



Gene Editing Rat Resource Center

Genotyping Your Model



Date:	04/13/2016		
Mutant Strain:	P2rx7-m8	Mutant Strain RGD ID:	12790679
Gene Targeted:	P2rx7	RGD Gene ID:	3241
Parental Strain:	PCK	RGD Parental Strain ID:	1580542
Mutation Description:	1 bp insertion in exon2		
Deletion:	NA		
Insertion	CTT ^a ATCAGCTCTGTGCACA lowercase 1-bp ins		
Mutation Region:	(RGSC 5.0/rn5) chr12:41,269,243-41,269,244		
Target Site:	GTGTGCACAGAGCTGATAAG		

Genotyping Primers:	
Forward:	CTAGCCAAGCATTCTACCAGTTGA
Reverse:	AGATTGCCATCAAGCAGCGG
WT PCR Size:	379 bp
Mutant Size:	380 bp

Derivation Information:

We injected pronuclear with the px330 plasmid containing the gRNA sequence (**GTGTGCACAGAGCTGATAAG**). This construct is available from addgene (px330). This construct is a dual expression plasmid expressing the gRNA by a U6 promoter, and the Cas9 by a CMV. We inject 5ng/ul of intact circular plasmid.

Suggested Genotyping:

Direct sequencing PCR products, or the Surveyor or T7 ce1 assay.

Funding Source:	
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Additional Notes:

Questions? Send an email to: mcwcustomrats@mcw.edu

PCR

5.0 μ l of template (approximately 200ng)

12.5 μ l of Accuprime Supermix II (Invitrogen, Carlsbad, CA)

1.0 μ l of primer set at 10 μ M

8.5 μ l of water

25 μ L / rxn

PCR cycling parameters:

95°C- 5 min

35 cycles of:

95°C- 30 sec

60°C- 30 sec

68°C- 45 sec

68°C- 5 min

4°C- forever