

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	37132
MMRRC Strain Name	B6N.129S1- <i>Ccl2</i> ^{tm1.1Tyos} /Mmnc
Gene Name(s)	chemokine (C-C motif) ligand 2 (Ccl2)
Breeding Protocol(s)	Sib-mating
Protocol Date	5/13/15

MMRRC #37132 PCR Reaction

	1X
ddH ₂ O	11
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM WT Primer F/R	1/1
10uM Mut Primer F/R	1/1
Taq	0.5
DNA	2

Thermal Cycler:

Step 1: 95°C for 5 min

Step 2: 95°C for 30 sec

Step 3: 58°C for 30 sec

Step 4: 72°C for 60 sec

Step 2 to 4 Cycles: 35

Step 5: 72°C for 7 min

Taq: **Apex Taq and Chromataq 5X Buffer**

Bands: WT: 615bp MUTANT: ~400bp

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

(37094)WTF: CCAACCCACAGTTTCTCTCTTC

(37094)WTRev: GATCACCCAGGTTCCCAAATAC

(37094)KOF: TGGGTACAACCTCTGCCAATCAGGA

(37094)KORev: AGCACAGACCTCTCTCTTGAGCTT

Run on 2% agarose gel

1. 100bp Marker
2. Homozygous Sample
3. Homozygous Sample
4. Homozygous Sample
5. Homozygous Sample
6. Homozygous Sample
7. Wild-type Control
8. Negative Control

