

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	30040
MMRRC Strain Name	129- <i>Htr1b</i> ^{tm1Rhn} /Mmnc
Gene Name(s)	5-hydroxytryptamine receptor 1B (Htr1b)
Breeding Protocol(s)	Sib-mating
Protocol Date	8/5/13

MMRRC #30040 PCR

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10 μM Primer F	1
10 μM Primer R	1
Taq	0.5
DNA	2

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 58 °C for 30 sec
 Step 4: 72°C for 45 sec
 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.

5ht1b-R: CCCATCAGCACCATGTACAC

5ht1bc-R: CTTCTATCGCCTTCTTGACG

5ht1b-F: GACTTGGTTCACGTACACAG

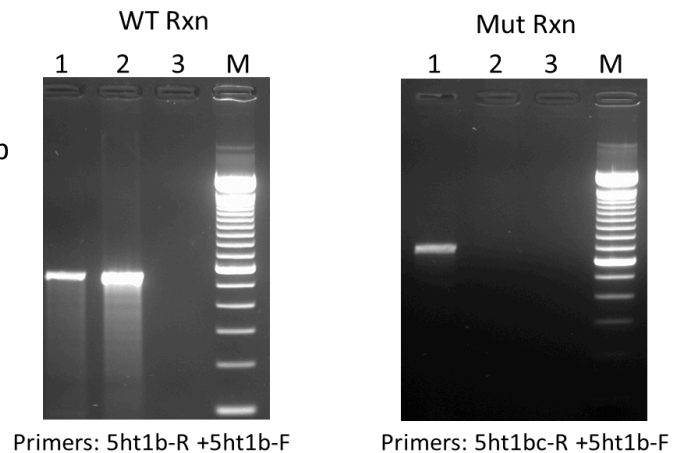
Bands expected:

WT: primers: 5ht1b-R +5ht1b-F: 561 bp

MUTANT: primers: 5ht1bc-R +5ht1b-F: ~ 680 bp

Run on 2 % agarose gel in TAE.

30040



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