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GemPharmatech

Pla2g4a Cas9-CKO Strategy

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Reviewer **Huan Wang**

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Project Overview

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Project Name	***Pla2g4a***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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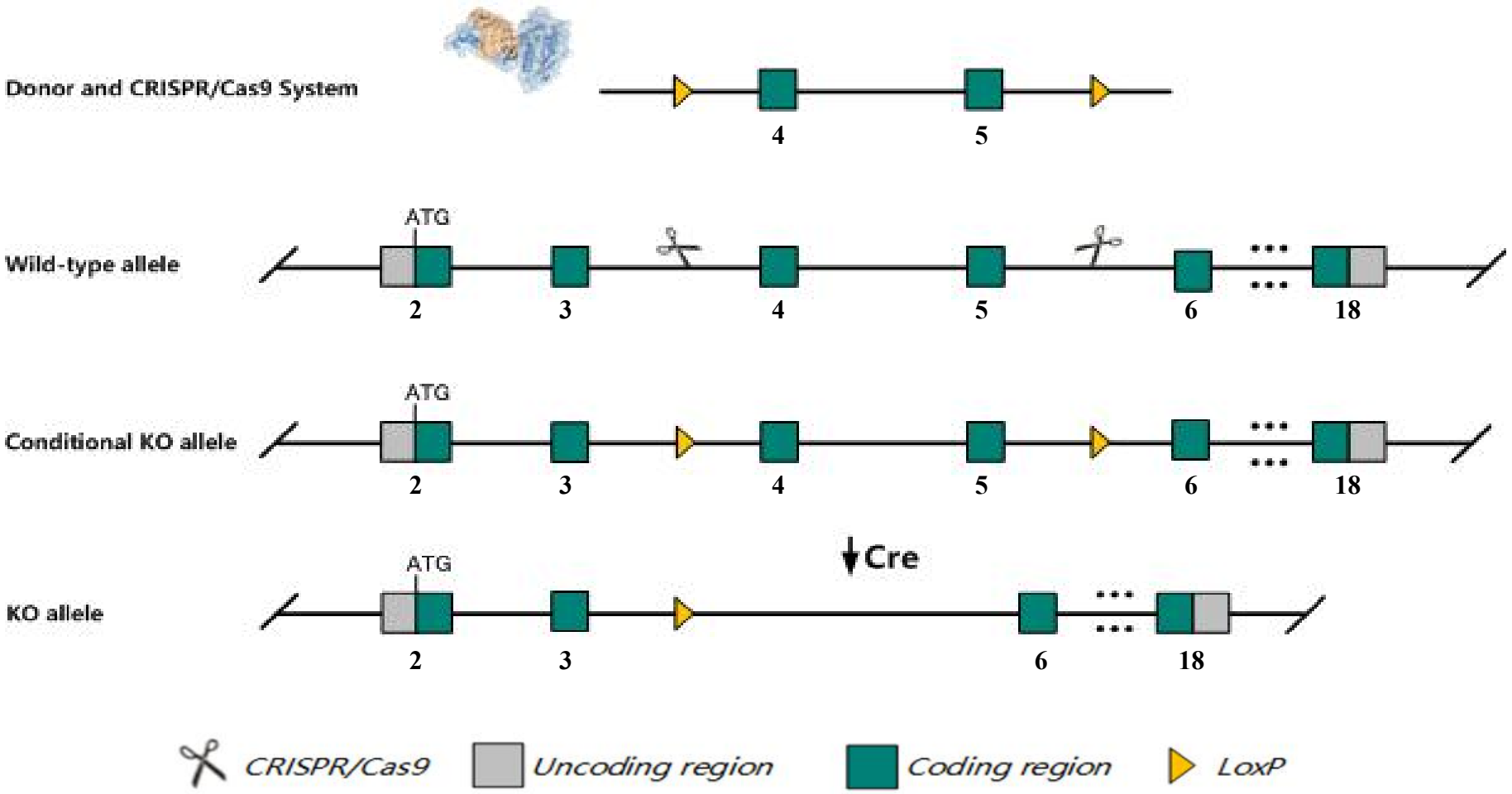
This model will use CRISPR/Cas9 technology to edit the *Pla2g4a* gene. The schematic diagram is as follows:



Technical routes

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The *Pla2g4a* gene has 6 transcripts. According to the structure of *Pla2g4a* gene, exon4-exon5 of *Pla2g4a-201* (ENSMUST00000070200.14) transcript is recommended as the knockout region.The region contains 263bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Pla2g4a* 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 homozygouse for disruptions in this gene display reduced allergic and autoimmune reactions. They also display an increased incidence of insulin and reduced female reproductive performance.

The *Pla2g4a* 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

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Pla2g4a phospholipase A2, group IVA (cytosolic, calcium-dependent) [Mus musculus (house mouse)]

Gene ID: 18783, updated on 12-Feb-2019

Summary

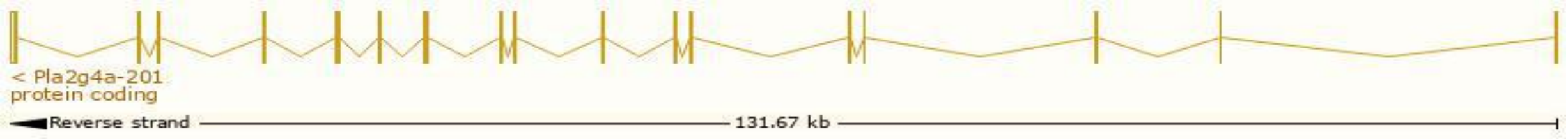
Official Symbol	Pla2g4a provided by MGI
Official Full Name	phospholipase A2, group IVA (cytosolic, calcium-dependent) provided by MGI
Primary source	MGI:MGI:1195256
See related	Ensembl:ENSMUSG00000056220
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Pla2g4, cPLA2, cPLA2-alpha, cPLA2alpha
Summary	The protein encoded by this gene is a member of the phospholipase A2 group IV family. This enzyme hydrolyzes membrane phospholipids, thereby releasing the polyunsaturated fatty acid, arachidonic acid. Arachidonic acid is further metabolized into eicosanoids such as leukotrienes, thromboxanes and prostaglandins, that play important roles in regulating diverse biological processes such as inflammatory responses, membrane and actin dynamics, and tumorigenesis. A rise in intracellular calcium levels results in binding of calcium to the C2 domain of this protein, and triggers the translocation from the cytosol to intracellular membranes, including the Golgi apparatus. Disruption of this gene in mice led to decreased levels of eicosonaoids and platelet-activating factor, decreased allergic symptoms, and impaired reproductive ability in females. Alternative splicing results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Mar 2015]
Expression	Biased expression in bladder adult (RPKM 17.0), placenta adult (RPKM 4.1) and 12 other tissues See more
Orthologs	human all

Transcript information Ensembl

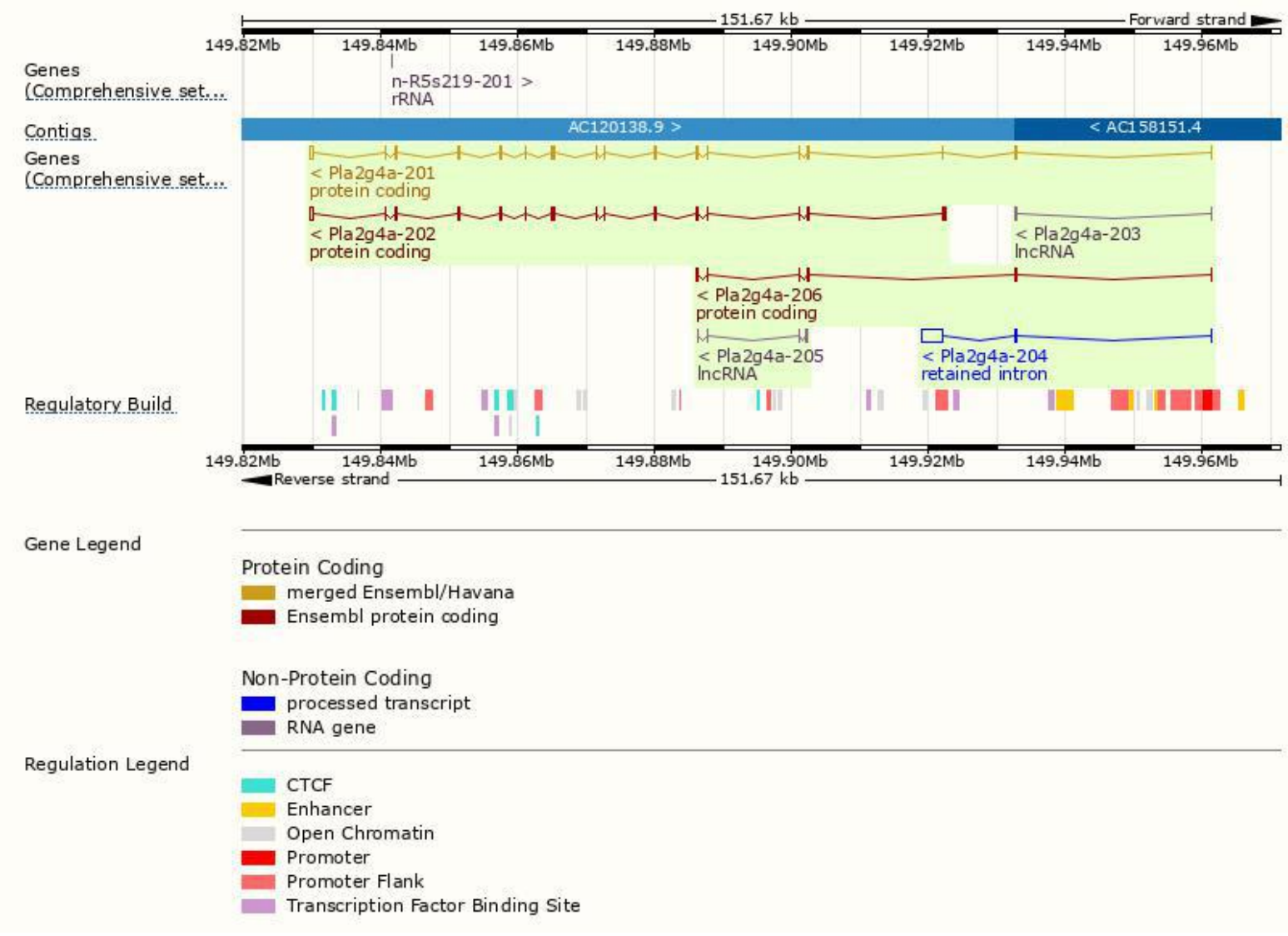
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pla2g4a-201	ENSMUST00000070200.14	2856	748aa	Protein coding	CCDS15352	P47713 Q3UMQ1	TSL:1 GENCODE basic APPRIS P1
Pla2g4a-202	ENSMUST00000111926.8	2835	740aa	Protein coding	CCDS78700	Q9DBX5	TSL:1 GENCODE basic
Pla2g4a-206	ENSMUST00000190507.1	613	149aa	Protein coding	-	A0A087WPN8	CDS 3' incomplete TSL:2
Pla2g4a-204	ENSMUST00000142040.1	3453	No protein	Retained intron	-	-	TSL:2
Pla2g4a-205	ENSMUST00000155438.1	332	No protein	lncRNA	-	-	TSL:3
Pla2g4a-203	ENSMUST00000134676.1	247	No protein	lncRNA	-	-	TSL:3

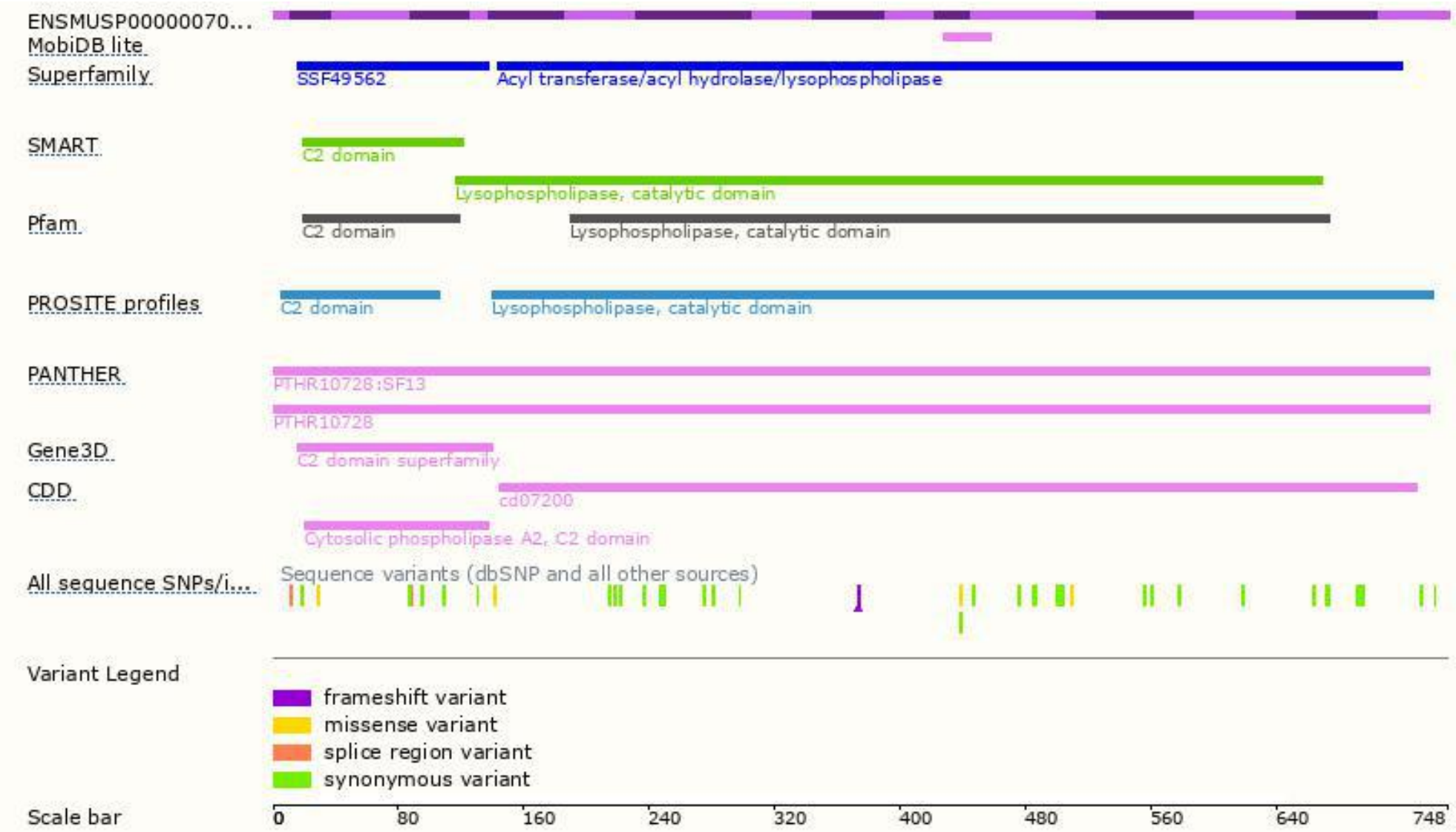
The strategy is based on the design of *Pla2g4a-201* transcript,The transcription is shown below



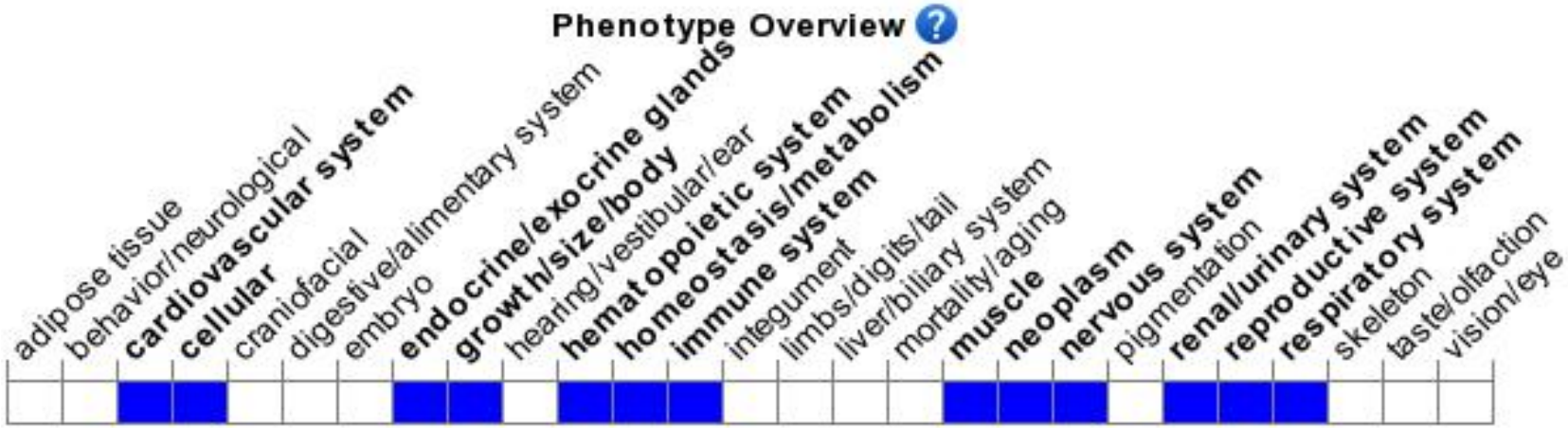
Genomic location distribution



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,Mice homozygouse for disruptions in this gene display reduced allergic and autoimmune reactions. They also display an increased incidence of insulin and reduced female reproductive performance.

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

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