

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	334
MMRRC Strain Name	B10.129- <i>Tcrd</i> ^{tm1Mom} /Mmnc
Gene Name(s)	T-cell receptor delta chain (Tcrd)
Breeding Protocol(s)	Sib-mating
Protocol Date	4/28/14

MMRRC #334 PCR Reaction

	<u>1X</u>
ddH ₂ O	12
5 × Buffer	5.0
25 mM MgCl ₂	2
10 mM dNTPs	0.5
10 μM oIMR6916 (340)	1
10 μM oIMR6917 (340)	1
10 μM oIMR8744 (340)	1
10 μM oIMR8745 (340)	1
Taq	0.5
DNA	2

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 58°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

Taq: Apex and Chromataq 5X Buffer

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

oIMR6916 (340): CTT GGG TGG AGA GGC TAT TC

oIMR6917 (340): AGG TGA GAT GAC AGG AGA TC

oIMR8744 (340): CAA ATG TTG CTT GTC TGG TG

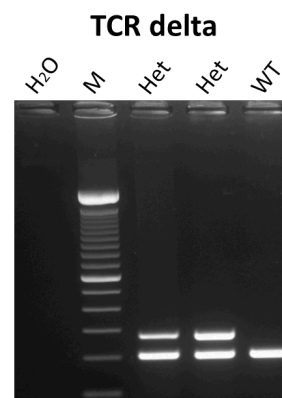
oIMR8745 (340): GTC AGT CGA GTG CAC AGT TT

Bands expected:

WT: [oIMR8744 (340) + oIMR8745 (340)]: 206 bp

MUTANT: [oIMR6916 (340) + oIMR6917 (340)]: 280 bp

Run on 2% agarose gel in TAE.



Primers: oIMR8744 (340) + oIMR8745 (340)
 oIMR6916 (340) + oIMR6917 (340)
 M: 100 bp DNA ladder (Invitrogen)