

***Plag1* Cas9-CKO Strategy**

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Design Date: 2019-7-22

Project Overview

Project Name

Plag1

Project type

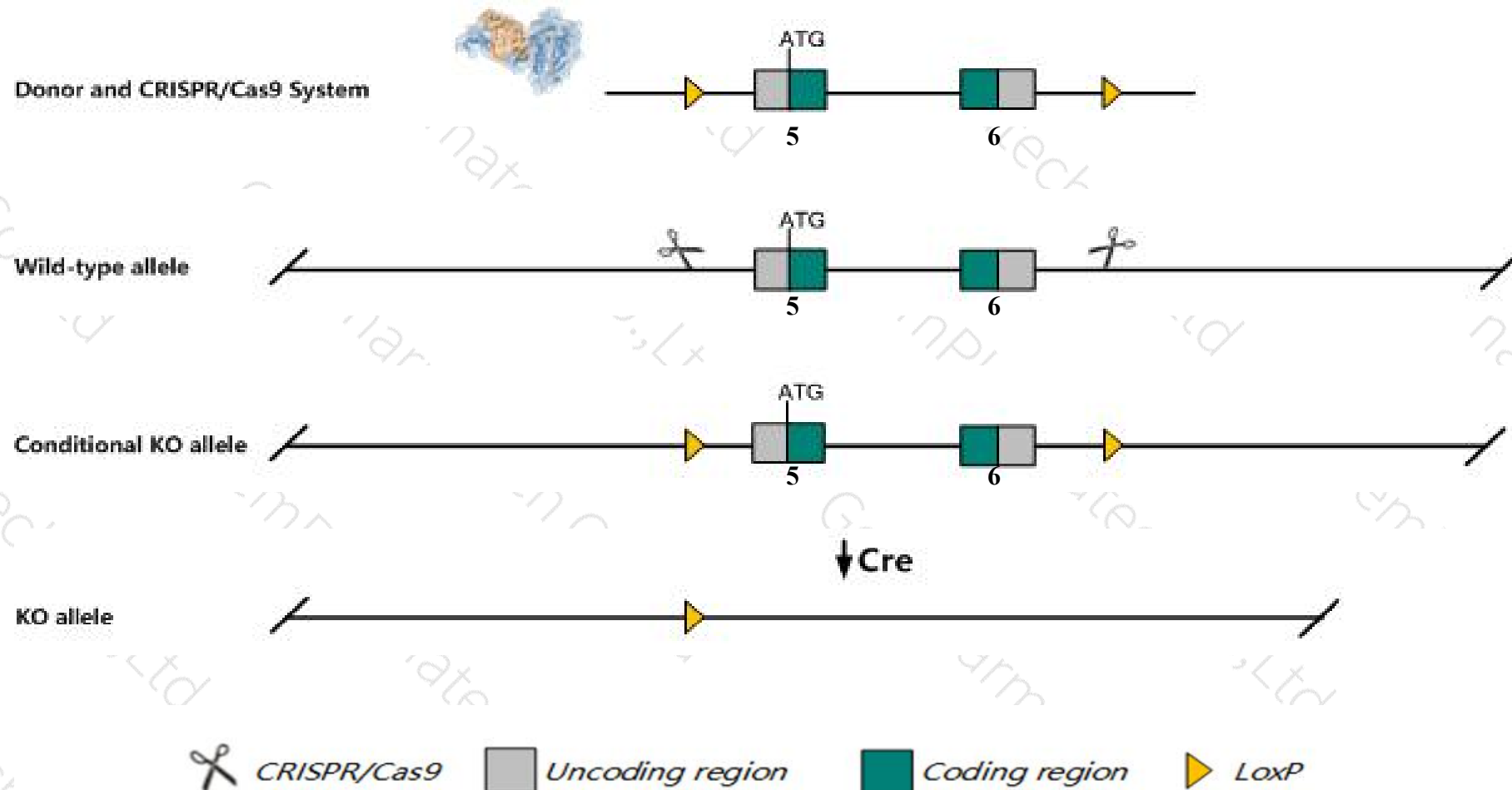
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Plagl1* gene. The schematic diagram is as follows:



- The *Plagl1* gene has 4 transcripts. According to the structure of *Plagl1* gene, exon5-exon6 of *Plagl1-201* (ENSMUST00000003369.9) transcript is recommended as the knockout region. The region contains all the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Plagl1* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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.

- According to the existing MGI data, Homozygous null mice display reduced male fertility, small seminal vesicles and ventral prostate, reduced litter size (females only), reduced embryonic and postnatal growth, and delayed eyelid opening.
- The *Plagl1* gene is located on the Chr4. 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.
- Transcript *Plagl1*-203 may not be affected .
- 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)

Plag1 pleiomorphic adenoma gene 1 [*Mus musculus* (house mouse)]

Gene ID: 56711, updated on 11-May-2019

Summary

Official Symbol Plag1 provided by [MGI](#)
Official Full Name pleiomorphic adenoma gene 1 provided by [MGI](#)
Primary source [MGI:MGI:1891916](#)
See related [Ensembl:ENSMUSG00000003282](#)
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
Expression Broad expression in limb E14.5 (RPKM 2.3), CNS E11.5 (RPKM 1.2) and 15 other tissues [See more](#)
Orthologs [human](#) [all](#)

Genomic context

Location: 4; 4 A1

See Plag1 in [Genome Data Viewer](#)

Exon count: 8

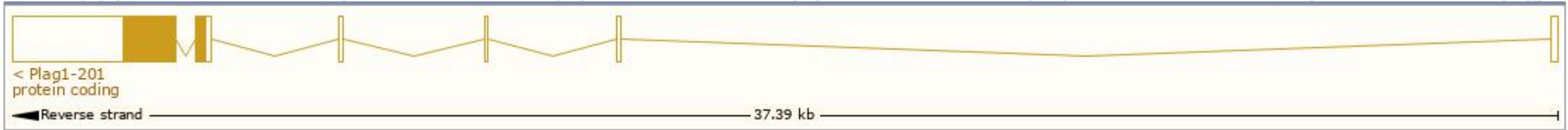
Annotation release	Status	Assembly	Chr	Location
106	current	GRCm38.p4 (GCF_000001635.24)	4	NC_000070.6 (3898769..3938435, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	4	NC_000070.5 (3828305..3865552, complement)

Transcript information (Ensembl)

The gene has 4 transcripts,all transcripts are shown below:

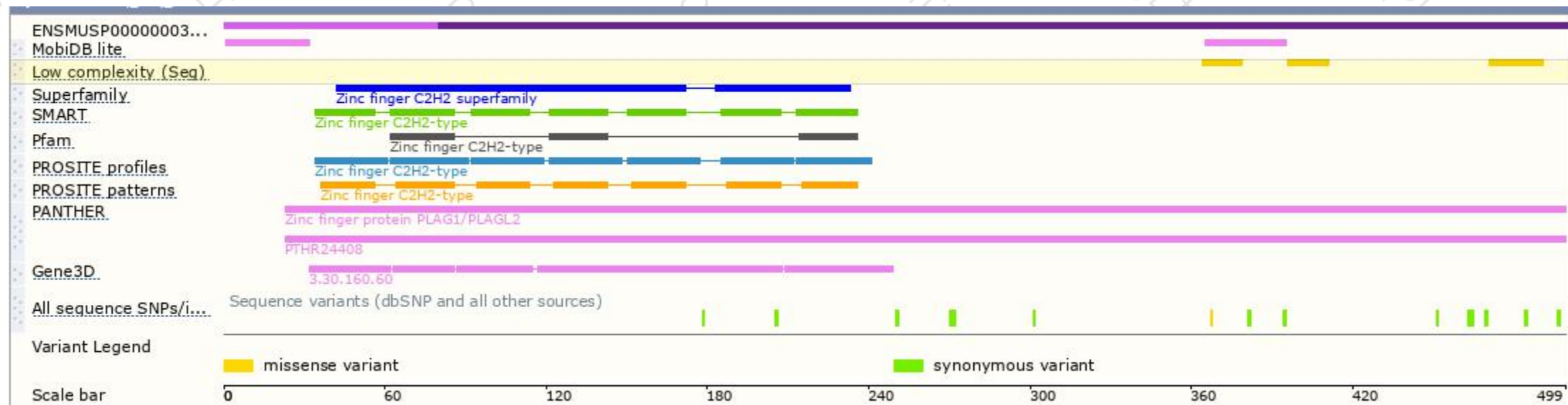
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Plag1-201	ENSMUST00000003369.9	4774	499aa	Protein coding	CCDS17941	Q9QYE0	TSL:1 GENCODE basic APPRIS P1
Plag1-202	ENSMUST00000137439.7	452	43aa	Nonsense mediated decay	-	D6RES7	TSL:2
Plag1-203	ENSMUST00000147035.1	4049	No protein	Retained intron	-	-	TSL:1
Plag1-204	ENSMUST00000151543.1	669	43aa	Nonsense mediated decay	-	D6RES7	TSL:3

The strategy is based on the design of *Plag1-201* transcript,The transcription is shown below

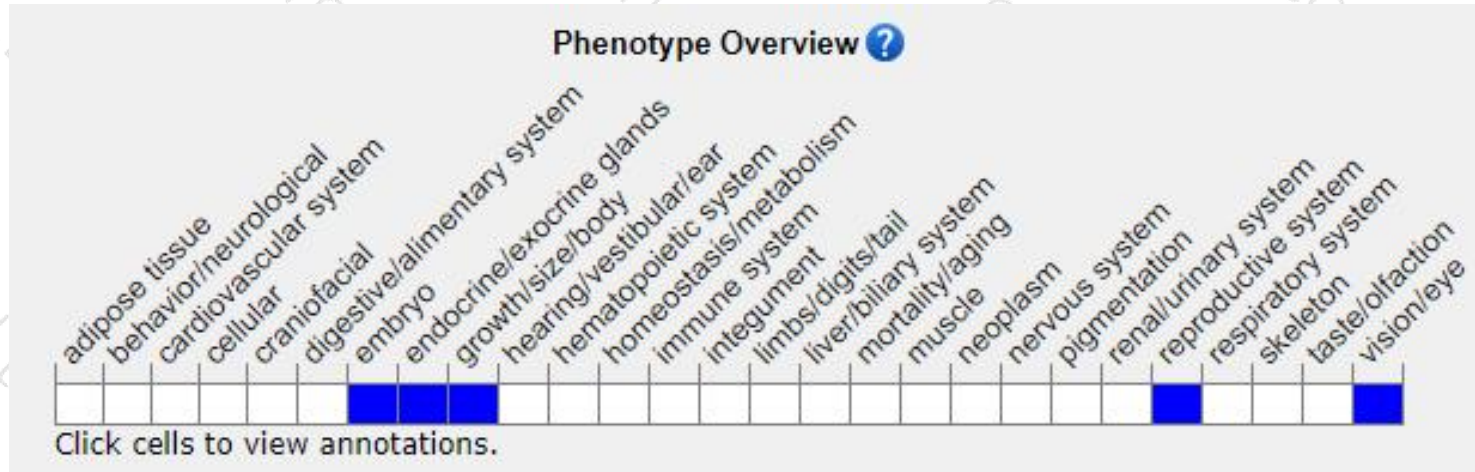


集萃药康
GemPharmatech

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/>).

Homozygous null mice display reduced male fertility, small seminal vesicles and ventral prostate, reduced litter size (females only), reduced embryonic and postnatal growth, and delayed eyelid opening.

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

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