Pld1 Cas9-CKO *Strategy*

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Reviewer： **JiaYu**

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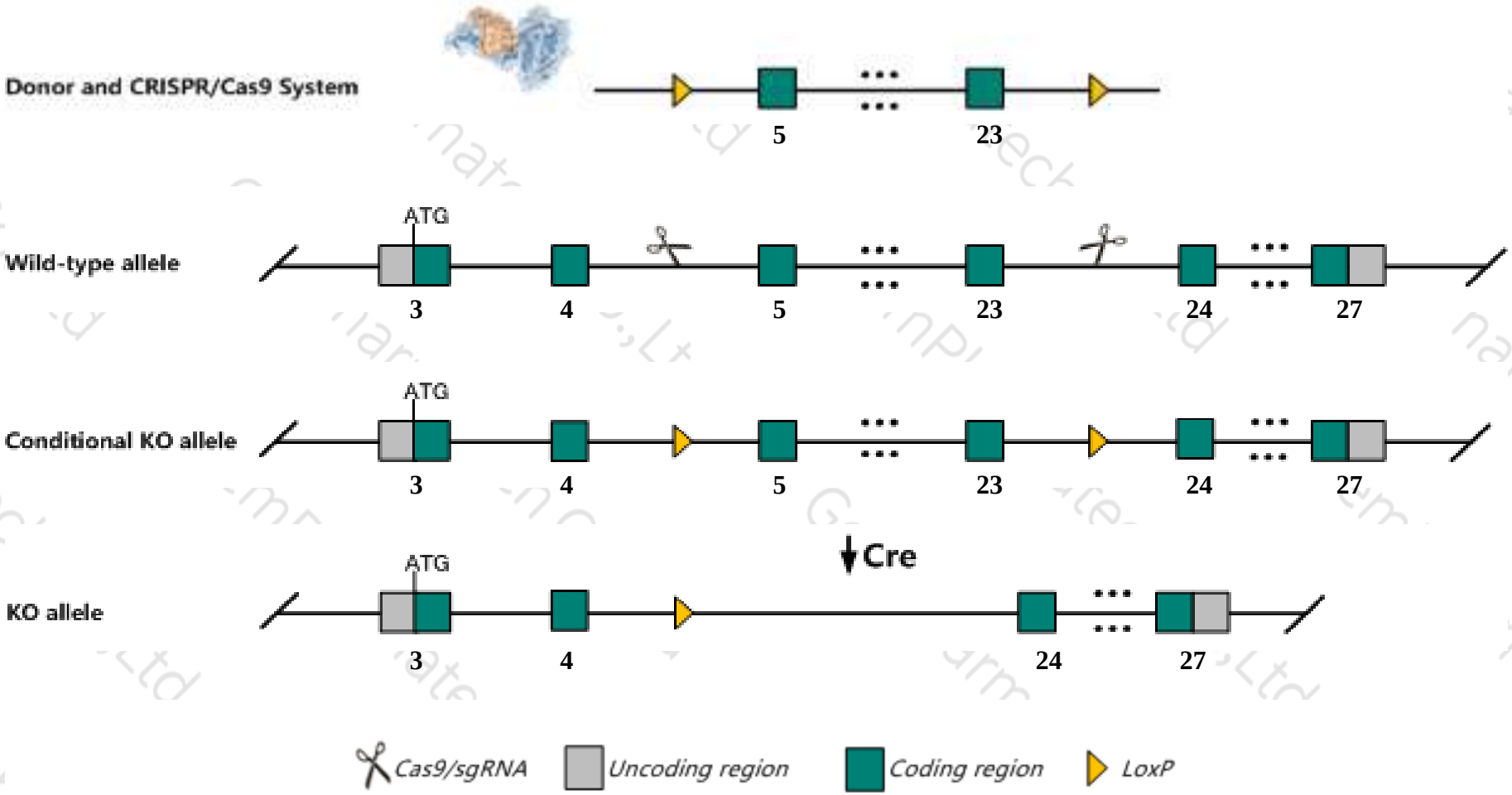
Project Overview

Project Name	***Pld1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pld1* gene. The schematic diagram is as follows:



Technical routes

- The *Pld1* gene has 10 transcripts. According to the structure of *Pld1* gene, exon5-exon23 of *Pld1-202* (ENSMUST00000120834.7) transcript is recommended as the knockout region.The region contains 2191bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Pld1* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector 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, Homozygotes for a null allele show reduced tumor growth and angiogenesis. Homozygotes for a second null allele show abnormal hepatic autophagy after food restriction. Homozygotes for a third null allele show altered platelet activation and protection from thrombosis and ischemic brain injury.

➢The insertion site of 5-terminal Loxp is in the regulatory region of *Pld1*, which may affect the regulation of *Pld1*.

➢ The *Pld1* gene is located on the Chr3. 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）

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Pld1 phospholipase D1 [*Mus musculus* (house mouse)]

Gene ID: 18805, updated on 14-Aug-2019

Summary

Official Symbol	Pld1 provided by MGI
Official Full Name	phospholipase D1 provided by MGI
Primary source	MGI:MGI:109585
See related	Ensembl:ENSMUSG00000027695
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	Pld1a; Pld1b; mPLD1; C85393; AA536939
Expression	Broad expression in large intestine adult (RPKM 8.9), bladder adult (RPKM 4.5) and 25 other tissues See more
Orthologs	human all

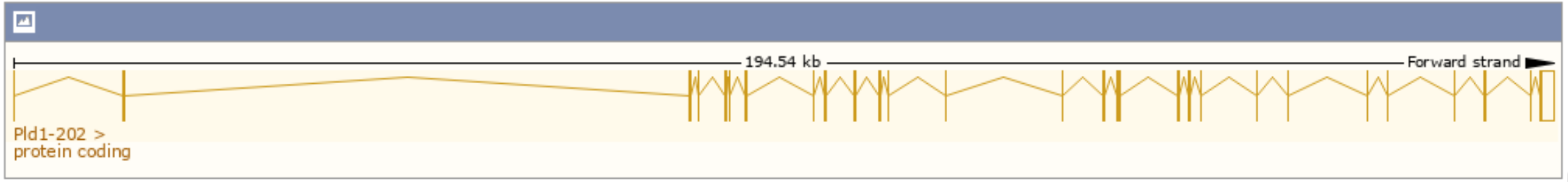
Transcript information（Ensembl）

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

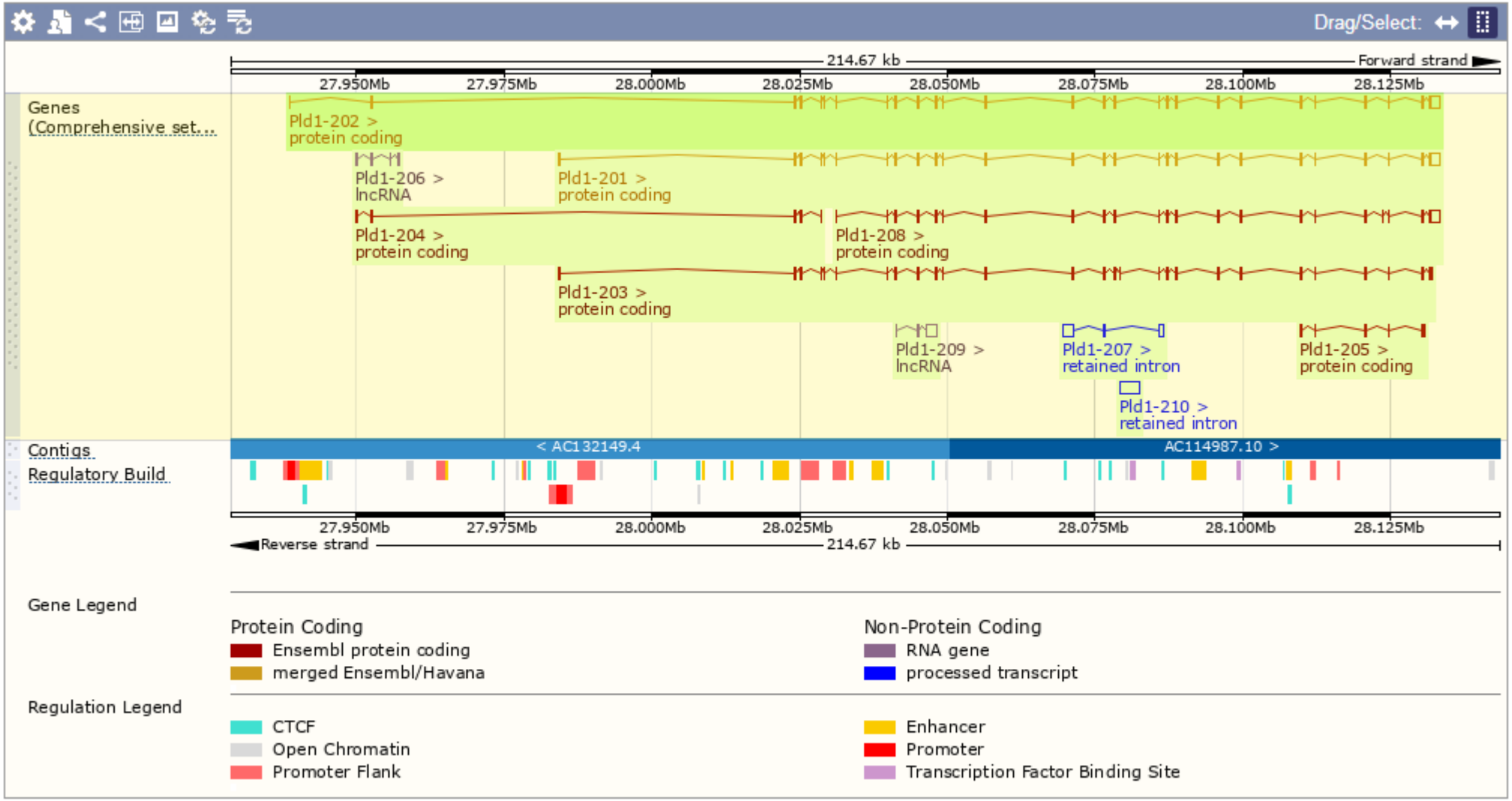
Show/hide columns (1 hidden) Filter

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pld1-202	ENSMUST00000120834.7	4849	1036aa	Protein coding	CCDS17275	Q6NVF2	TSL:1 GENCODE basic APPRIS P2
Pld1-201	ENSMUST00000067757.10	4804	1036aa	Protein coding	CCDS17275	Q6NVF2	TSL:1 GENCODE basic APPRIS P2
Pld1-208	ENSMUST00000148827.7	4239	871aa	Protein coding	-	F6WKY8	CDS 5' incomplete TSL:1
Pld1-203	ENSMUST00000123539.7	3732	1074aa	Protein coding	-	D6RH77	TSL:5 GENCODE basic APPRIS ALT2
Pld1-204	ENSMUST00000125338.7	653	126aa	Protein coding	-	D3Z2V2	CDS 3' incomplete TSL:3
Pld1-205	ENSMUST00000126594.2	603	201aa	Protein coding	-	F6QJR6	CDS 5' and 3' incomplete TSL:2
Pld1-210	ENSMUST00000195622.1	3319	No protein	Retained intron	-	-	TSL:NA
Pld1-207	ENSMUST00000131842.1	2865	No protein	Retained intron	-	-	TSL:1
Pld1-209	ENSMUST00000149017.2	1824	No protein	lncRNA	-	-	TSL:5
Pld1-206	ENSMUST00000127139.7	555	No protein	lncRNA	-	-	TSL:1

The strategy is based on the design of *Pld1-202* transcript,The transcription is shown below

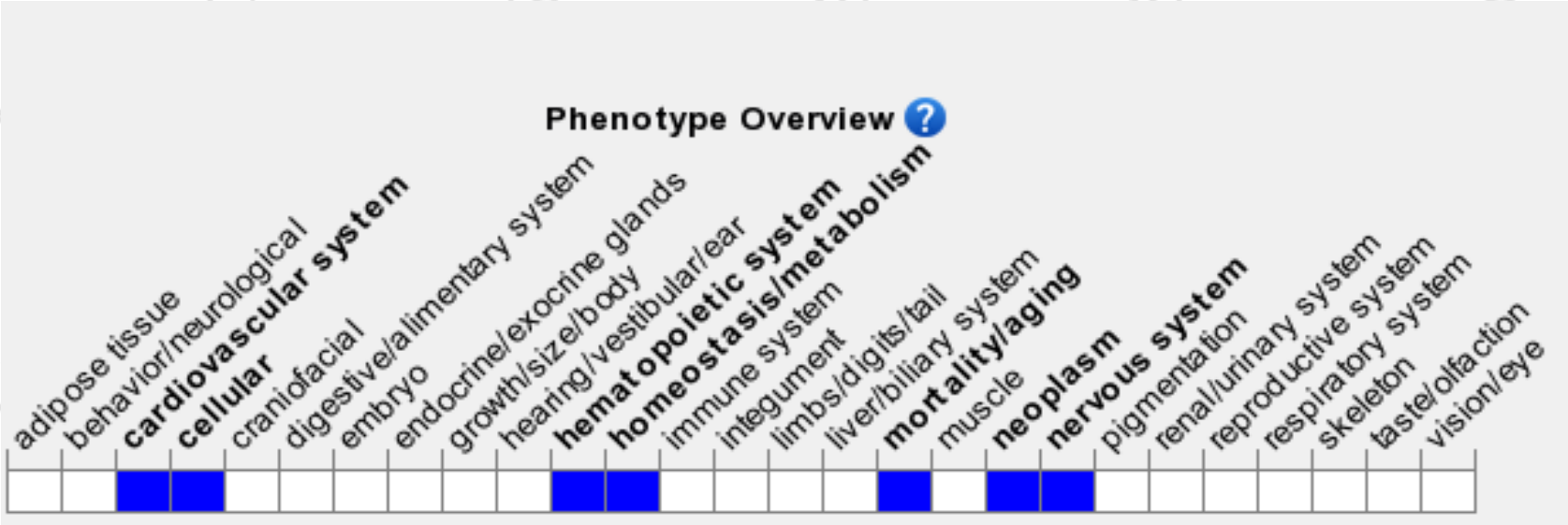


Genomic location distribution



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, Homozygotes for a null allele show reduced tumor growth and angiogenesis. Homozygotes for a second null allele show abnormal hepatic autophagy after food restriction. Homozygotes for a third null allele show altered platelet activation and protection from thrombosis and ischemic brain injury.

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

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