

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	34679
MMRRC Strain Name	B6.129- <i>Adarb1</i> ^{tm1Phs} <i>Gria2</i> ^{tm1.1Phs} /Mmmh
Gene Name(s)	adenosine deaminase, RNA-specific, B1 (<i>Adarb1</i>) glutamate receptor, ionotropic, AMPA2 (alpha 2) (<i>Gria2</i>)
Breeding Protocol(s)	Sib-mating (homozygous x homozygous)
Protocol Date	1/23/14

MMRRC# 34679 PCR Reactions

The genotyping for this strain requires two PCR reactions: One for ADARB KO and one for GluR-BR/R.

ADARB-KO Reaction

Thermal Cycler:

Step 1: 94°C for 5min
 Step 2: 94°C for 30 sec
 Step 3: 65°C for 30 sec
 Step 4: 72°C for 30 sec
 Step 2 to 4 Cycles: 35
 Step 5: 72°C for 7 min

Taq: **Apex and Chromataq 5X Buffer**

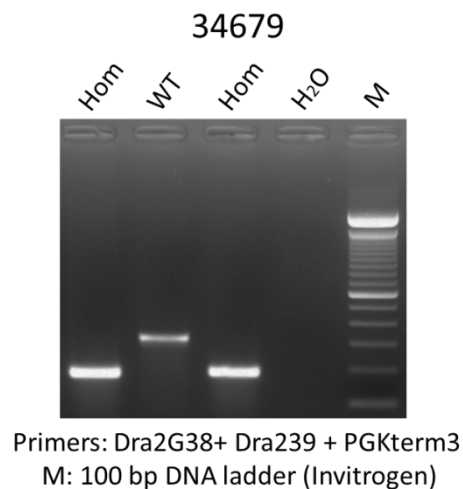
Bands: MUTANT: 194bp
 WT: 328bp

	<u>1X</u>
ddH ₂ O	12
5X Buffer	5.0
25mM MgCl ₂	2.5
10mM dNTPs	0.5
10 uM Primer 1	1
10 uM Primer 2	1
10 uM Primer 3	1
Taq	0.5
DNA	2

Primer sequences 5' to 3':

Dra2G38: CAGCTCCCTTCGCATGTTCACCTC
 Dra2G39: GATCGGAGACATAAAGACACATG
 PGKterm3: CGAGATCAGCAGCCTCTGTTCCAC

Run on 2.0% agarose gel in TAE.



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GluR-BR/R Knock-In Reaction

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

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 2 to 4 Cycles: 35

Step 5: 72°C for 7min

Taq: **Apex and Chromataq 5X Buffer**

Bands: MUTANT: 294bp

WT: 200bp

Primer sequences 5' to 3':

MH50: GACCCTGTAGGAAAAATCTAACC

MH50a: GAATGTTGATCATGTGTTTCCCTG

Run on 2.0% agarose gel in TAE.

