Lama5 Cas9-CKO Strategy

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Design Date: 2019-9-11
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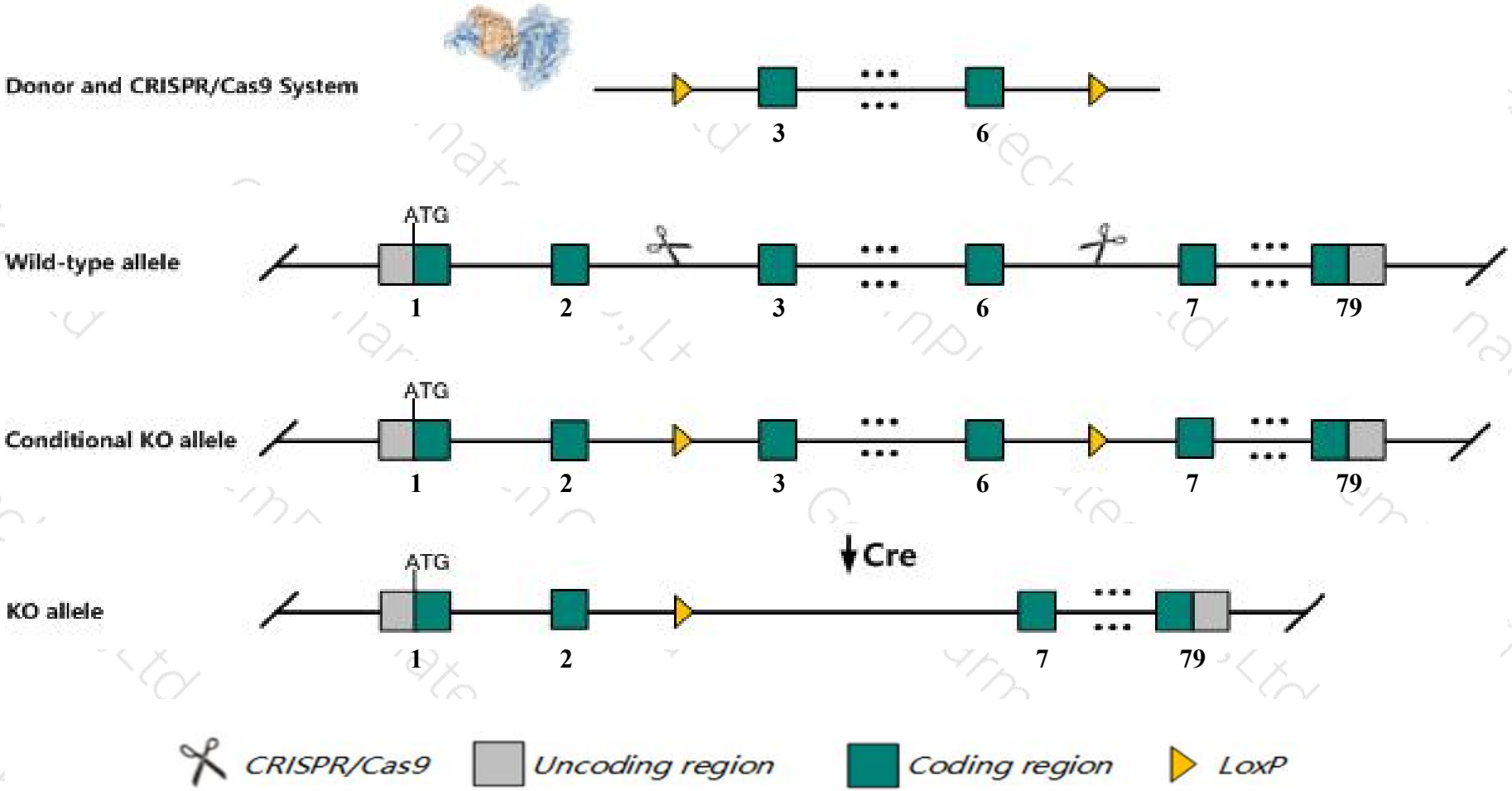
Project Overview

Project Name	***Lama5***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Lama5* gene. The schematic diagram is as follows:



Technical routes

- The *Lama5* gene has 4 transcripts. According to the structure of *Lama5* gene, exon3-exon6 of *Lama5-201* (ENSMUST00000015791.5) transcript is recommended as the knockout region.The region contains 506bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Lama5* 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,Homozygotes for a targeted null mutation exhibit disrupted basal laminae leading to exencephaly, syndactyly, placentopathy, kidney defects, abnormal lobar septation with absence of a visceral pleural membrane, and lethality in late gestation.

➢ The *Lama5* gene is located on the Chr2. 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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Lama5 laminin, alpha 5 [Mus musculus (house mouse)]

Gene ID: 16776, updated on 12-Mar-2019

Summary

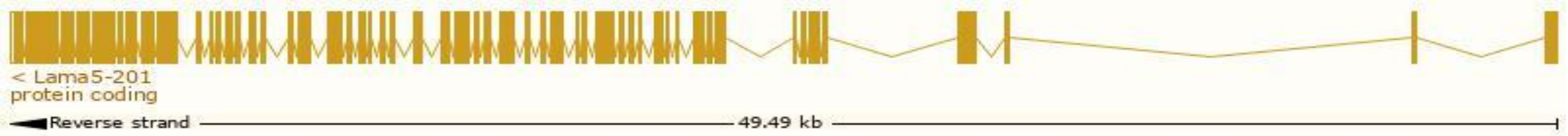
Official Symbol	Lama5 provided by MGI
Official Full Name	laminin, alpha 5 provided by MGI
Primary source	MGI:MGI:105382
See related	Ensembl:ENSMUSG00000015647
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AA408760, AA408762, AI853660, [a]5, laminin-511, mKIAA0533
Expression	Broad expression in lung adult (RPKM 40.7), mammary gland adult (RPKM 30.3) and 16 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

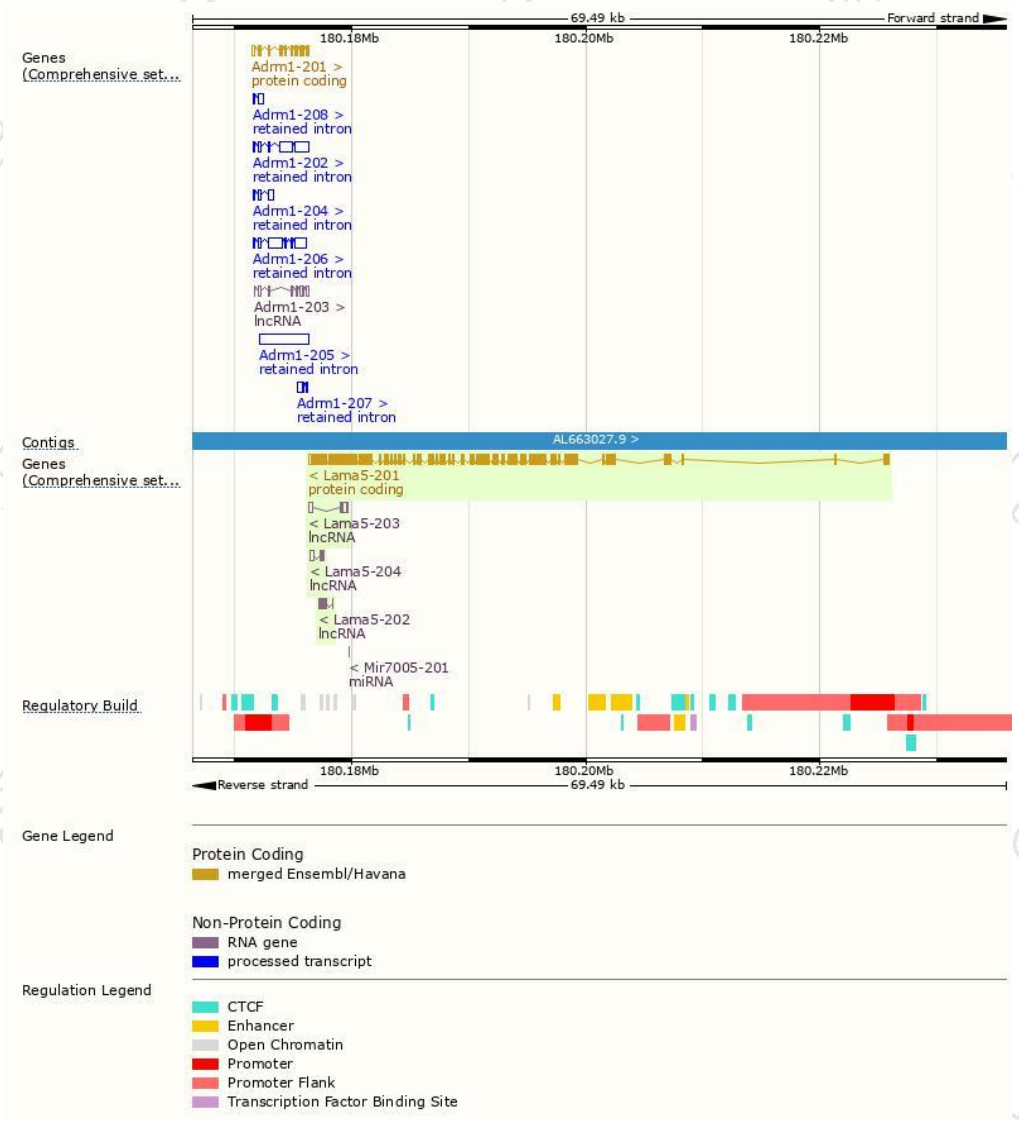
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lama5-201	ENSMUST00000015791.5	11388	3718aa	Protein coding	CCDS38375	Q61001	TSL:1 GENCODE basic APPRIS P1
Lama5-203	ENSMUST00000130796.1	734	No protein	lncRNA	-	-	TSL:5
Lama5-204	ENSMUST00000149812.1	478	No protein	lncRNA	-	-	TSL:5
Lama5-202	ENSMUST00000123906.1	451	No protein	lncRNA	-	-	TSL:5

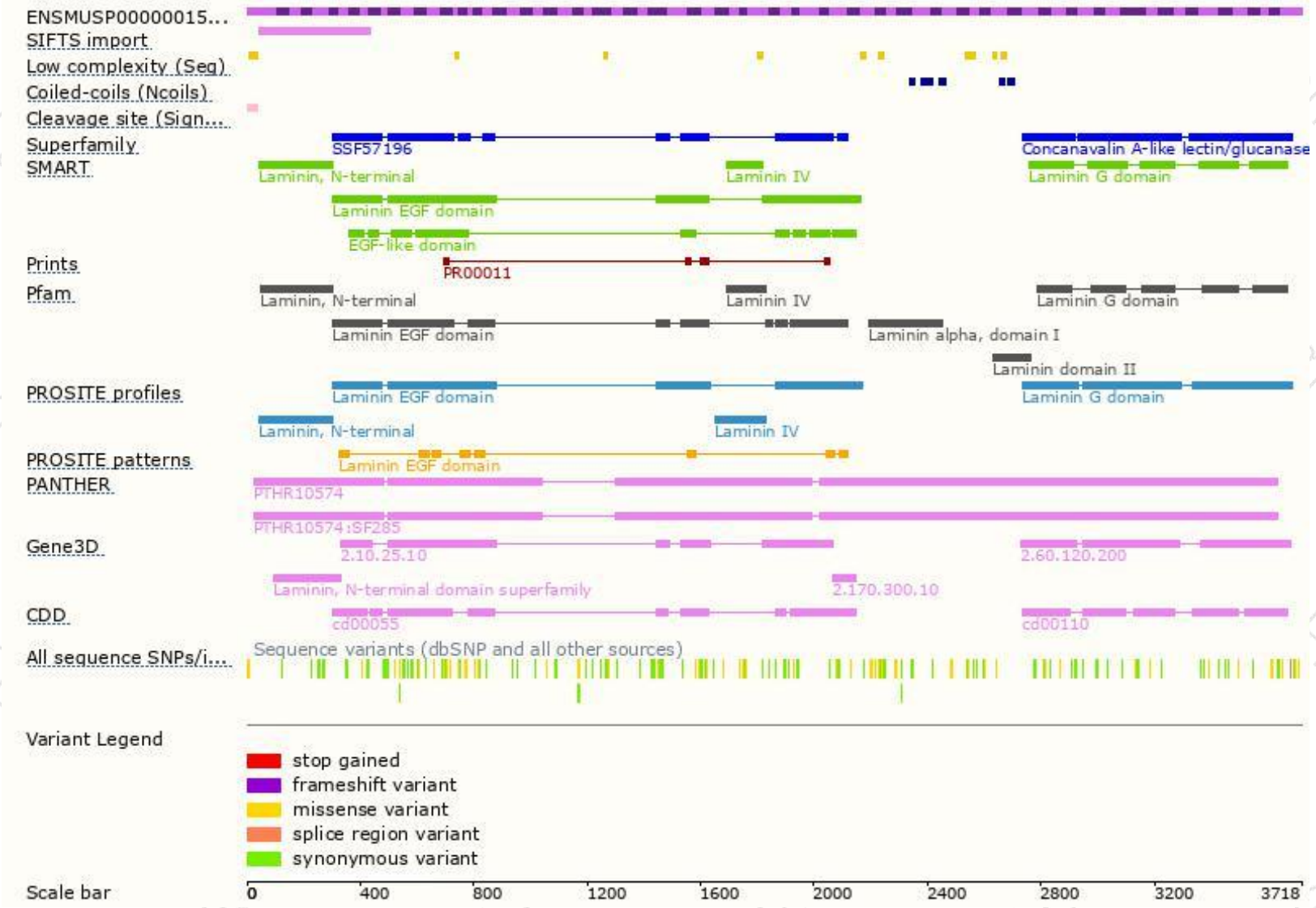
The strategy is based on the design of *Lama5-201* transcript,The transcription is shown below



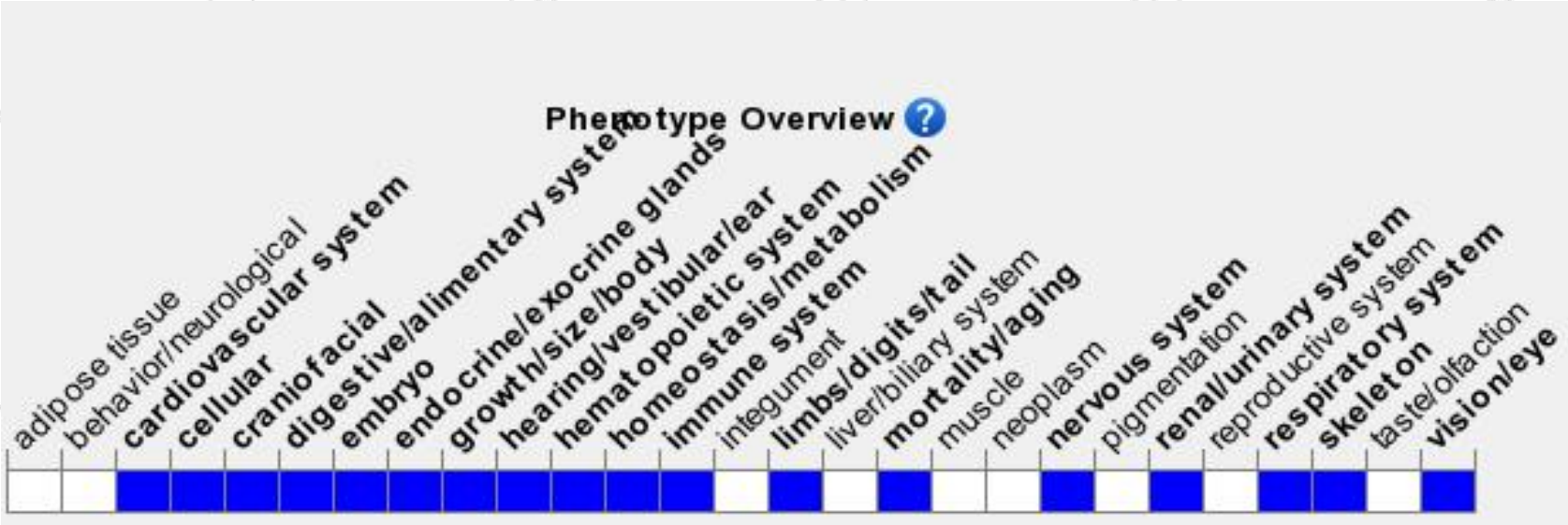
Genomic location distribution



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/).

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

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