

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	333
MMRRC Strain Name	B10.Cg- <i>Tcrb</i> ^{tm1^{Mom}} /Mmnc
Gene Name(s)	T-cell receptor beta chain (<i>Tcrb</i>)
Breeding Protocol(s)	Sib-mating
Protocol Date	3/17/14

MMRRC #333 PCR Reactions

Mutant Reaction: primers pgk OUT/FOR and *Tcrb* REV1 reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25 mM MgCl ₂	2
10 mM dNTPs	0.5
10 μM Primer F	1
10 μM Primer R	1
Taq	0.5
DNA	2

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: 35x from step 2 to step 4
 Step 6: 72°C for 7 min

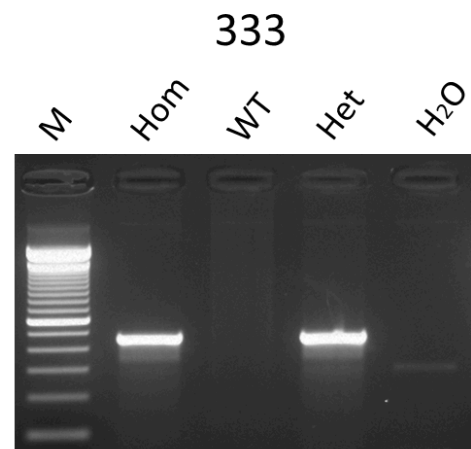
Taq: Apex and Chromataq 5X Buffer

Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

pgk OUT/FOR: TAC CGG TGG ATG TGG AAT GT

Tcrb REV1: CCG TGG CAT CTA TTC TGT CA

Bands expected: targeted allele 450 bp



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

Wild-Type Reaction: Primers Tcrb FOR1 and TcrbREV1 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: 30x from step 2 to step 4

Step 6: 72°C for 7 min

Taq: Apex and Chromataq 5X Buffer

Primer sequences 5' to 3': Primers are 10 µM with respect to each primer.

Tcrb FOR1: ATG AGT CAT TTG TCT GAA GGG C

Tcrb REV1: CCG TGG CAT CTA TTC TGT CA

Bands expected: Wild-type allele 539 bp

Run on 2% agarose gel in TAE.

