

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11625
MMRRC Strain Name	B6;129P2- <i>Gria4</i> ^{tm1Dgen} /Mmnc
Gene Name(s)	glutamate receptor, ionotropic, AMPA4 (alpha 4) (<i>Gria4</i>)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	7/22/13

MMRRC #11625 PCR Reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25 mM MgCl ₂	2
10 mM 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: 60 °C for 30 sec
 Step 4: 72°C for 30 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.

GriaWT-F: TAATTGTGTGTGCTTGCGGTTGGAG

GriaWT-R: CAAGGAAGGGTGAAGGAAGCAAAG

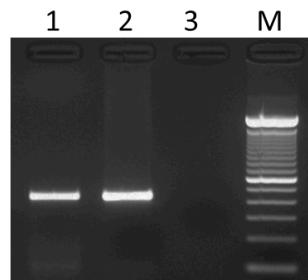
Neo-Mut-F: GGGTGGGATTAGATAAATGCCTGCTCT

Bands expected: WT primers (GriaWT-F + GriaWT-R): 436 bp

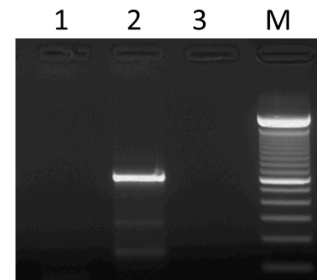
Mutant primers (GriaWT-R + Neo-Mut-F): 577 bp

Run on 2% agarose gel in TAE.

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WT, primers GriaWT-F + GriaWT-R



Mut, primers GriaWT-R + Neo-Mut-F

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