

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11634
MMRRC Strain Name	B6.129P2- <i>Opr1</i> ^{tm1Dgen} /Mmnc
Gene Name(s)	opioid receptor-like 1 (<i>Opr1</i>)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	5/19/15

MMRRC #11634 PCR Reaction

	<u>1X</u>
ddH ₂ O	12
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
10uM Primer 3	1
Taq	0.5
DNA	2

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C for 45 sec

Step 3: 60°C for 45 sec

Step 4: 72°C for 90 sec

Step 2 to 4 Cycles: 35

Step 5: 72°C for 7 min

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

Bands: WT: 211bp MUTANT: 295bp

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

NIH0990-GS1: GGCACACCAAGATGAAGACTGCTAC

NIH0990-GS2: CGGTCTACTCATGGCAGTCAAAG

NIH00909-Neo: GGGCCAGCTCATTCTCCCACTCAT



1. 100bp Marker
2. Heterozygous Sample
3. Wild-type Control
4. Negative Control