

Pld5 Cas9-CKO Strategy

Designer: Longyun Hu

Reviewer: Rui Xiong

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Project Overview



Project Name Pld5

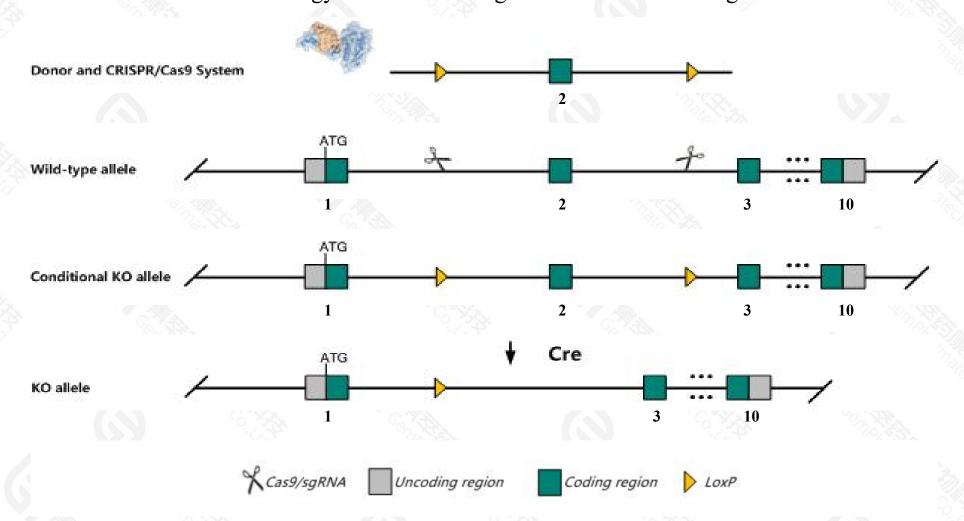
Project type Cas9-CKO

Strain background C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Pld5* gene. The schematic diagram is as follows:



Technical routes



- > The *Pld5* gene has 6 transcripts. According to the structure of *Pld5* gene, exon2 of *Pld5-201*(ENSMUST00000065967.14) transcript is recommended as the knockout region. The region contains 137bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Pld5* 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.

Notice



- > According to the existing MGI data, no abnormal phenotype was observed in a high-throughput screen, nor in a pathology assessment.
- > Transcript *Pld5-202* may not be affected.
- > The *Pld5* 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)



Pld5 phospholipase D family, member 5 [Mus musculus (house mouse)]

Gene ID: 319455, updated on 17-Dec-2020

Summary

☆ ?

Official Symbol Pld5 provided by MGI

Official Full Name phospholipase D family, member 5 provided by MGI

Primary source MGI:MGI:2442056

See related Ensembl: ENSMUSG00000055214

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 B230365F16Rik

Expression Biased expression in cerebellum adult (RPKM 5.0), whole brain E14.5 (RPKM 0.7) and 5 other tissuesSee more

Orthologs <u>human all</u>

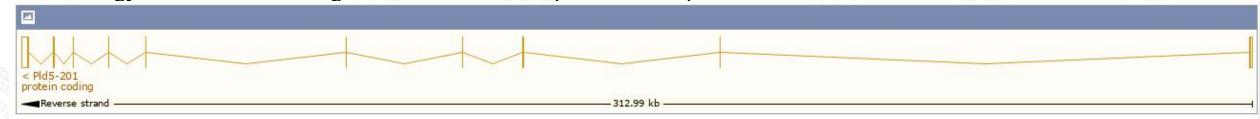
Transcript information (Ensembl)



The gene has 6 transcripts, all transcripts are shown below:

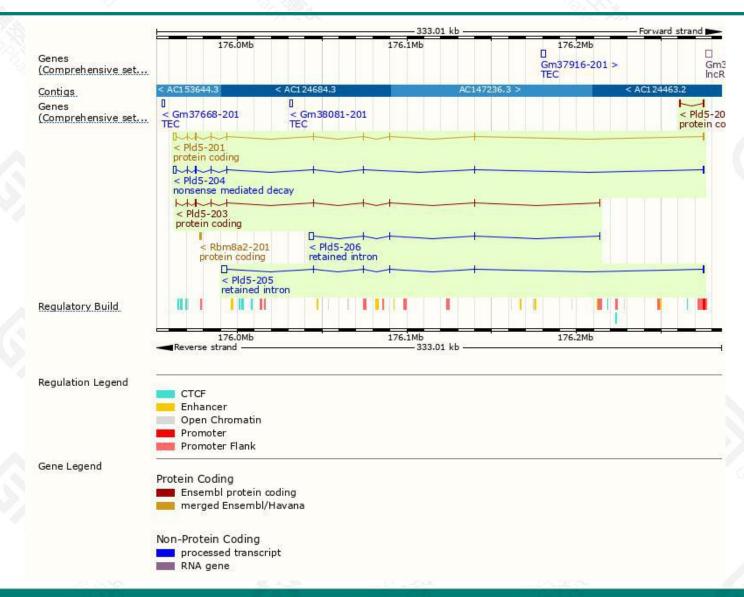
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pld5-201	ENSMUST00000065967.14	3557	536aa	Protein coding	CCDS15552		TSL:1 , GENCODE basic , APPRIS P3 ,
Pld5-203	ENSMUST00000111167.2	1861	474aa	Protein coding	CCDS56660		TSL:1 , GENCODE basic , APPRIS ALT2
Pld5-202	ENSMUST00000111166.2	1370	<u>67aa</u>	Protein coding	-		TSL:1 , GENCODE basic ,
Pld5-204	ENSMUST00000125404.8	3283	109aa	Nonsense mediated decay	-		TSL:1,
Pld5-205	ENSMUST00000144340.8	4215	No protein	Retained intron	2		TSL:5,
Pld5-206	ENSMUST00000156184.2	3274	No protein	Retained intron	25		TSL:1,

The strategy is based on the design of *Pld5-201* transcript, the transcription is shown below:



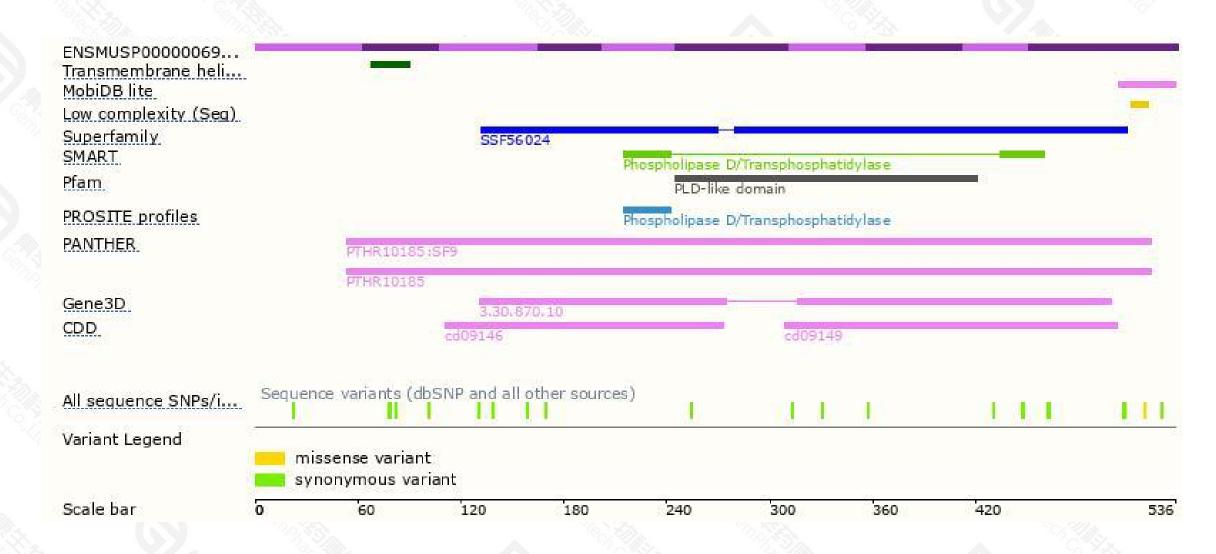
Genomic location distribution





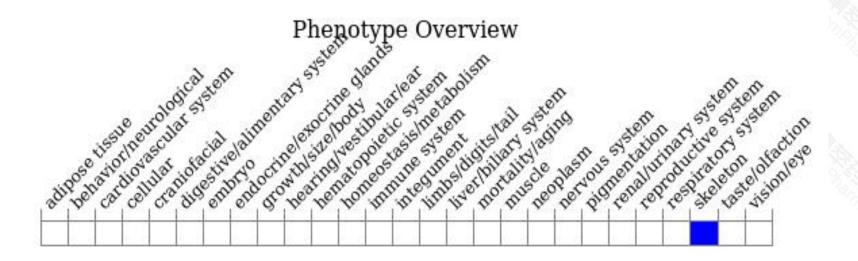
Protein domain





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, no abnormal phenotype was observed in a high-throughput screen, nor in a pathology assessment.



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

Tel: 025-5864 1534





