

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	12
MMRRC Strain Name	B6.129P2- <i>Ccr2</i> ^{tm1Mae} /Mmnc
Gene Name(s)	CC chemokine receptor 2 (CCR2)
Breeding Protocol(s)	Intra-strain mating (homo x homo)
Protocol Date	10/7/13

MMRRC# 12 PCR Reaction

	1X
DNA	1.0
5X Buffer	5.0
10mM dNTPs	0.5
20 uM Primer F/R	1.0
Taq	1.0
ddH ₂ O	14.0
25mM MgCl ₂	2.0

NOTE: Run two 2-primer reactions: (F5 & B4) and (F5 and Neo27)

Thermal Cycler:

94°C for 45 sec
 55°C for 45 sec
 72°C for 60 sec
 35x

Taq: **Apex and Chromataq 10X Buffer**

Bands: MUTANT: ~650bp
 WT: 210bp

Primers are 10uM with respect to each primer (20uM total).

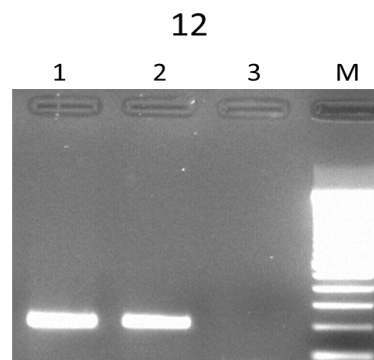
Primer sequences 5' to 3':

CCR2 F5 (12): CTACGATGATGGTGAGCCTTGTC

CCR2 B4 (12): AATGTGAGCAGGAAGAGCAGGTC

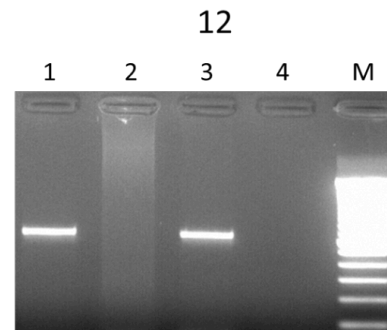
CCR2 Neo 27 (12): ACCGCTTCCTCGTGCTTTAC

Run on 2.0% agarose gel in TAE.



Primers: F5 + B4

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



Primers: F5 + Neo27

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