1 (341) and TcrbREV1 (341) reaction

Thermal Cycler:
Step 1: 94°C for 5 min
Step 2: 94°C for 30 sec
Step 3: 50°C for 30 sec
Step 4: 72°C for 45 sec
Step 5: 30 × from step 2 to step 4
Step 6: 72°C for 7 min

Primer sequences 5' to 3': Primers are 10 µM with respect to each primer.
Tcrb FOR1 (341): ATG AGT CAT TTG TCT GAA GGG C
Tcrb REV1 (341): CCG TGG CAT CTA TTC TGT CA

Bands expected: Wild-type allele 539 bp

Run on 2% agarose gel in TAE.



Primers: Tcrb FOR1 (341) + Tcrb REV1 (341)
M: 100 bp DNA ladder (Invitrogen)

The Mutant Mouse Resource and Research Center – University of North Carolina
CB# 7519, Chapel Hill, NC 27599
Phone: (919)966-4570 Fax: (919)843-1818
www.med.unc.edu/mmrrc www.mmrrc.org

gld (FasL mutation) genotyping

Note: gld genotyping requires *Stu*I restriction digestion.

Thermal Cycler:
Step 1: 94°C for 5 min
Step 2: 94°C for 30 sec
Step 3: 60°C for 30 sec
Step 4: 72°C for 45 sec
Step 5: 34 × from step 2 to step 4
Step 6: 72°C for 7 min

Primer sequences 5' to 3': Primers are 10 µM with respect to each primer.
Gld-F (336): TCTCAACTCTCTCTGATCAATTTTGAGGAATCTAAGGCC
Gld-R (336): TTGGACCTTGCGGTCATGAGGTCTTTGTGGCTCATGTA

Bands expected: Both WT and MUTANT show 203 bp

*Stu*I restriction digestion

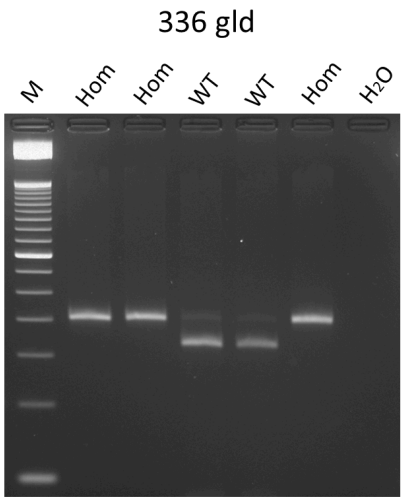
Restriction digestion mix for each PCR sample

Digestion total volume 25 µl
10.5 µl H_2O
2.5 µl NEB Buffer 4, 10 ×
2 µl *Stu*I

Add 15µl Mix to each 10 ul PCR product: incubate at 37°C for overnight. Then 65°C for 20 minutes

Expected Results:
gld MUTANT: 203 bp
WT: 166 bp and 37 bp (cannot be seen)

Run on 4% agarose gel.



Primers: Gld-F (336) + Gld-R (336); *Stu*I digestion
M: 50 bp DNA ladder (Invitrogen)

The Mutant Mouse Resource and Research Center – University of North Carolina
CB# 7519, Chapel Hill, NC 27599
Phone: (919)966-4570 Fax: (919)843-1818
www.med.unc.edu/mmrrc www.mmrrc.org