

***Plcd4* Cas9-CKO Strategy**

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Project Overview

Project Name

Plcd4

Project type

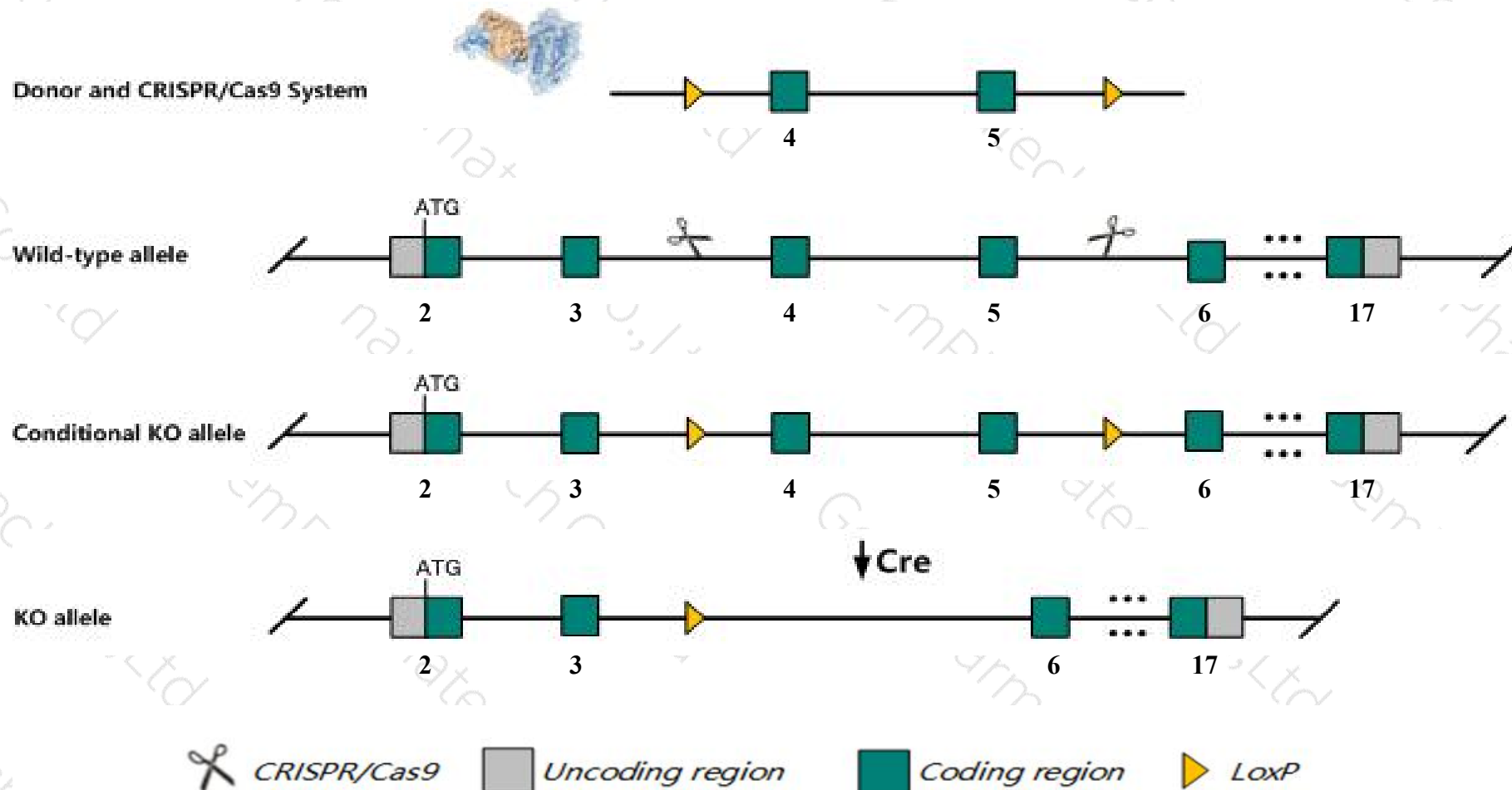
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Plcd4* gene. The schematic diagram is as follows:



Technical routes

- The *Plcd4* gene has 11 transcripts. According to the structure of *Plcd4* gene, exon4-exon5 of *Plcd4-201*(ENSMUST00000027362.13) transcript is recommended as the knockout region. The region contains 359bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Plcd4* 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, males homozygous for a targeted null mutation are subfertile or sterile. Sperm from mutant males fail to initiate the acrosome reaction.
- The *Plcd4* gene is located on the Chr1. 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)

Plcd4 phospholipase C, delta 4 [Mus musculus (house mouse)]

Gene ID: 18802, updated on 13-Mar-2020

Summary



Official Symbol [Plcd4](#) provided by [MGI](#)

Official Full Name [phospholipase C, delta 4](#) provided by [MGI](#)

Primary source [MGI:MGI:107469](#)

See related [Ensembl:ENSMUSG00000026173](#)

Gene type [protein coding](#)

RefSeq status [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 [4921507K24Rik](#)

Expression [Biased expression in testis adult \(RPKM 22.6\), cerebellum adult \(RPKM 4.2\) and 3 other tissues](#)[See more](#)

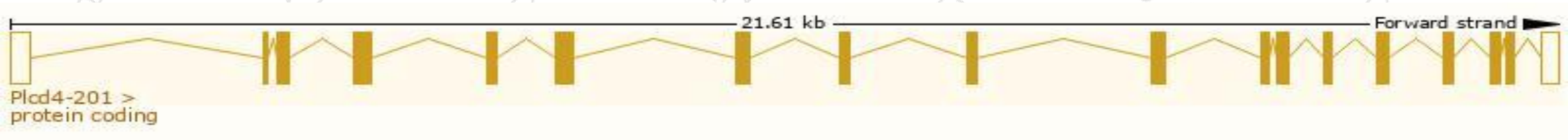
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

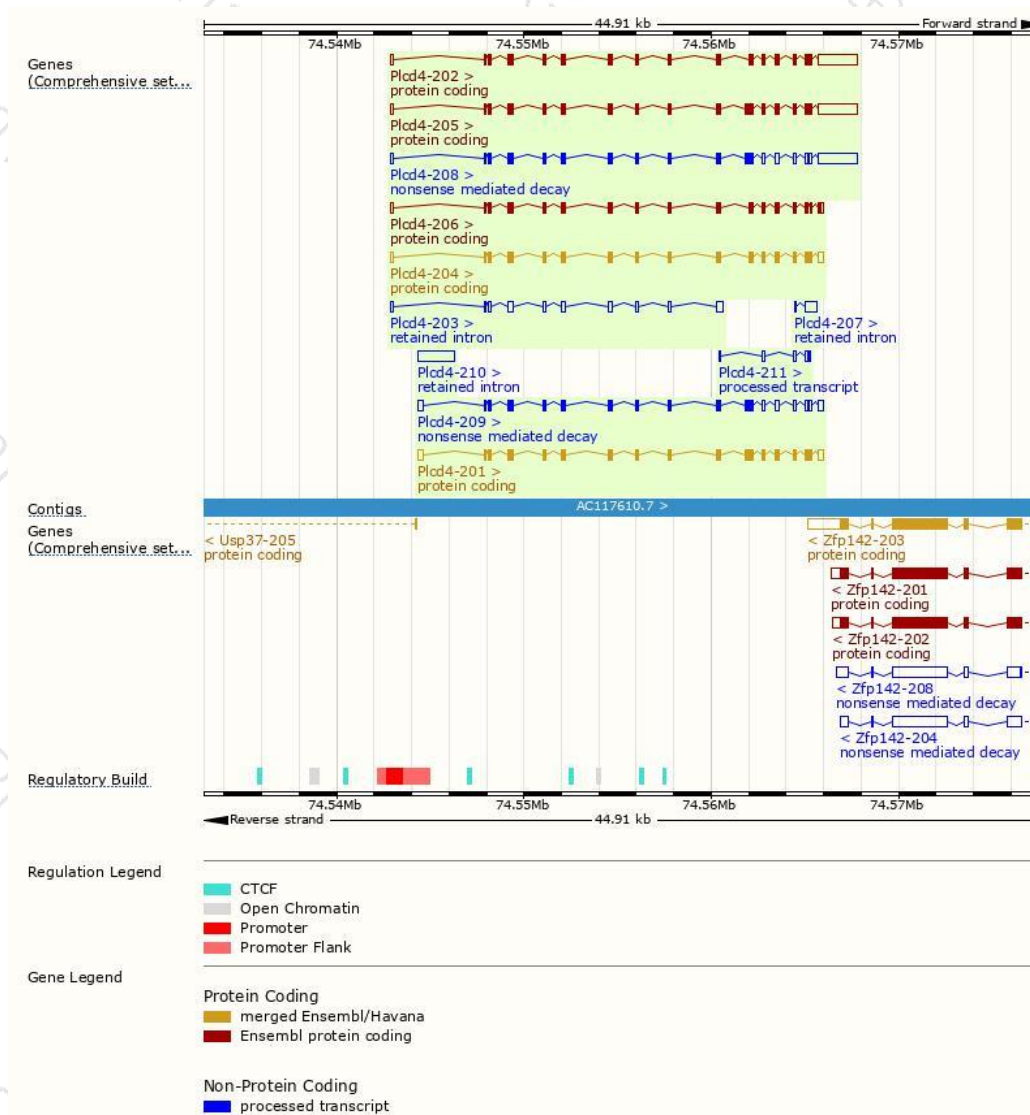
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Plcd4-201	ENSMUST00000027362.13	2977	807aa	Protein coding	CCDS35616	Q8K3R3	TSL:1 GENCODE basic APPRIS P4
Plcd4-204	ENSMUST000000113747.7	2720	775aa	Protein coding	CCDS35617	Q8K3R3	TSL:1 GENCODE basic APPRIS ALT2
Plcd4-205	ENSMUST000000113749.7	4648	807aa	Protein coding	-	Q8K3R3	TSL:5 GENCODE basic APPRIS ALT2
Plcd4-202	ENSMUST000000067916.12	4552	775aa	Protein coding	-	Q8K3R3	TSL:5 GENCODE basic APPRIS ALT2
Plcd4-206	ENSMUST000000113750.7	2692	798aa	Protein coding	-	Q8K3R3	TSL:1 GENCODE basic APPRIS ALT2
Plcd4-208	ENSMUST000000141412.7	4682	574aa	Nonsense mediated decay	-	D6RGN2	TSL:1
Plcd4-209	ENSMUST000000152707.7	3011	574aa	Nonsense mediated decay	-	D6RGN2	TSL:5
Plcd4-211	ENSMUST000000189956.1	512	No protein	Processed transcript	-	-	TSL:3
Plcd4-210	ENSMUST000000185411.1	1912	No protein	Retained intron	-	-	TSL:NA
Plcd4-203	ENSMUST000000113745.7	1749	No protein	Retained intron	-	-	TSL:1
Plcd4-207	ENSMUST000000135111.1	671	No protein	Retained intron	-	-	TSL:3

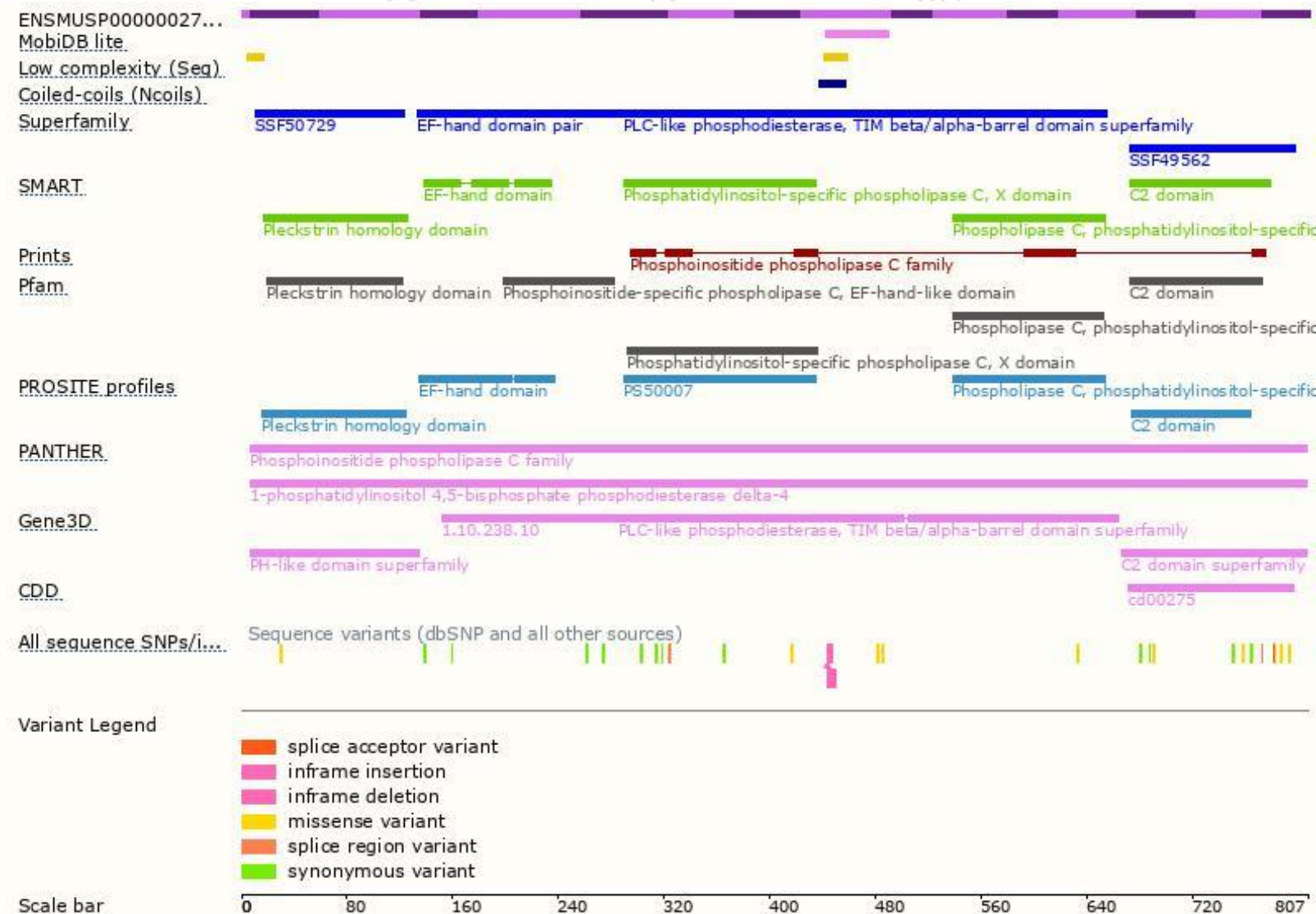
The strategy is based on the design of *Plcd4-201* transcript,the transcription is shown below:



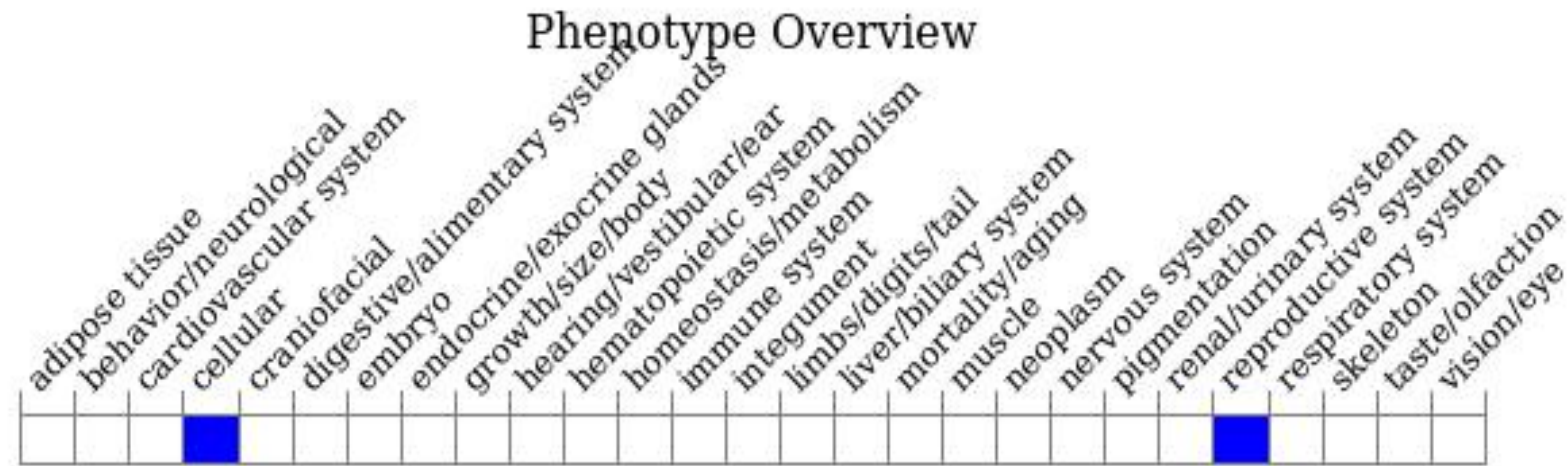
Genomic location distribution



Protein domain



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, males homozygous for a targeted null mutation are subfertile or sterile. Sperm from mutant males fail to initiate the acrosome reaction.

If you have any questions, you are welcome to inquire.

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