

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	337
MMRRC Strain Name	B6.129- <i>Tcrb</i> ^{tm1Mom} <i>Ifng</i> ^{tm1Ts} /Mmnc
Gene Name(s)	T-cell receptor beta chain (<i>Tcrb</i>), Interferon gamma (<i>Ifng</i>)
Breeding Protocol(s)	Sib-mating
Protocol Date	10/28/13

MMRRC #337 PCR Reactions

This strain needs to genotype for TCR beta and IFN-gamma using four 2-primer reactions.

	<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

IFNg Reaction

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 65°C for 30 sec
 Step 4: 72°C for 45 sec
 Step 5: 32x 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.

p126_ (337): AGAAGTAAGTGGGAAGGGCCCAAGAAG

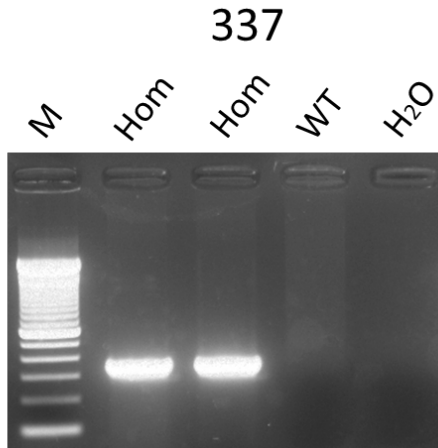
p127_ (337): AGGGAAACTGGGAGAGGAGAAATAT

p128_ (337): TCAGCGCAGGGGCGCCCGGTTCTTT

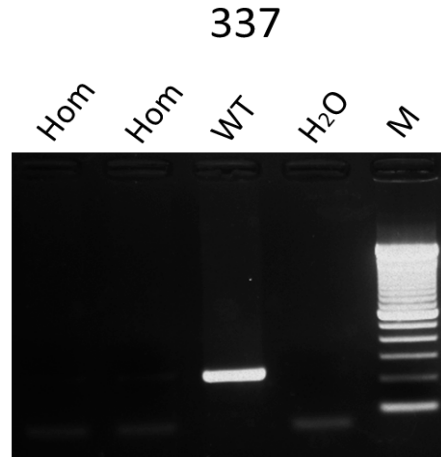
p129_ (337): ATCGACAAGACCGGCTTCCATCCGA

Bands expected: WT: Primers p126_ (337) + p127_ (337), 230 bp

Mut: primers p128_ (337) + p129_ (337), 375 bp



Primers: p128_ (337) + p129 _ (337)
 M: 100 bp DNA ladder (Invitrogen)



Primers: p126_ (337) + p127 _ (337)
 M: 100 bp DNA ladder (Invitrogen)

TCR beta Reaction

Mut reaction: primers pgk OUT/FOR and Tcrb REV1 reaction

Thermal Cycler:

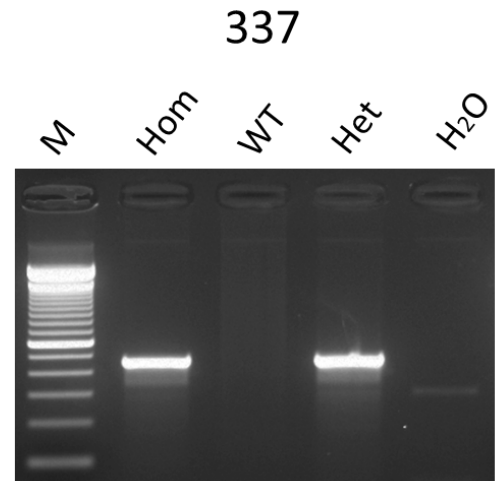
Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 61°C for 30 sec
 Step 4: 72°C for 45 sec
 Step 5: 35 x from step 2 to step 4
 Step 6: 72°C for 7 min

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

pgk OUT/FOR: 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)

WT 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: 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: 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.

