

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	13
<b>MMRRC Strain Name</b>	B6.129P2- <i>Npr1</i> <sup>tm1(Npr1)Unc</sup> /Mmnc
<b>Gene Name(s)</b>	Natriuretic peptide receptor 1 (Npr1)
<b>Breeding Protocol(s)</b>	Backcross to C57BL/6J
<b>Protocol Date</b>	8/19/13

### MMRRC #13 PCR Reaction

	<u>1X</u>
ddH <sub>2</sub> O	11
5X Buffer	5.0
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10 uM Primer 1	2.0
10 uM Primer 2	1.0
10 uM Primer 3	1
Taq	0.5
DNA	2

**Thermal Cycler:**

Step 1: 94°C for 5 min  
 Step 2: 94°C for 30 sec  
 Step 3: 60 °C for 30 sec  
 Step 4: 72°C for 1 min  
 Step 5: 35x from step 2 to step 4  
 Step 6: 72°C for 7 min

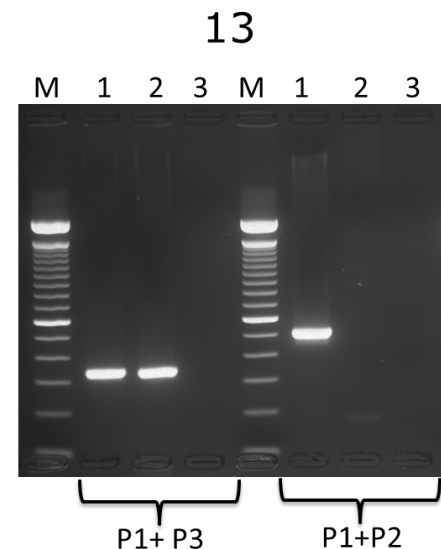
**Taq: Apex and Chromataq 5X Buffer**

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

P1: GCA TGG TTC AGC TCT AAG AC  
 P2: CTA ACC CTG TGA ACT GTA AGC  
 P3: CCT TCA GTT ATC TAC ATC TGC

**Bands expected:** (P1 + P3) WT 339bp  
 (P1 + P2) Mut ~500bp

Run on 2% agarose gel in TAE.



Lane 1: Het; Lane 2: WT; Lane 3: H<sub>2</sub>O  
 M: 100 bp DNA ladder Invitrogen