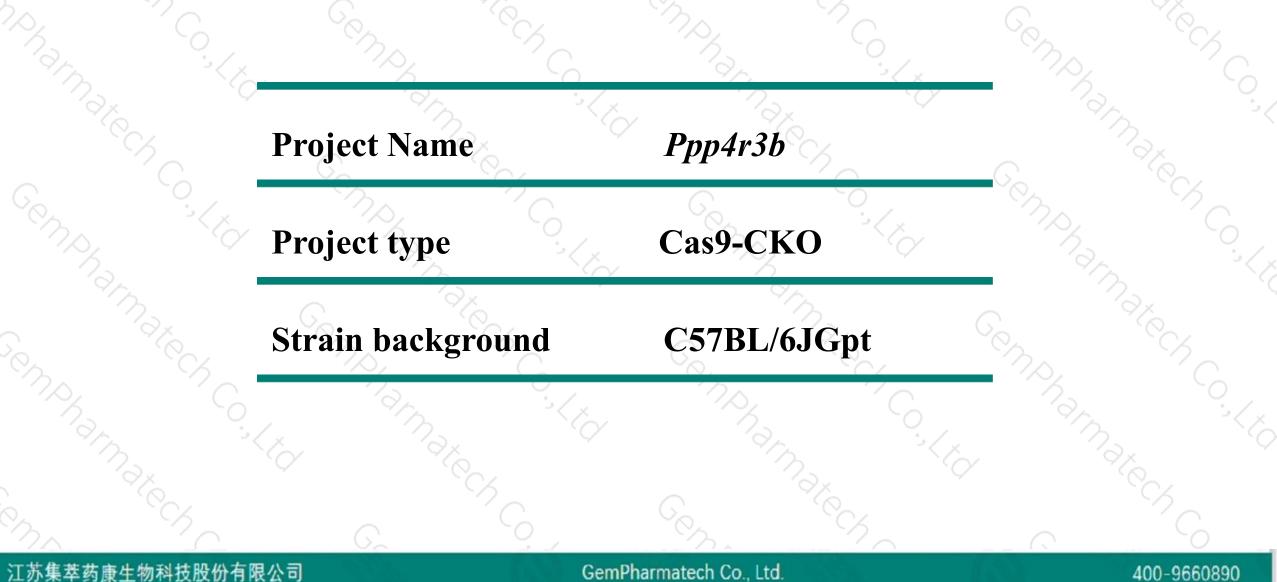


# **Ppp4r3b** Cas9-CKO Strategy

Designer: Reviewer: Design Date: JiaYu Xiaojing Li 2020-3-5

# **Project Overview**



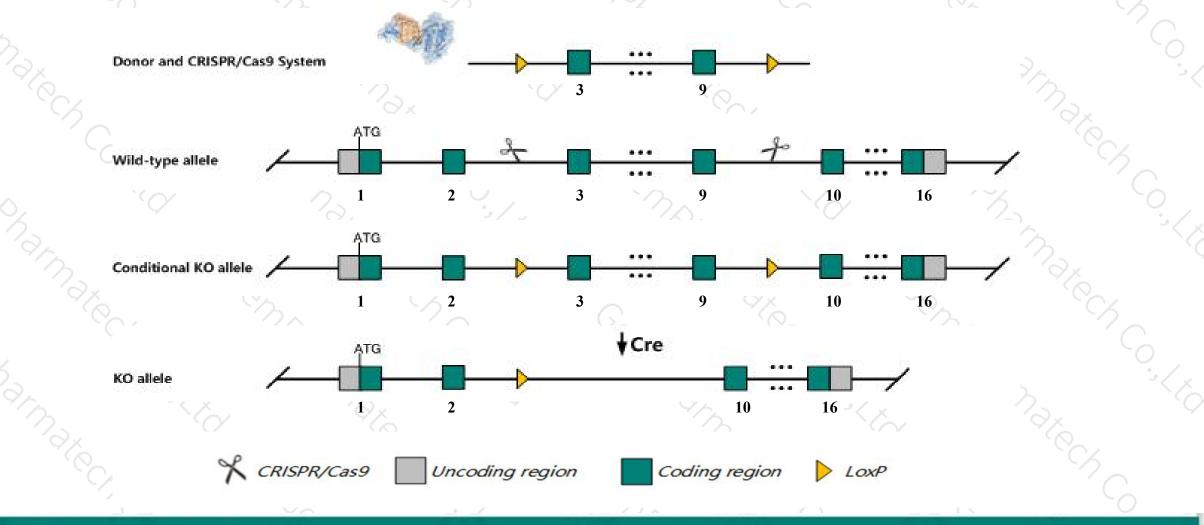


## **Conditional Knockout strategy**



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This model will use CRISPR/Cas9 technology to edit the *Ppp4r3b* gene. The schematic diagram is as follows:



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The Ppp4r3b gene has 7 transcripts. According to the structure of Ppp4r3b gene, exon3-exon9 of Ppp4r3b-201 (ENSMUST0000020755.11) transcript is recommended as the knockout region. The region contains 1270bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Ppp4r3b* gene. The brief process is as follows:CRISPR/Cas9 system 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 will be 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.



- The Ppp4r3b gene is located on the Chr11. 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.
- ≻Transcript 206 CDS 5' incomplete the influences is unknown. Transcript 203 CDS 5' and 3' incomplete the influences is unknown.
- 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.

# **Gene information (NCBI)**



\$ ?

### Ppp4r3b protein phosphatase 4 regulatory subunit 3B [Mus musculus (house mouse)]

Gene ID: 104570, updated on 31-Jan-2019

#### Summary

Official Symbol	Ppp4r3b provided by MGI
Official Full Name	protein phosphatase 4 regulatory subunit 3B provided by MGI
Primary source	MGI:MGI:2144474
See related	Ensembl:ENSMUSG0000020463
Gene type	protein coding
<b>RefSeq status</b>	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
	Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW011752, AW557776, Smek2, mKIAA1387
Expression	Ubiquitous expression in CNS E11.5 (RPKM 9.9), CNS E14 (RPKM 7.1) and 27 other tissues See more
Orthologs	human all

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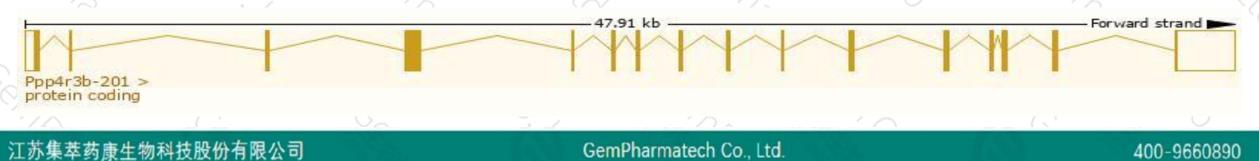
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## The gene has 7 transcripts, all transcripts are shown below:

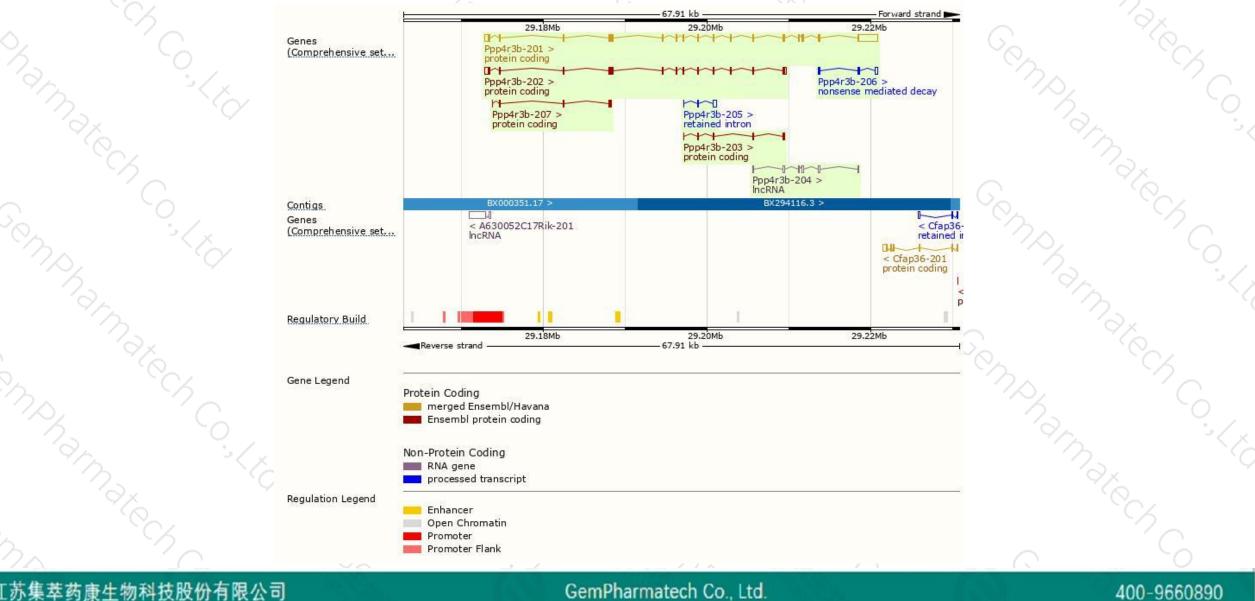
		(2017 Ma)		1 1 1				28 170.
	ot Flags	UniProt	CCDS	Biotype	Protein	bp	Transcript ID	Name
21	TSL:1 GENCODE basic APPRIS P1	<u>Q922R5</u>	CCDS24492	Protein coding	<u>820aa</u>	5125	ENSMUST00000020755.11	Ppp4r3b-201
	TSL:1 GENCODE basic	<u>Q922R5</u>		Protein coding	<u>616aa</u>	2410	ENSMUST00000102856.8	Ppp4r3b-202
	CDS 3' incomplete TSL:3	Q5RJB9	8 <del>4</del>	Protein coding	<u>194aa</u>	616	ENSMUST00000156280.1	Ppp4r3b-207
end of the CDS. CDS 5' and 3' incomplete TSL:	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' i	<u>Q5M6W0</u>	1 (1 <u>2</u>	Protein coding	<u>192aa</u>	576	ENSMUST00000127621.2	Ppp4r3b-203
	93 CDS 5' incomplete TSL:2	<u>M0QW93</u>		Nonsense mediated decay	<u>89aa</u>	657	ENSMUST00000148759.1	Ppp4r3b-206
	TSL:5	-		Retained intron	No protein	611	ENSMUST00000139283.1	Ppp4r3b-205
	TSL:5	-	8 <del>4</del>	IncRNA	No protein	827	ENSMUST00000127818.1	Ppp4r3b-204
and a second sec	M0       5' and 3' truncations in transcript evidence prevent annotation of the start and the         93       CDS 5' incomplete TSL:2         TSL:5	<u>Q5M6W0</u> <u>M0QW93</u>		Protein coding Nonsense mediated decay Retained intron	<u>192aa</u> <u>89aa</u> No protein	576 657 611	ENSMUST00000127621.2 ENSMUST00000148759.1 ENSMUST00000139283.1	Ppp4r3b-203 Ppp4r3b-206 Ppp4r3b-205

The strategy is based on the design of Ppp4r3b-201 transcript, The transcription is shown below



## **Genomic location distribution**





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## **Protein domain**



ENSMUSP00000020		1				•		
MobiDB lite								
Low complexity (Seg) Superfamily	SSF50729	Armad	lillo-type fold					
Pfam		Domain	of unknown	function DUF625				
PANTHER	PTHR23318:SF18							
	PTHR23318							21
Gene3D								
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If you have any questions, you are welcome to inquire. Tel: 400-9660890



