Lamb3 Cas9-CKO Strategy

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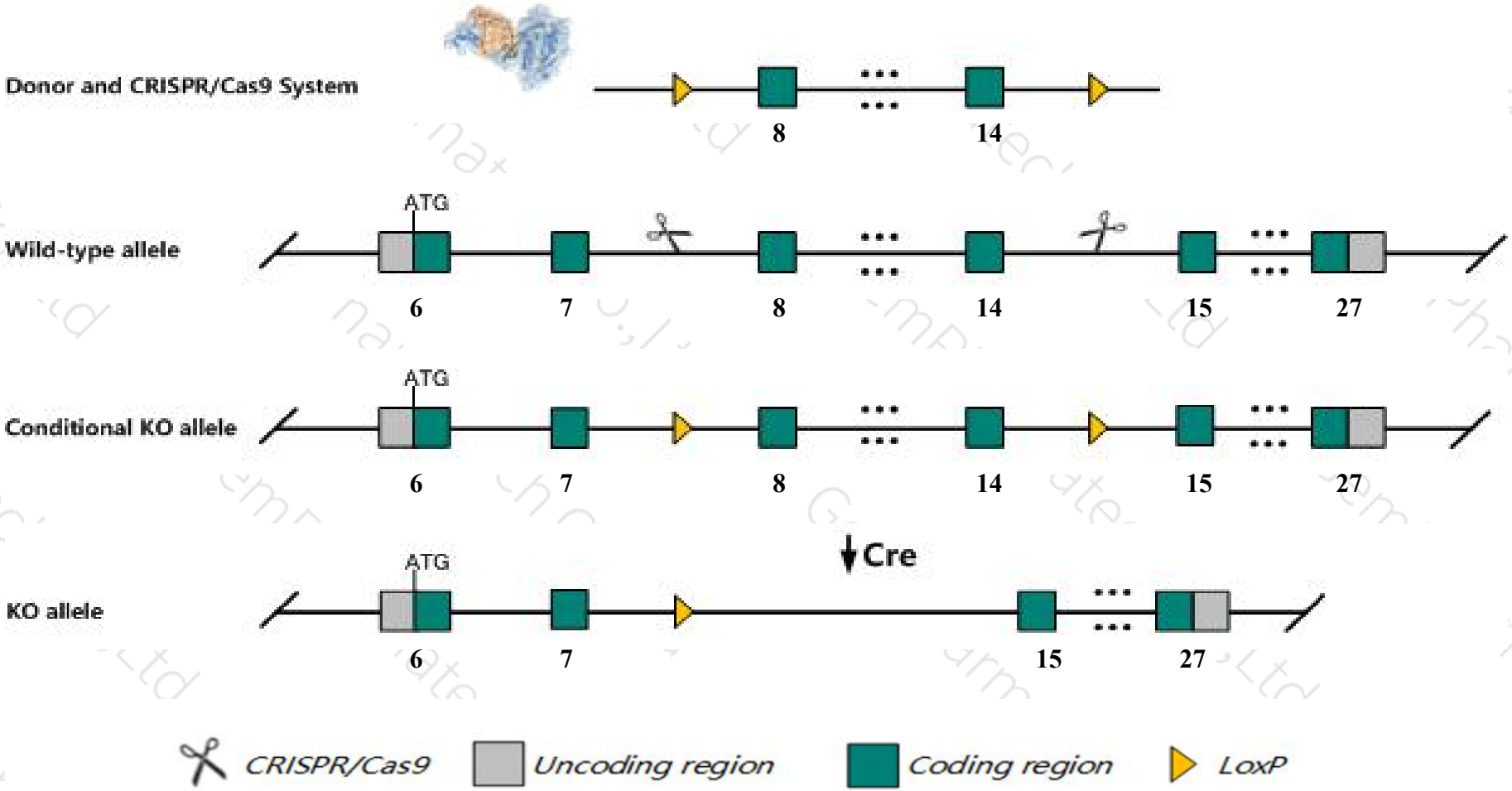
Project Overview

Project Name	***Lamb3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Lamb3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Lamb3* gene has 9 transcripts. According to the structure of *Lamb3* gene, exon8-exon14 of *Lamb3-209*(ENSMUST00000194677.5) transcript is recommended as the knockout region.The region contains 940bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Lamb3* 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 spontaneous intracisternal A particle sequence insertion exhibit blistering of the skin and mucosal surfaces with abnormal hemidesmosomes. Mutants die neonatally, usually without feeding.

➢ The *Lamb3* 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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Lamb3 laminin, beta 3 [Mus musculus (house mouse)]

Gene ID: 16780, updated on 13-Mar-2020

Summary

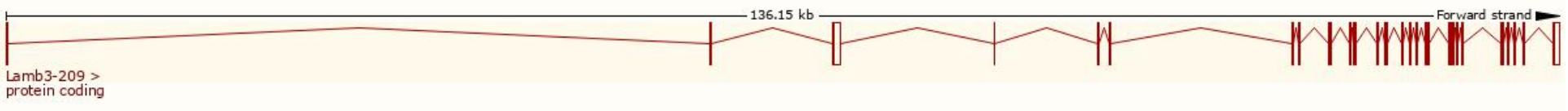
Official Symbol	Lamb3 provided by MGI
Official Full Name	laminin, beta 3 provided byMGI
Primary source	MGI:MGI:99915
See related	Ensembl:ENSMUSG00000026639
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Biased expression in colon adult (RPKM 41.0), lung adult (RPKM 26.5) and 11 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

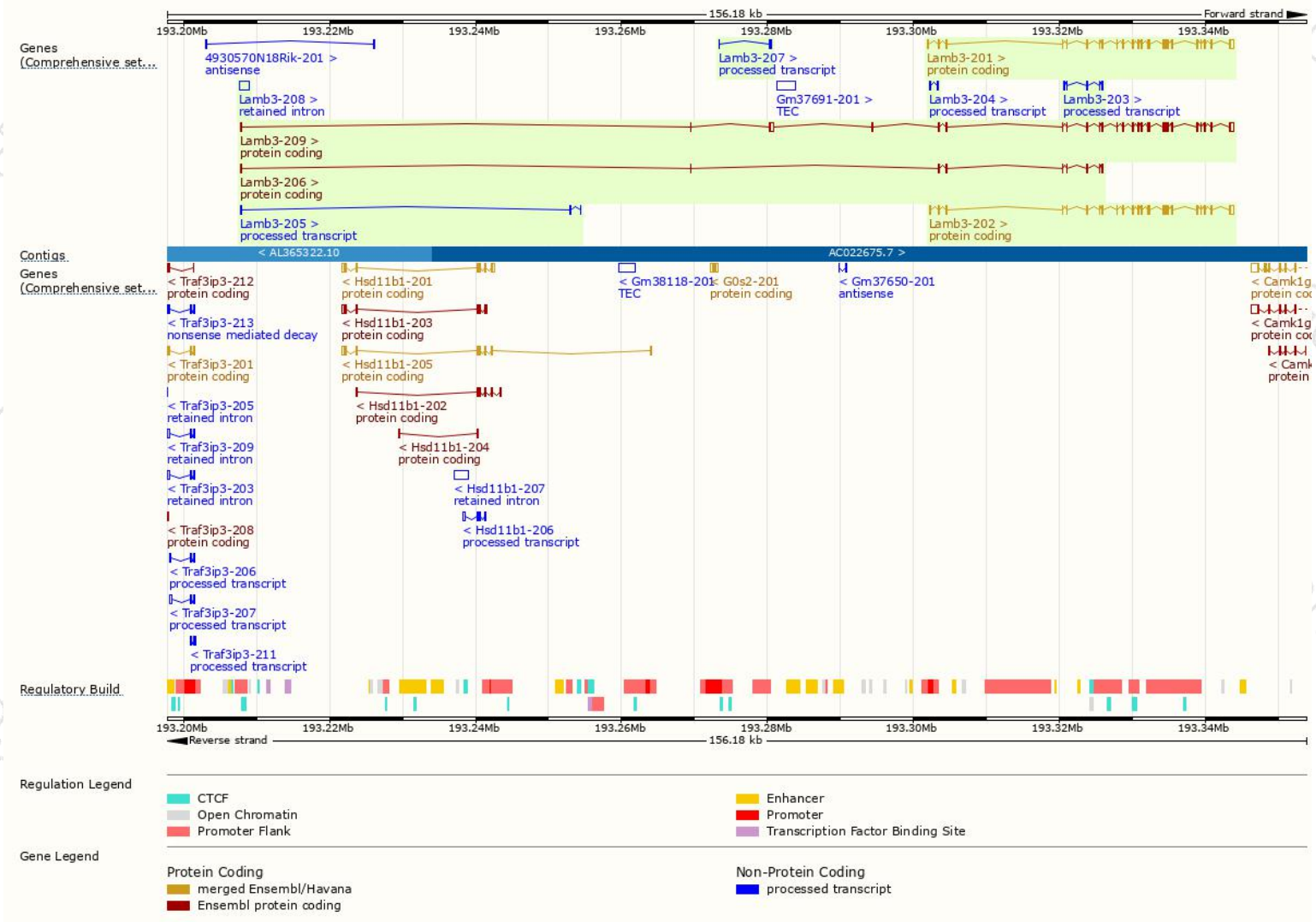
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein ▲	Biotype	CCDS	UniProt	Flags
Lamb3-206	ENSMUST00000192322.5	1029	244aa	Protein coding	-	A0A0A6YVX1	CDS 3' incomplete TSL:5
Lamb3-209	ENSMUST00000194677.5	4867	1168aa	Protein coding	CCDS15637	Q61087	TSL:1 GENCODE basic APPRIS P1
Lamb3-201	ENSMUST00000016315.15	4056	1168aa	Protein coding	CCDS15637	Q61087	TSL:1 GENCODE basic APPRIS P1
Lamb3-202	ENSMUST00000159955.1	4013	1168aa	Protein coding	CCDS15637	Q61087	TSL:1 GENCODE basic APPRIS P1
Lamb3-203	ENSMUST00000161526.2	461	No protein	Processed transcript	-	-	TSL:3
Lamb3-207	ENSMUST00000193128.1	355	No protein	Processed transcript	-	-	TSL:2
Lamb3-204	ENSMUST00000162180.2	260	No protein	Processed transcript	-	-	TSL:5
Lamb3-205	ENSMUST00000162977.1	258	No protein	Processed transcript	-	-	TSL:1
Lamb3-208	ENSMUST00000194407.1	1240	No protein	Retained intron	-	-	TSL:NA

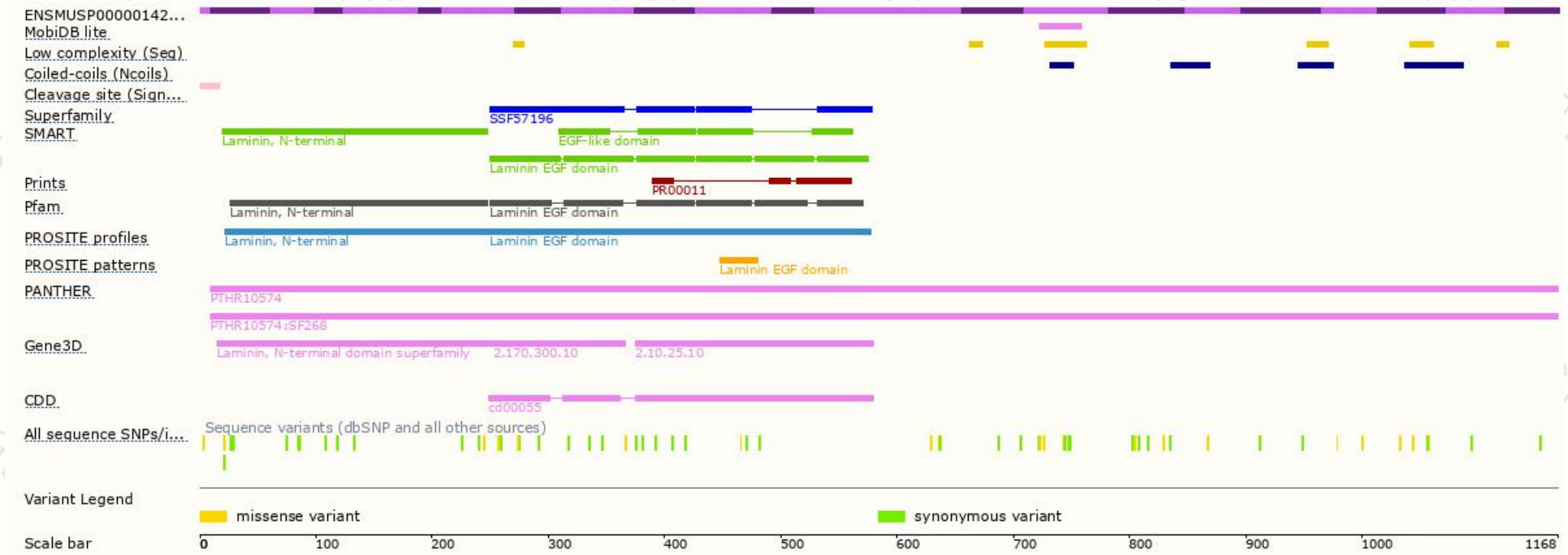
The strategy is based on the design of *Lamb3-209* transcript,the transcription is shown below:



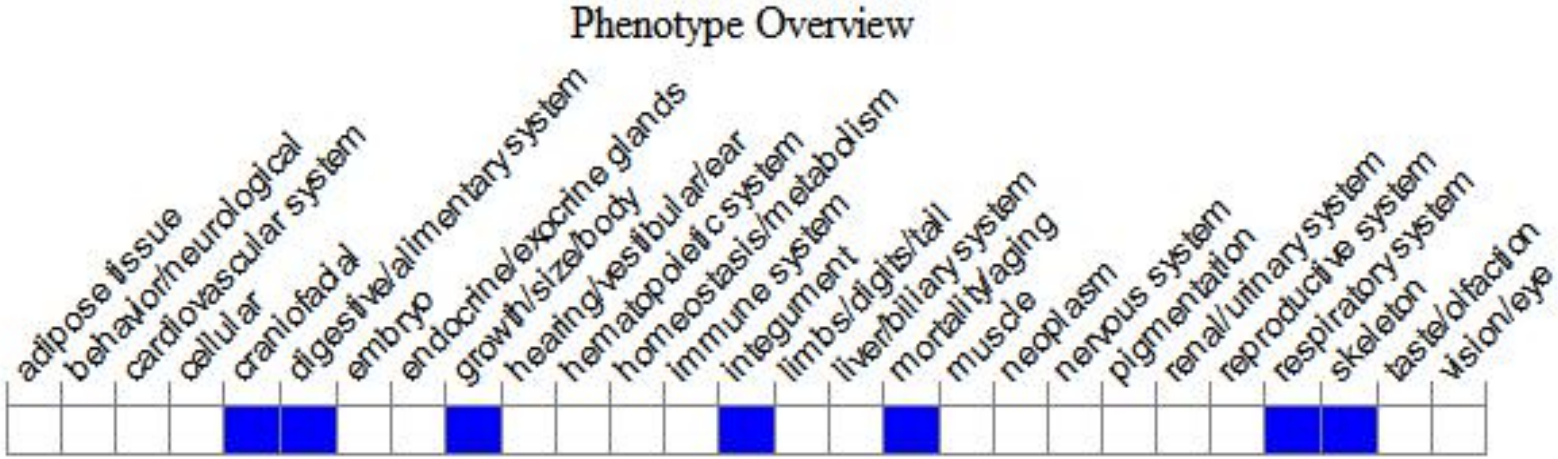
Genomic location distribution



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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