Pnpla2 Cas9-CKO Strategy

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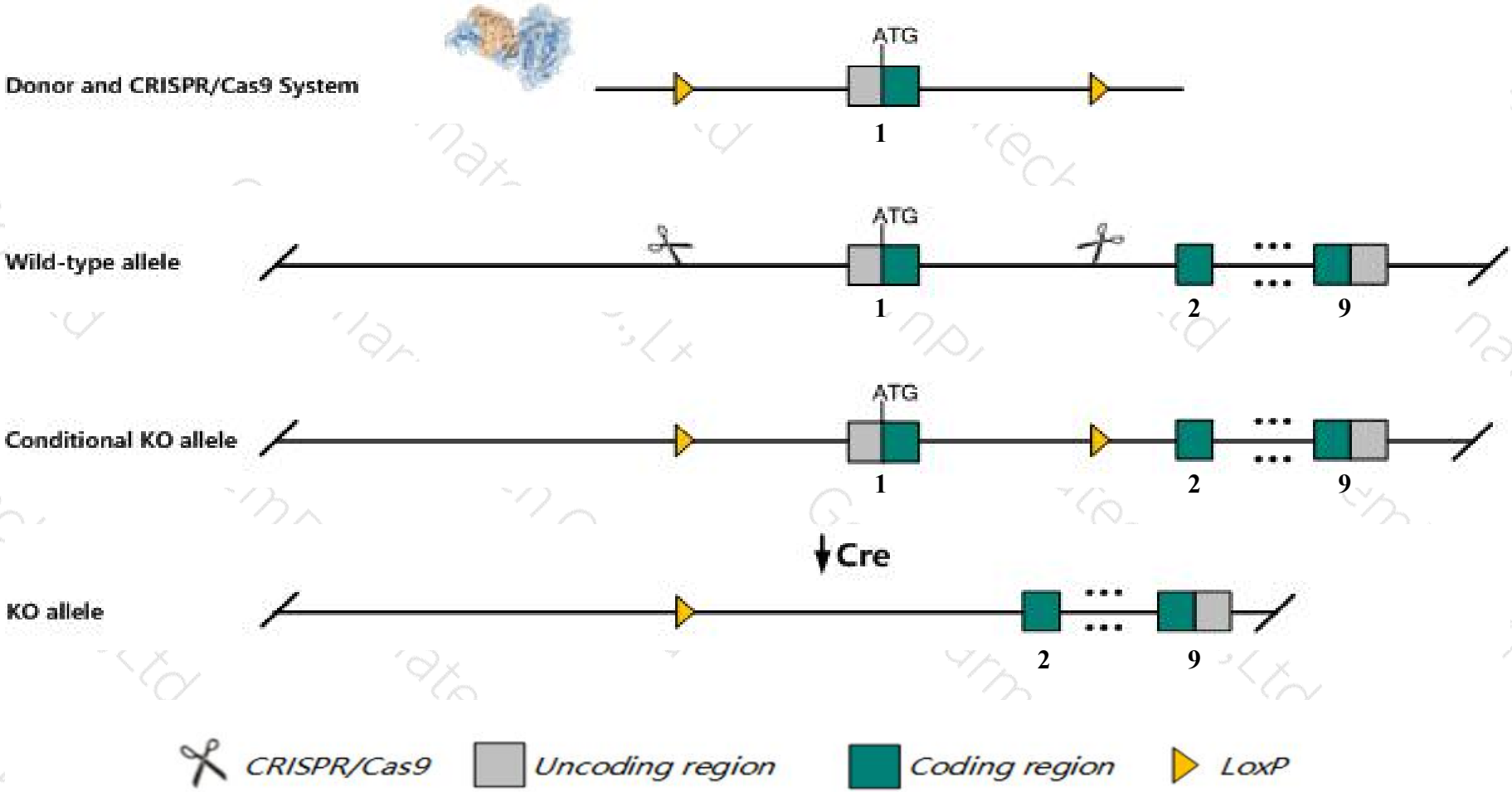
Design Date: 2019-9-28

Project Overview

Project Name	***Pnpla2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pnpla2* gene. The schematic diagram is as follows:



Technical routes

- The *Pnpla2* gene has 7 transcripts. According to the structure of *Pnpla2* gene, exon1 of *Pnpla2-203* (ENSMUST00000164016.7) transcript is recommended as the knockout region.The region contains start codon ATG. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Pnpla2* 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.

Notice

➢ According to the existing MGI data,Mice homozygous for this targeted mutation have defects in lipolysis and altered energy metabolism. Triglyceride accumulation in cardiac muscle leads to severe cardiac dysfunction and mortality.

➢ The *Pnpla2* gene is located on the Chr7. 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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Pnpla2 patatin-like phospholipase domain containing 2 [Mus musculus (house mouse)]

Gene ID: 66853, updated on 5-Feb-2019

Summary

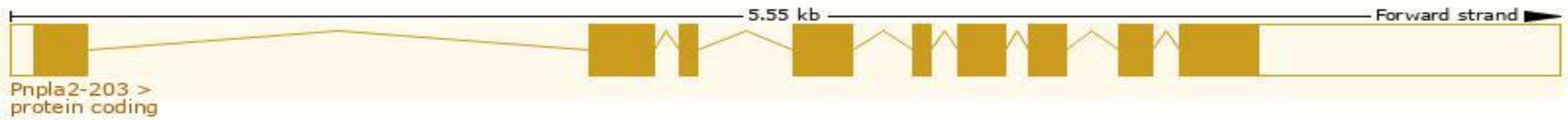
Official Symbol	Pnpla2 provided by MGI
Official Full Name	patatin-like phospholipase domain containing 2 provided byMGI
Primary source	MGI:MGI:1914103
See related	Ensembl:ENSMUSG00000025509
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	0610039C21Rik, 1110001C14Rik, Atgl, TTS-2.2
Expression	Biased expression in mammary gland adult (RPKM 647.4), subcutaneous fat pad adult (RPKM 559.9) and 14 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

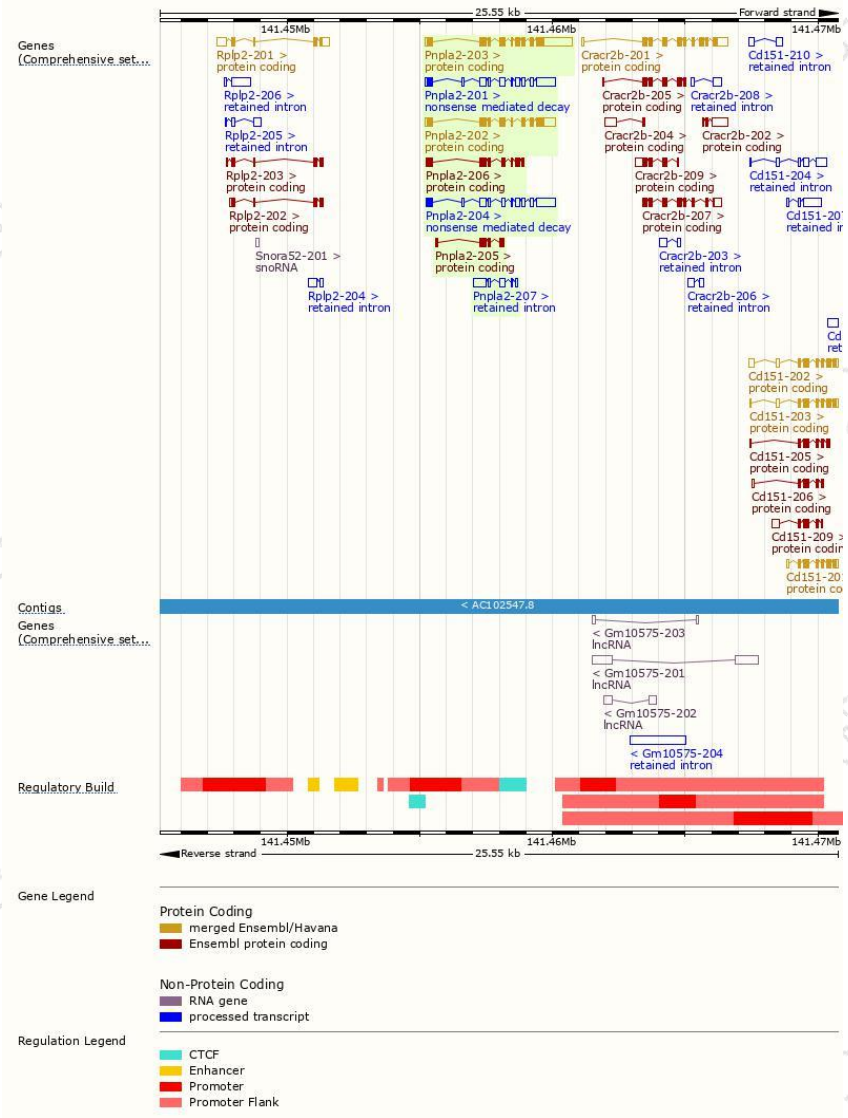
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pnpla2-203	ENSMUST00000164016.7	2625	486aa	Protein coding	CCDS52445	Q8BJ56	TSL:1 GENCODE basic APPRIS ALT2
Pnpla2-202	ENSMUST00000064151.12	1799	430aa	Protein coding	CCDS22015	Q8BJ56	TSL:1 GENCODE basic APPRIS P3
Pnpla2-206	ENSMUST00000169665.7	985	305aa	Protein coding	-	E9PZA4	CDS 3' incomplete TSL:5
Pnpla2-205	ENSMUST00000165487.1	504	158aa	Protein coding	-	E9Q323	CDS 3' incomplete TSL:3
Pnpla2-201	ENSMUST00000026583.14	2048	70aa	Nonsense mediated decay	-	H3BIT5	TSL:1
Pnpla2-204	ENSMUST00000164924.7	1957	70aa	Nonsense mediated decay	-	H3BIT5	TSL:1
Pnpla2-207	ENSMUST00000169723.1	920	No protein	Retained intron	-	-	TSL:2

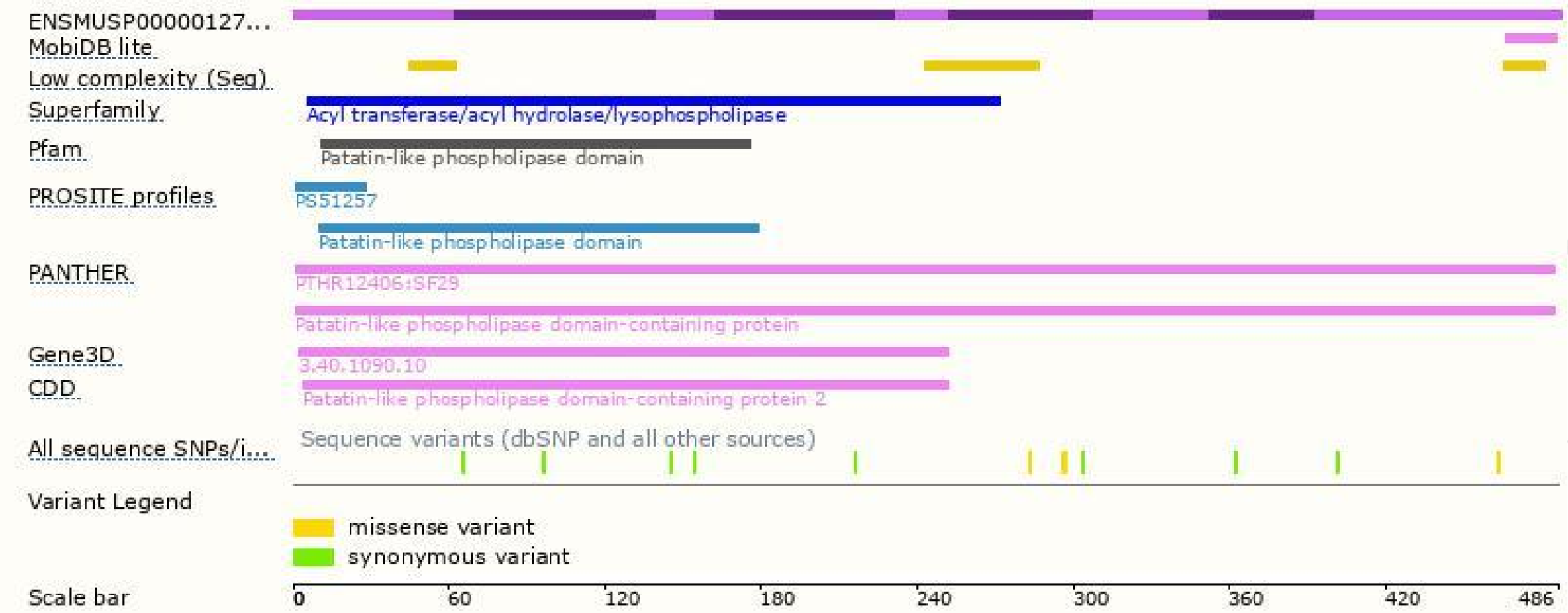
The strategy is based on the design of *Pnpla2-203* transcript,The transcription is shown below



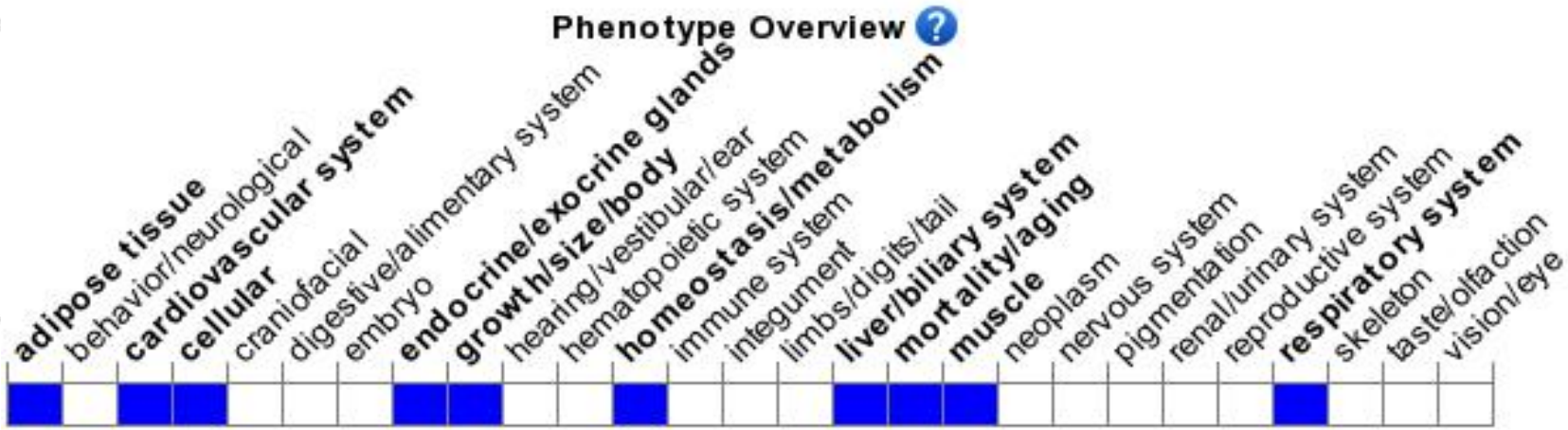
Genomic location distribution



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/).

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

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