

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11624
MMRRC Strain Name	B6.129P2- <i>Gria3</i> ^{tm1Dgen} /Mmnc
Gene Name(s)	glutamate receptor, ionotropic, AMPA3 (alpha 3) (<i>Gria3</i>)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	7/16/13

MMRRC #11624 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 7 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.

GS1: AGCTGATATAGCTGTTGCTCCACTC

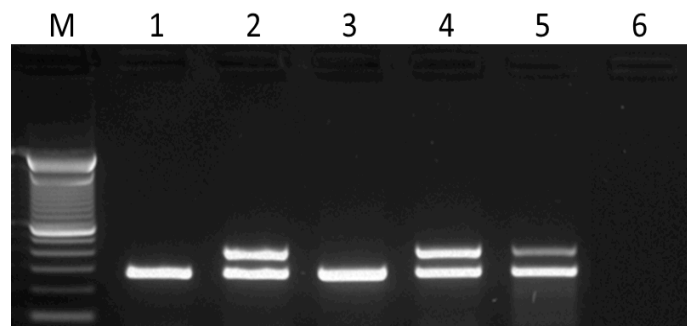
GS2: GGTCACGAGGTTCTTCATTGTTGTC

Neo: GGGTGGGATTAGATAAATGCCTGCTCT

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Bands expected: MUTANT: 380bp
 WT: 275bp

Run on 2.0% agarose gel in TAE.



Lane 1, 3: WT; Lane 2, 4 and 5: Het; Lane 6: H₂O;
 M: 100 bp DNA ladder Invitrogen