Pld5 Cas9-KO Strategy

Designer: Longyun Hu

Reviewer: Rui Xiong

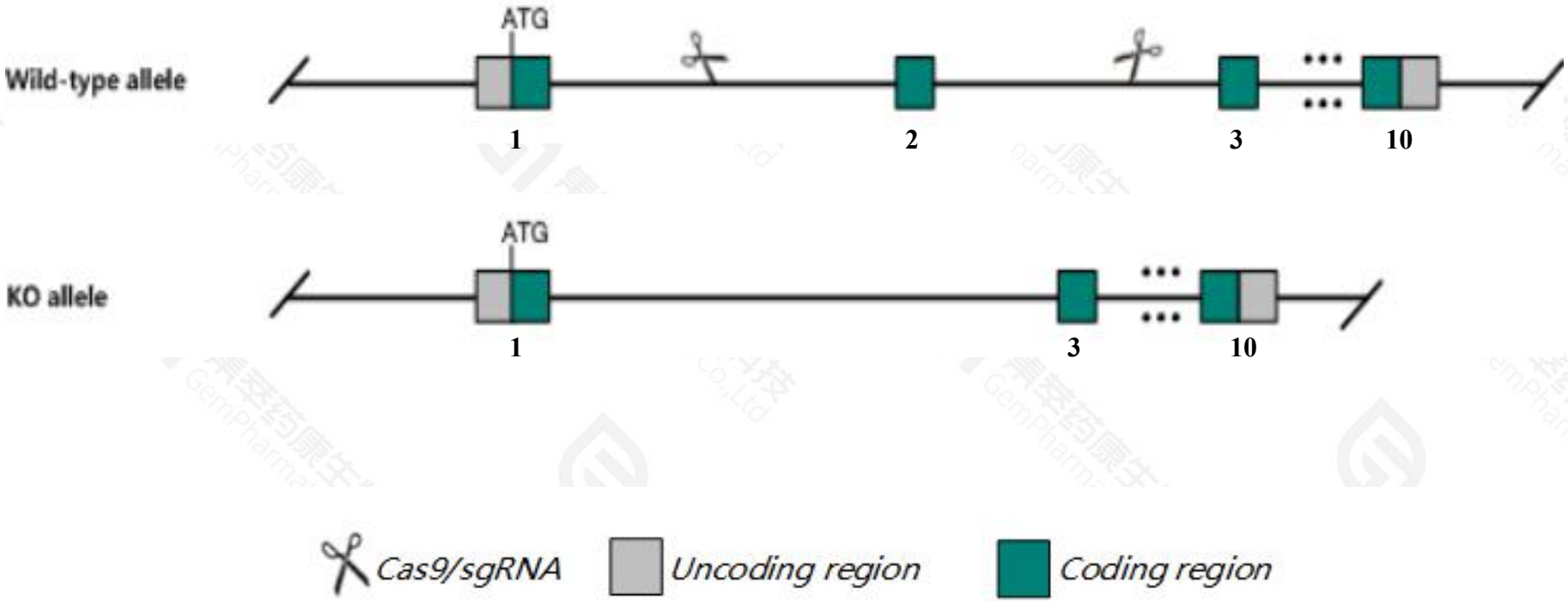
Design Date: 2021-3-22

Project Overview

Project Name	***Pld5***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

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.Cas9 and sgRNA 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.

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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

集萃药康 GemPharmatech

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

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

Summary

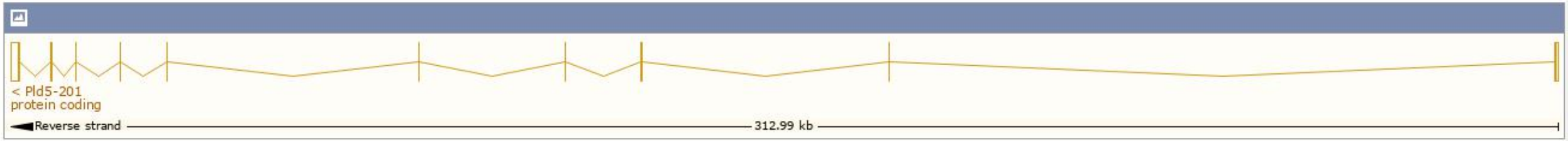
Official Symbol	Pld5 provided by MGI
Official Full Name	phospholipase D family, member 5 provided byMGI
Primary source	MGI:MGI:2442056
See related	Ensembl:ENSMUSG00000055214
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; 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	human all

Transcript information（Ensembl）

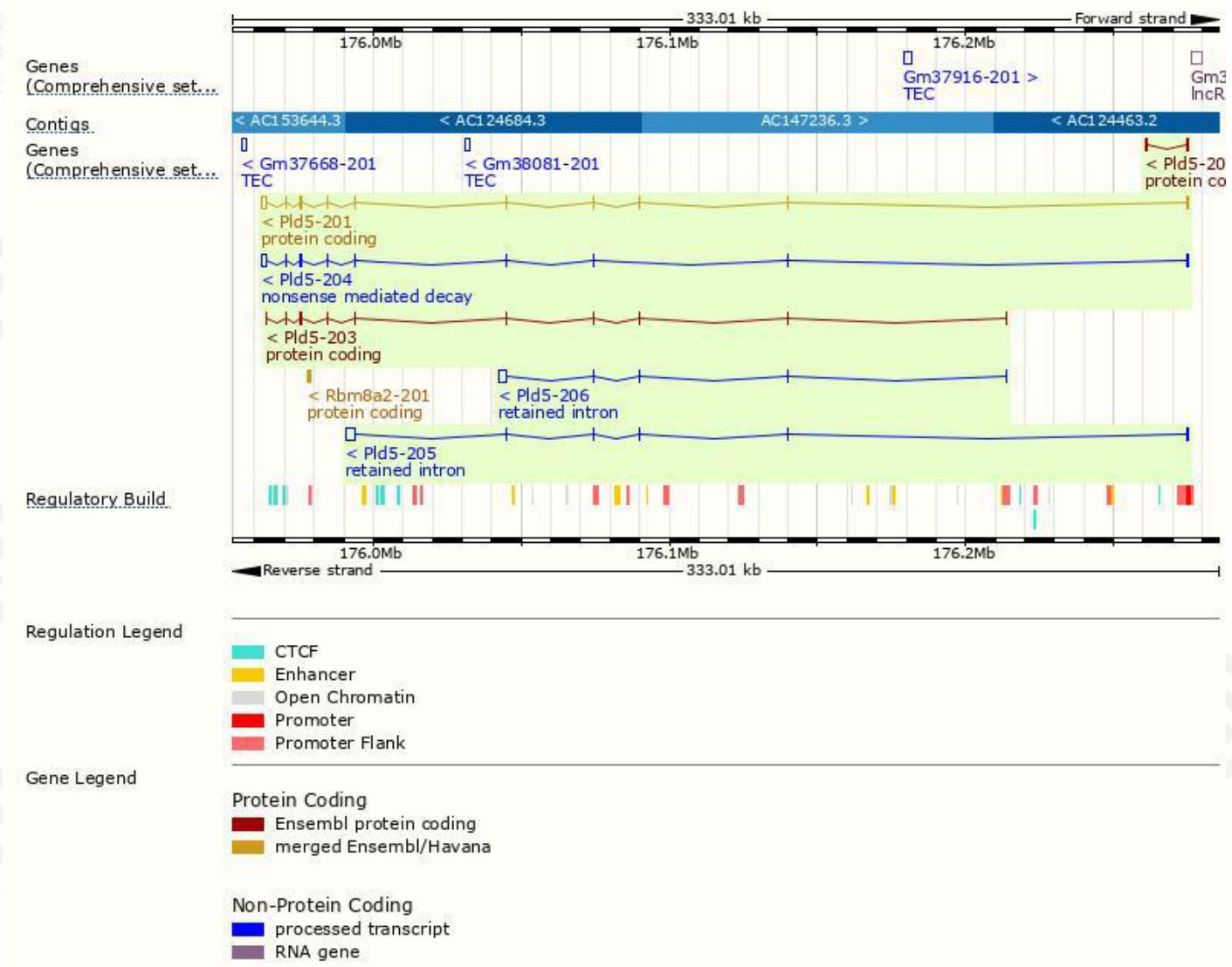
The gene has 6 transcripts,all transcripts are shown below:

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	67aa	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	-		TSL:5 ,
Pld5-206	ENSMUST00000156184.2	3274	No protein	Retained intron	-		TSL:1 ,

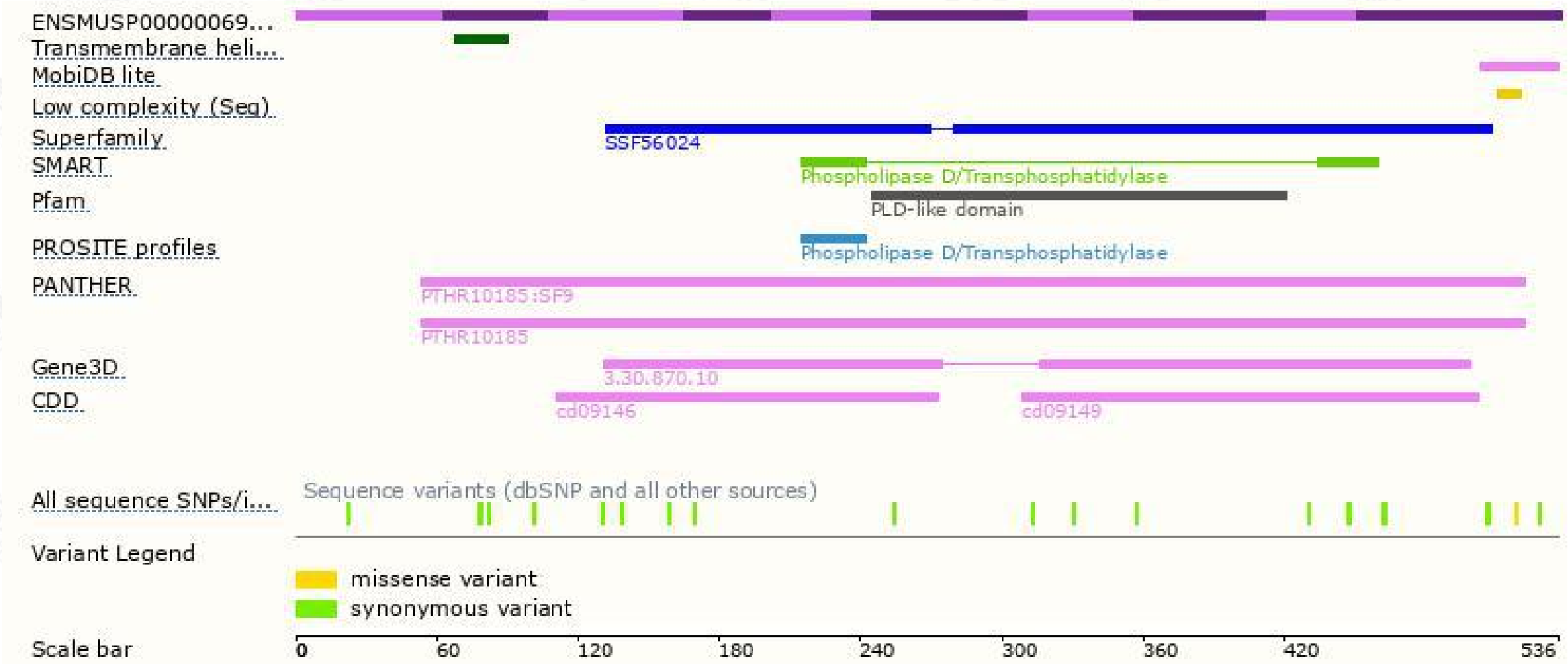
The strategy is based on the design of *Pld5-201* transcript,the transcription is shown below:



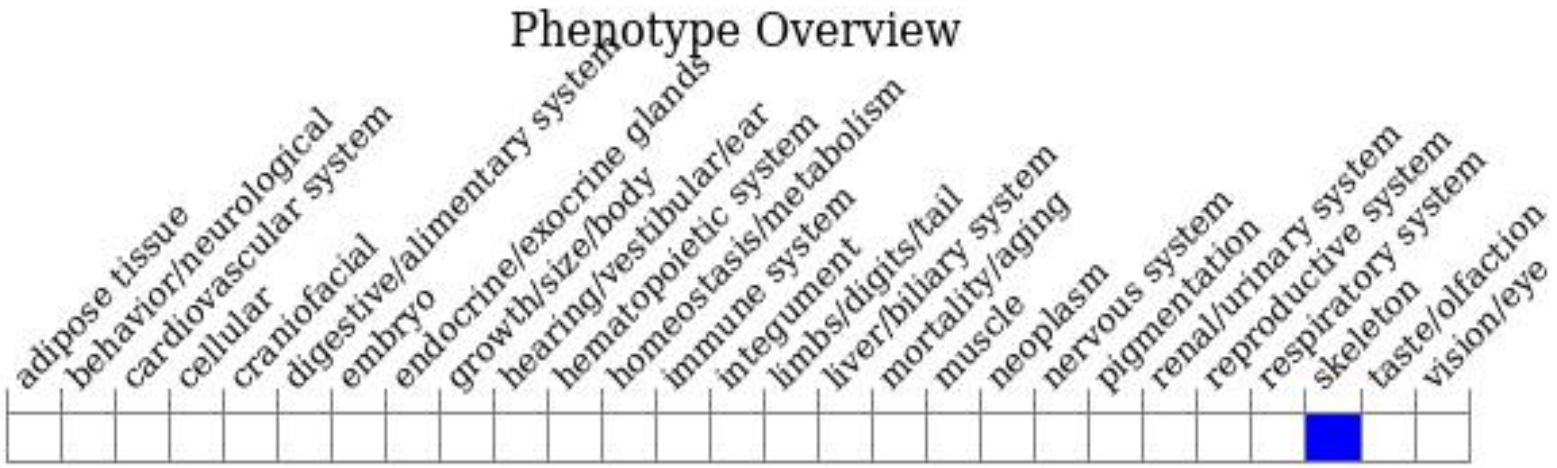
Genomic location distribution



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

集萃药康生物科技
GemPharmatech Co.,Ltd