

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11623
MMRRC Strain Name	B6.129P2- <i>Gpr3</i> ^{tm1Dgen} /Mmnc
Gene Name(s)	G-protein coupled receptor 3 (Grp3)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	7/29/13

MMRRC #11623 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: 35
 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-0688 GS1: TATCCA CTCTCCAAGA ACCATCTGG

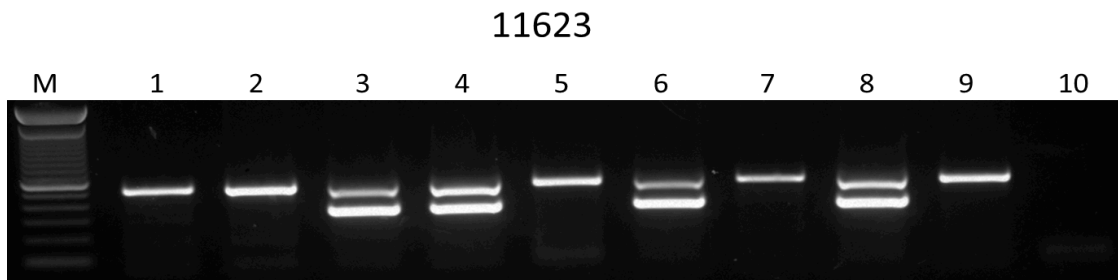
NIH-0688 GS2: GGAATTAAGCCCTGGTGGACCTAAC

NIH-0688 Neo: GGGCCAGCTCATTCTCCCACTCAT

Bands expected: MUT 338 bp

WT 505 bp

Run on 2.0% agarose gel in TAE.



Lane 1, 2, 5, 7 and 9: WT; Lane 3, 4, 6 and 8: Het; Lane 10: H₂O; M: 100 bp DNA ladder (Invitrogen)

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