MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	339
MMRRC Strain Name	B6.Cg-*Tcrb*^{tm1Mom} Tg(UBC-GFP)30Scha/Mmnc
Gene Name(s)	T-cell receptor beta chain (Tcrb), Ubiquitin C (UBC human), GFP
Breeding Protocol(s)	Sib-mate
Protocol Date	9/3/13

MMRRC #339 PCR Reactions

Genotyping for this strain includes TCR beta and EGFP reactions.

TCR beta genotyping

	1X
ddH_2O	13
5X 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

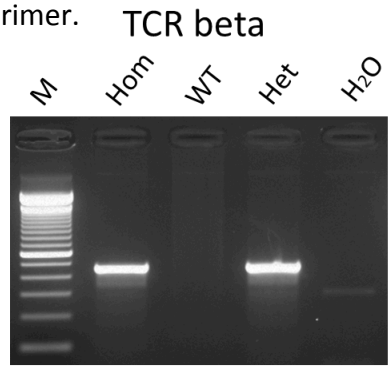
Mut reaction: primers pgk OUT/FOR (341) and Tcrb REV1 (341) reaction

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 x 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.
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)

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

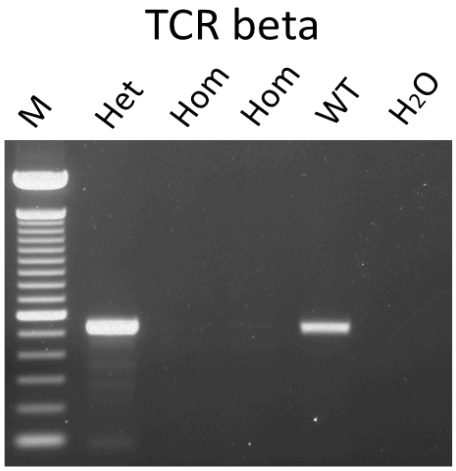
WT reaction: Primers Tcrb FOR1 (341) and TcrbREV1 (341) reaction

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 x 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)

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MMRRC at UNC
Mutant Mouse Resource and Research Center supported by NIH

EGFP PCR reaction

	1X
ddH_2O	13.8
5X Buffer	5.0
25mM $MgCl_2$	2
10mM dNTPs	0.5
50 µM each EGFP F/R	0.4
50 µM each Actin F/R	0.8
Taq	0.5
DNA	2

Thermal Cycler:
Step 1: 95°C for 5 min
Step 2: 95°C for 45 sec
Step 3: 60°C for 45 sec
Step 4: 72°C for 1 min
Step 5: 30 x from step 2 to step 4
Step 6: 72°C for 10 min

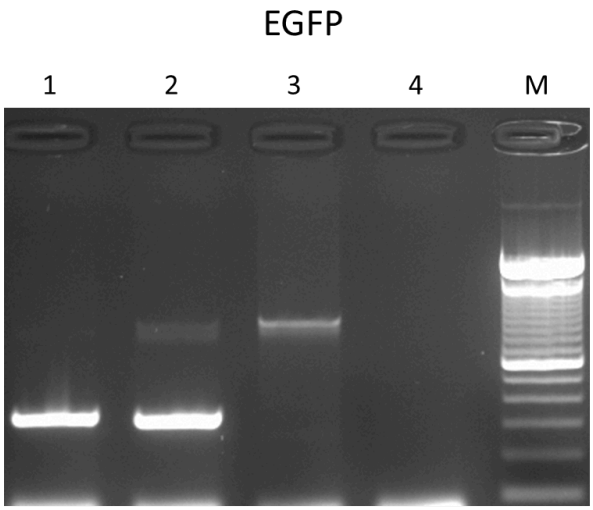
Taq: Apex and Chromataq 5X Buffer

Primer sequences 5' to 3': Primers are 50 µM with respect to each primer
EGFP F: CCT ACG GCG TGC AGT GCT TCA GC
EGFP R: CGG CGA GCT GCA CGC TGC GTC CTC
Actin PCR control/forward primer: GAT GAC GAT ATC GCT GCG CTG GTC G
Actin PCR control/reverse primer: GCC TGT GGT ACG ACC AGA GGC ATA CAG

Bands expected: Actin ~1kb (DNA control), EGFP ~300bp

Run on 1% agarose gel.

Note: 1 kb band of Actin was not shown in some reactions.



Lane 1, 2: EGFP+; Lane 3: WT; Lane 4: H_2O; M: 100 bp DNA ladder (Invitrogen)

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