

Ppp2r5d Cas9-CKO Strategy

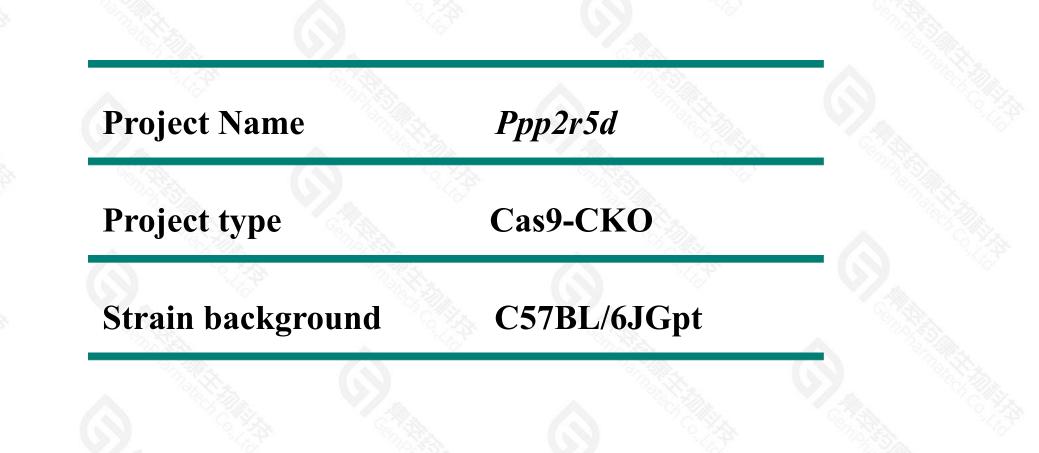
Designer: Huan Wang

Reviewer: Yumeng Wang

Design Date: 2021-7-14

Project Overview



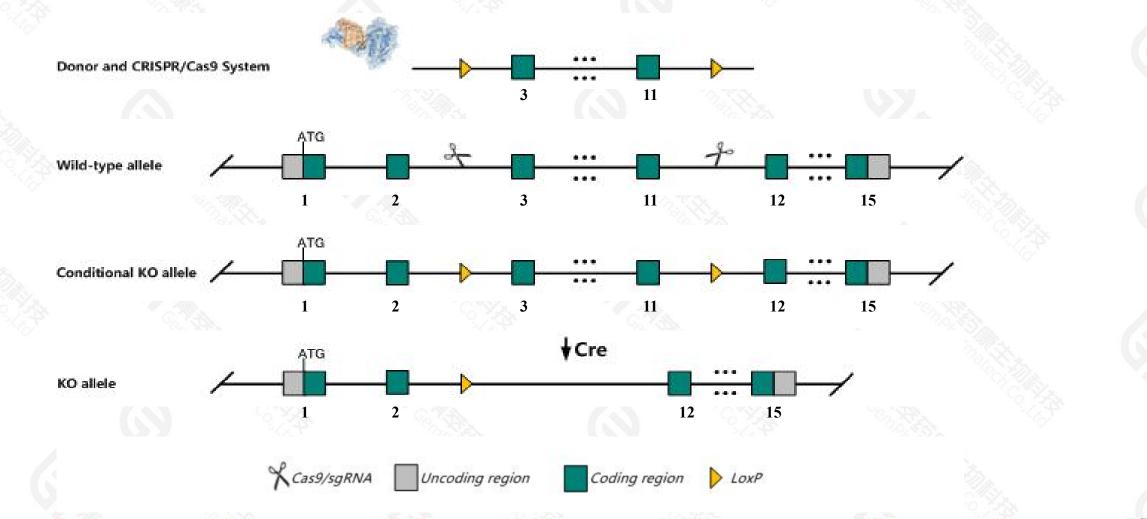


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Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ppp2r5d* gene. The schematic diagram is as follows:



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Technical routes



> The Ppp2r5d gene has 4 transcripts. According to the structure of Ppp2r5d gene, exon3-exon11 of Ppp2r5d-201(ENSMUST0000002839.9) transcript is recommended as the knockout region. The region contains 1250bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Ppp2r5d* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor was constructed.Cas9, sgRNA and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

> The flox mice was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell

types.



- > According to the existing MGI data,mice homozygous for a gene-trap allele exhibit lethality, while heterozygous mice display decreased prepulse inhibition. Mice homozygous for a targeted knock-out allele exhibit decreased thermal nociception threshold, impaired coordination, and increased latency to removing an adhesive sticker.
- ➤ The Intron11 is only 535bp,loxp insertion may affect mRNA splicing.
- > The Ppp2r5d gene is located on the Chr17. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- > This strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes,all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

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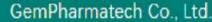
Gene information (NCBI)

Ppp2r5d protein phosphatase 2, regulatory subunit B', delta [Mus musculus (house mouse)]

Gene ID: 21770, updated on 13-Mar-2020

- Summary

Ppp2r5d provided by MGI
protein phosphatase 2, regulatory subunit B', delta provided by MGI
MGI:MGI:2388481
Ensembl:ENSMUSG00000059409
protein coding
VALIDATED
Mus musculus
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
Muroidea; Muridae; Murinae; Mus; Mus
B'delta, TEG-271, Tex271
Ubiquitous expression in large intestine adult (RPKM 36.1), CNS E11.5 (RPKM 34.1) and 28 other tissues See more
human all





\$?

Transcript information (Ensembl)



The gene has 4 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ppp2r5d-201	ENSMUST0000002839.8	2926	<u>594aa</u>	Protein coding	CCDS28836	<u>Q91V89</u>	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P2
Ppp2r5d-204	ENSMUST00000233988.1	3038	<u>595aa</u>	Protein coding	-	Q7TNL5	GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Ppp2r5d-202	ENSMUST00000233082.1	1587	No protein	Retained intron	-	20	
Ppp2r5d-203	ENSMUST00000233757.1	485	No protein	Retained intron	2	25	

The strategy is based on the design of *Ppp2r5d-201* transcript, the transcription is shown below:



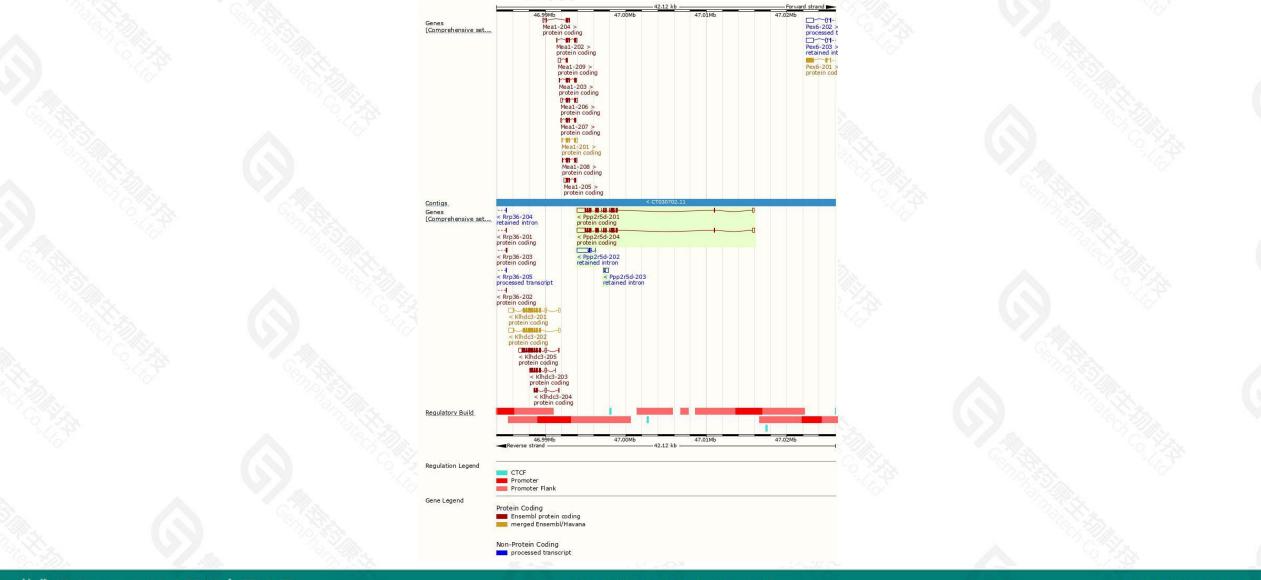
22.01 kb

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Genomic location distribution





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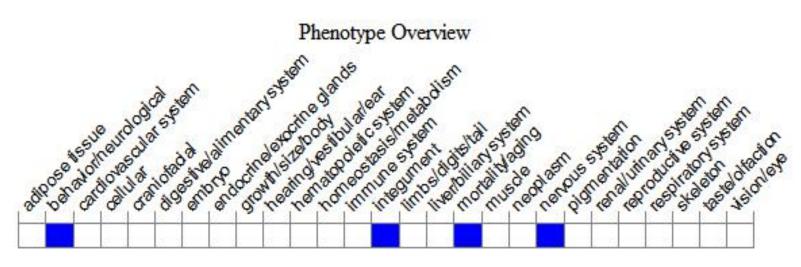
Protein domain



ENSMUSP00000002 MobiDB lite Low complexity (Seg) Superfamily		Armadillo-ty	/pe fold	त्रो. <u> </u>		.16			
<u>Pfam</u>		Protein phosph	atase 2A, reg	ulatory B su	bunit, B56	_	_		
PIRSF	Protein phosphatas								_
PANTHER	PTHR102571SF89								
	Protein phosphatase	e 2A, regulatory	B subunit, B5	16					
Gene3D		Armadillo-like	e helical						
All sequence SNPs/i	Sequence variant	s (dbSNP and	all other sou	rces)		110	0.00	111	an as r
Variant Legend	missense va								
		120	180	240	300	360	420	480	594

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Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data,mice homozygous for a gene-trap allele exhibit lethality, while heterozygous mice display decreased prepulse inhibition. Mice homozygous for a targeted knock-out allele exhibit decreased thermal nociception threshold, impaired coordination, and increased latency to removing an adhesive sticker.

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If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



