

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	227
MMRRC Strain Name	B6.129S6- <i>Il4</i> ^{tm1Rao} /Mmnc
Gene Name(s)	Interleukin 4 (Il4)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	10/7/13

MMRRC #227 PCR 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: 58°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.

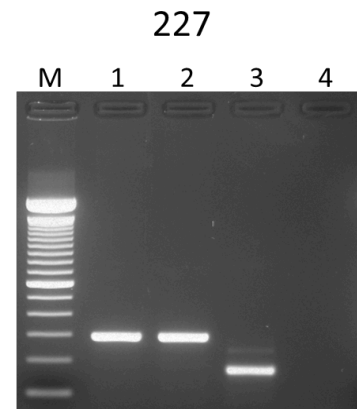
WT-F (227): TGGGTAGTCAAGGAGTGGGC

WT-R (227): CCCACAGCATGAAAGCAGAC

Mut (227): GGATGTCTTCACGGTTTCCA

Bands expected: WT: WT-F (227) + WT-R (227): 173 bp
 MUTANT: WT-F (227) + Mut (227): 295 bp

Run on 2% agarose gel in TAE.



Lane 1, 2: Hom; Lane 3: WT; Lane 4: H₂O
 M: 100 bp DNA ladder (Invitrogen)