



# Gene Editing Rat Resource Center

## Genotyping Your Model



Date:	04/06/2020		
Mutant Strain:	LE-Grin2b <sup>em1Mcwi</sup>	Mutant Strain RGD ID:	14394515
Gene Targeted:	Grin2b	RGD Gene ID:	2738
Parental Strain:	Long Evans	RGD Parental Strain ID:	2308852
Mutation Description:	Deletion of Grin2b exon3		
Deletion:	Exon3 of rat Grin2b		
Insertion	None		
Mutation Region:	(RGSC 6.0/rn6) chr4:169,751,704-169,752,731		
Target Site:	TATCCCCACACTTGATGTGG and CTCATCGACAACATACCCTC		

Genotyping Primers:	
Forward:	GGATTCCAAGCACAAAGTCTCCT
Reverse:	CTCCTGCCCTACTGTGAGCTTGG and CCAAAGTGAAGAAGCATGGAGG
WT PCR Size:	437 bp
Mutant Size:	263 bp

### Derivation Information:

This strain was produced by injecting 2 Crisprs targeting the sequences **TATCCCCACACTTGATGTGG** and **CTCATCGACAACATACCCTC** into Long Evans rat embryos. This allele was made by Crispr mutagenesis. The resulting mutation is the deletion of exon 3.

### Suggested Genotyping:

PCR and run on an agarose gel

Funding Source:	
-----------------	--

Questions? Send an email to: [mcwcustomrats@mcw.edu](mailto:mcwcustomrats@mcw.edu)

**Additional Notes:** This rat has a larger deletion. A 3 primer PCR is run to determine WT and deletion alleles.

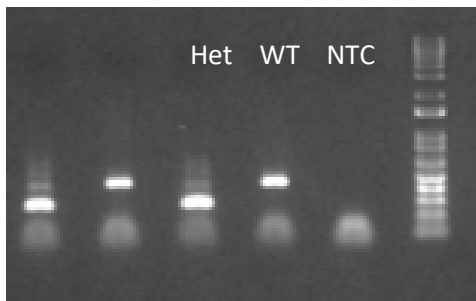
Grin2b\_upGeno\_F1: GGATTCCAAGCACAAAGTCTCCT

Grin2b\_upGeno\_R1: CCAAAGTGAAGAACATGGAGG

Grin2b\_dnsGeno\_R2: CTCCTGCCCTACTGTGAGCTTGG

WT: 437bp product

Deletion: about 263bp product



## PCR

5.0 ul of template (approximately 200ng)

12.5 µl of Accuprime Supermix II (Invitrogen, Carlsbad, CA)

1.0 µl of primer set at 10 µM

8.5 µl of water

-----

25 uL / rxn

## PCR cycling parameters:

95°C- 5 min

35 cycles of:

95°C- 30 sec

60°C- 30 sec

68°C- 45 sec

Questions? Send an email to: [mcwcustomrats@mcw.edu](mailto:mcwcustomrats@mcw.edu)

68°C- 5 min

4°C- forever