

Plcb3 Cas9-CKO Strategy

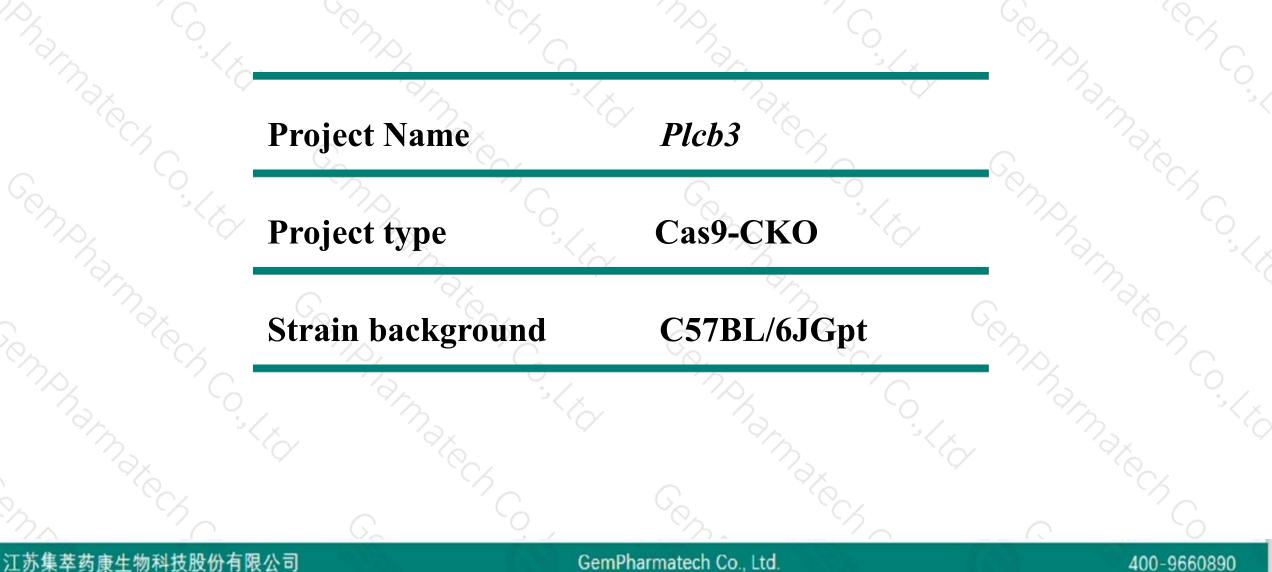
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Reviewer: Daohua Xu

Design Date: 2020-7-20

Project Overview



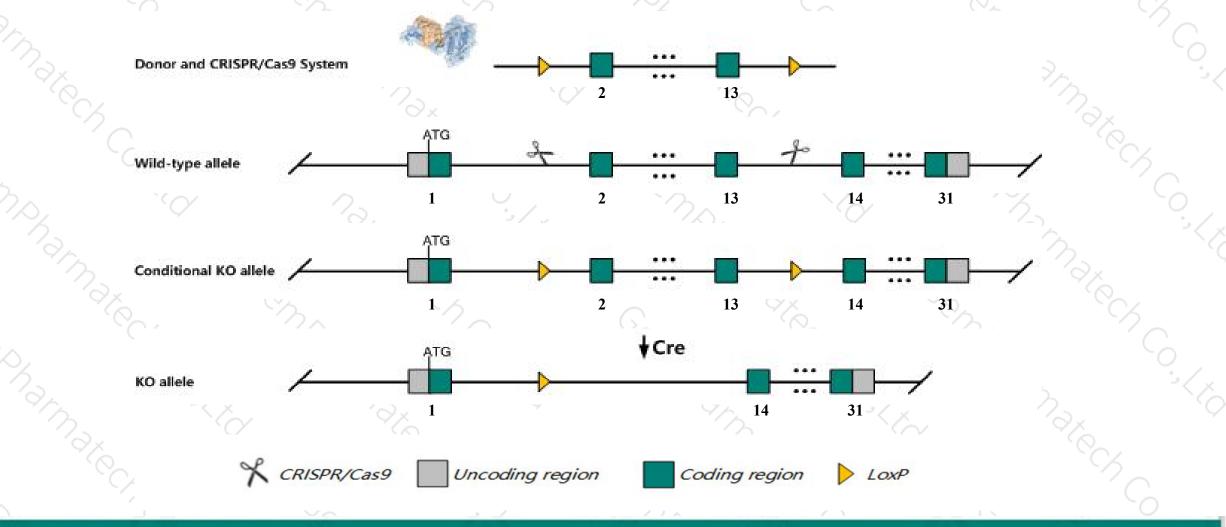


Conditional Knockout strategy



400-9660890

This model will use CRISPR/Cas9 technology to edit the *Plcb3* gene. The schematic diagram is as follows:



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The *Plcb3* gene has 10 transcripts. According to the structure of *Plcb3* gene, exon2-exon13 of *Plcb3*-201(ENSMUST00000025912.9) transcript is recommended as the knockout region. The region contains 1426bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Plcb3* 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.



- > According to the existing MGI data, mice homozygous for one targeted mutation die at E2.5 and exhibit poor embryonic organization and hypocellularity. Mice homozygous for a second targeted mutation survive to adulthood and exhibit an increased antinocieptive response to opioids.
- ➤ The effect on transcript *Plcb3*-207&210 is unknown.
- ➤ Transcript *Plcb3*-203&205 may not be affected.
- > The *Plcb3* gene is located on the Chr19. 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.

Gene information (NCBI)



☆ ?

Plcb3 phospholipase C, beta 3 [Mus musculus (house mouse)]

Gene ID: 18797, updated on 13-Mar-2020

- Summary

Official Symbol Plcb3 provided by MGI Official Full Name phospholipase C, beta 3 provided byMGI Primary source MGI:MGI:104778 See related Ensembl:ENSMUSG0000024960 Gene type protein coding RefSeg status VALIDATED Organism Mus musculus Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Lineage Muroidea; Muridae; Murinae; Mus; Mus Also known as mKIAA4098 Expression Broad expression in large intestine adult (RPKM 158.9), duodenum adult (RPKM 122.8) and 16 other tissuesSee more Orthologs human all

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Transcript information (Ensembl)



The gene has 10 transcripts, all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|-----------|----------------------|------|---------------|----------------------|-----------|---------------|--|
| Plcb3-201 | ENSMUST00000025912.9 | 4257 | <u>1234aa</u> | Protein coding | CCDS70925 | P51432 | 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 P |
| Picb3-210 | ENSMUST00000237808.1 | 1986 | <u>378aa</u> | Protein coding | - | <u>Q8C810</u> | CDS 5' incomplete |
| Plcb3-202 | ENSMUST00000235352.1 | 1665 | <u>508aa</u> | Protein coding | | A0A494B9C1 | CDS 3' incomplete |
| Plcb3-207 | ENSMUST00000237074.1 | 636 | <u>191aa</u> | Protein coding | 2 | A0A494BAY4 | CDS 3' incomplete |
| Plcb3-208 | ENSMUST00000237446.1 | 887 | No protein | Processed transcript | - | - | |
| Plcb3-206 | ENSMUST00000236961.1 | 660 | No protein | Processed transcript | - | - | |
| Plcb3-209 | ENSMUST00000237726.1 | 4558 | No protein | Retained intron | | - | |
| Plcb3-204 | ENSMUST00000235832.1 | 1268 | No protein | Retained intron | 2 | 2: | |
| Plcb3-203 | ENSMUST00000235560.1 | 921 | No protein | Retained intron | - | - | |
| Picb3-205 | ENSMUST00000236597.1 | 604 | No protein | Retained intron | | - | |
| 1 | | | | | | 1 | |

The strategy is based on the design of *Plcb3-201* transcript, the transcription is shown below:



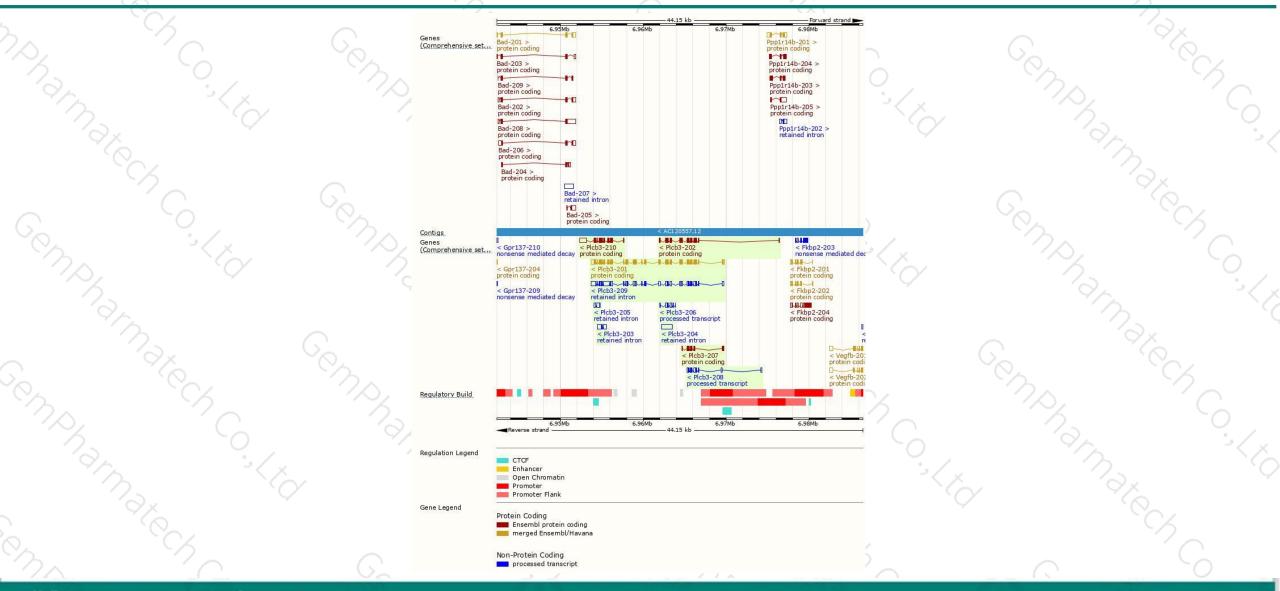
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Reverse strand

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Genomic location distribution



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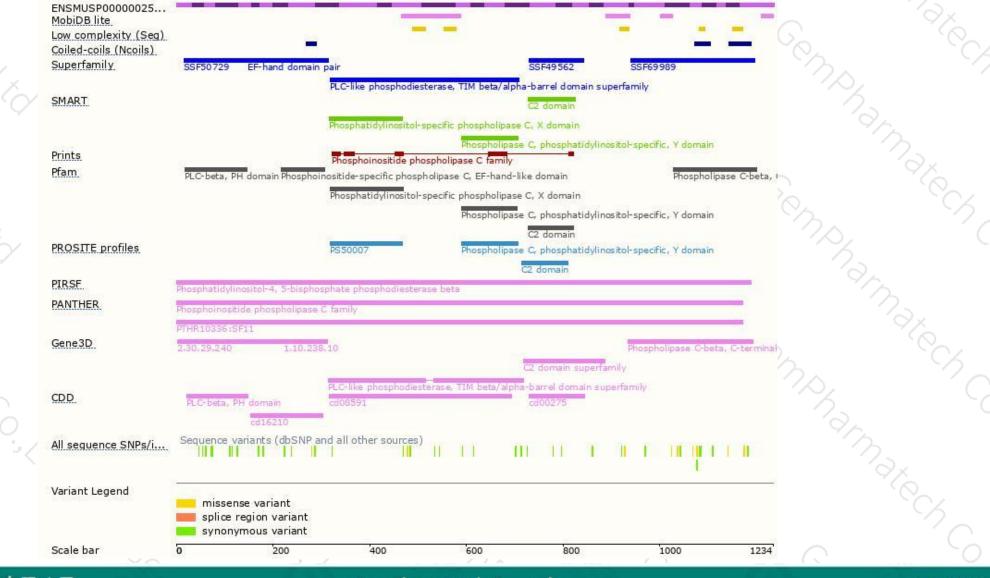
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Protein domain



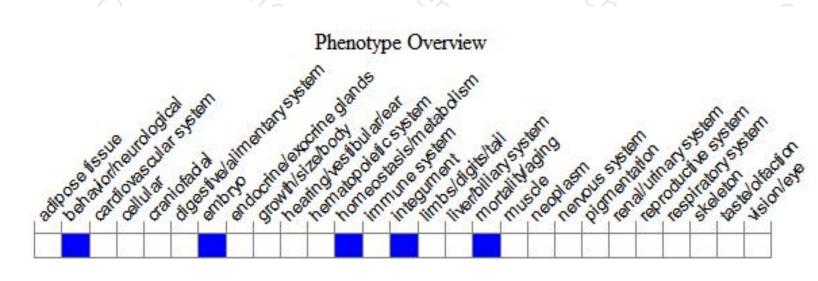


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Mouse phenotype description(MGI)





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 one targeted mutation die at E2.5 and exhibit poor embryonic organization and hypocellularity. Mice homozygous for a second targeted mutation survive to adulthood and exhibit an increased antinocieptive response to opioids.

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If you have any questions, you are welcome to inquire. Tel: 400-9660890



