

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11620
MMRRC Strain Name	B6.129P2- <i>Gabraq^{tm1Dgen}</i> /Mmnc
Gene Name(s)	gamma-aminobutyric acid (GABA) A receptor, subunit theta
Breeding Protocol(s)	Backcross to B6J
Protocol Date	7/10/19

PCR Reaction

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



1. Wild-type Sample
2. Heterozygous Sample
3. Heterozygous Sample
4. Heterozygous Sample
5. Heterozygous Sample
6. Wild-type Control
7. Negative Control
8. 100bp Marker (Invitrogen)

Thermal Cycler:

Step 1: 94C, 5min
 Step 2: 94C, 45sec
 Step 3: 60C, 45sec
 Step 4: 72C, 90sec
 Step 2 to 4 Cycles: 35
 Step 5: 72C, 7min

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

Bands: WT: 210bp MUTANT: 409bp

Primer sequences 5' to 3': Primers are 10uM with respect to each primer

NIH0627(11620)1	CTCCATCTGCTTGCAGCCTGAAAAC
NIH0627(11620)2	TCCAGGAGAGAATGGTGGTGAGGAC
NIH0627(11620)N	GGTGGGATTAGATAAATGCCTGCTCT