

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11628
MMRRC Strain Name	B6.129P2- <i>Htr2b</i> ^{tm1Dgen} /Mmnc
Gene Name(s)	5-hydroxytryptamine (serotonin) receptor 2B (Htr2b)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	7/16/13

MMRRC #11628 PCR Reaction

	<u>1X</u>
ddH ₂ O	13.5
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10 μM Primer each (3-primer mix)	1.5
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 5: Step 2 to 4; Cycles 36
 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.

NIH-0010 GS1: GCACATTTTACAGAAGACATGTGATCAC

NIH-0010 GS2: TCCAACCAGCAAATCTGCTATCGCC

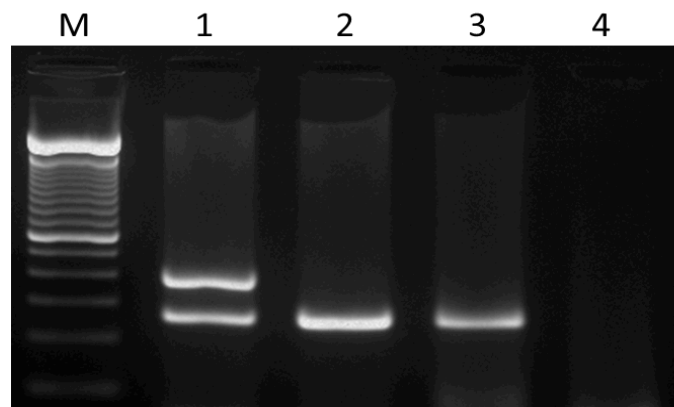
NIH-0010 Neo: GGGTGGGATTAGATAAATGCCTGCTCT

11628

Bands expected: MUT ANT: 403bp

WT: 265bp

Run on 2.0% agarose gel in TAE.



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