Limd1 Cas9-CKO Strategy

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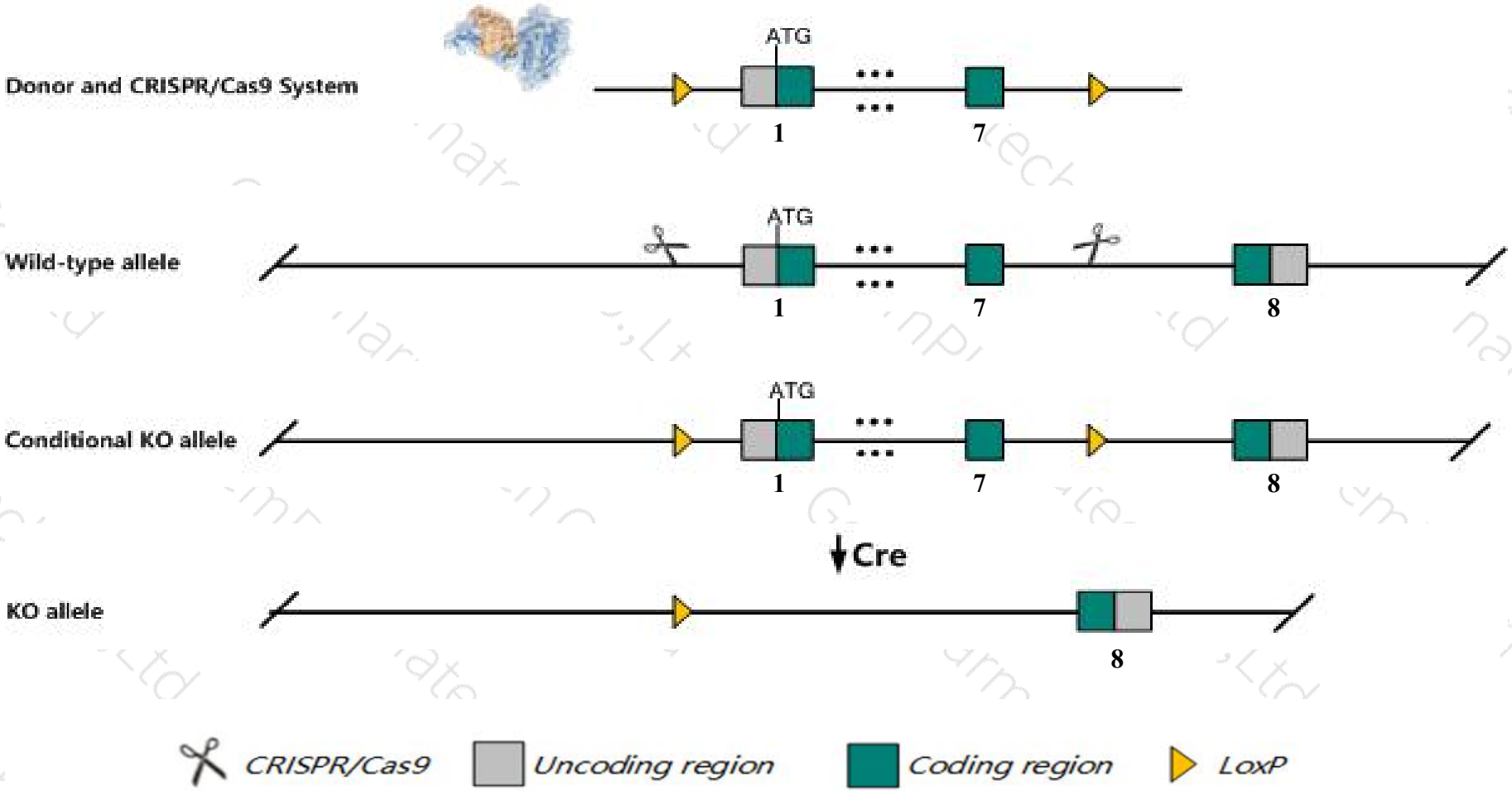
Project Overview

Project Name	***Limd1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Limd1* gene. The schematic diagram is as follows:



Technical routes

- The *Limd1* gene has 5 transcripts. According to the structure of *Limd1* gene, exon1-exon7 of *Limd1-201* (ENSMUST00000026269.3) 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 *Limd1* 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 a knock-out allele exhibit normal basal bone osteoclast numbers and bone density but are resistant to physiological and pathologic osteoclastogenic stimuli.

- The *Limd1* gene is located on the Chr9. 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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Limd1 LIM domains containing 1 [*Mus musculus* (house mouse)]

Gene ID: 29806, updated on 10-Oct-2019

Summary

Official Symbol	Limd1 provided by MGI
Official Full Name	LIM domains containing 1 provided by MGI
Primary source	MGI:MGI:1352502
See related	Ensembl:ENSMUSG00000025239
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	AW822033; D9Ertd192e
Expression	Ubiquitous expression in large intestine adult (RPKM 22.1), spleen adult (RPKM 19.7) and 28 other tissues See more
Orthologs	human all

Genomic context

Location: 9 F4; 9 74.0 cM

See Limd1 in Genome Data Viewer

Exon count: 9

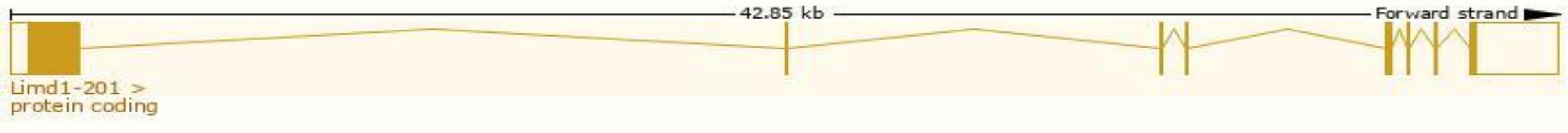
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	9	NC_000075.6 (123478661..123521552)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	9	NC_000075.5 (123387819..123430670)

Transcript information（Ensembl）

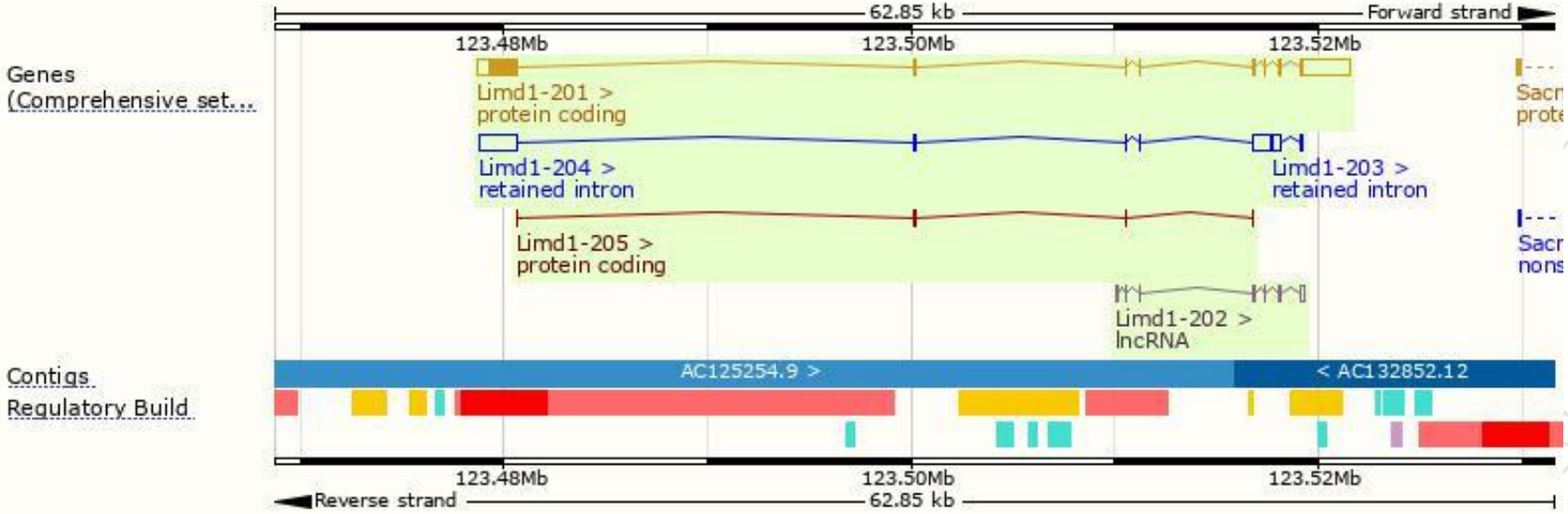
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Limd1-201	ENSMUST00000026269.3	4848	668aa	Protein coding	CCDS23660	Q9QXD8	TSL:1 GENCODE basic APPRIS P1
Limd1-205	ENSMUST00000217639.1	216	72aa	Protein coding	-	A0A1L1SUR8	CDS 5' and 3' incomplete TSL:5
Limd1-202	ENSMUST00000213278.1	699	No protein	Processed transcript	-	-	TSL:3
Limd1-204	ENSMUST00000216352.1	2986	No protein	Retained intron	-	-	TSL:1
Limd1-203	ENSMUST00000214309.1	580	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Limd1-201* transcript,The transcription is shown below



Genomic location distribution



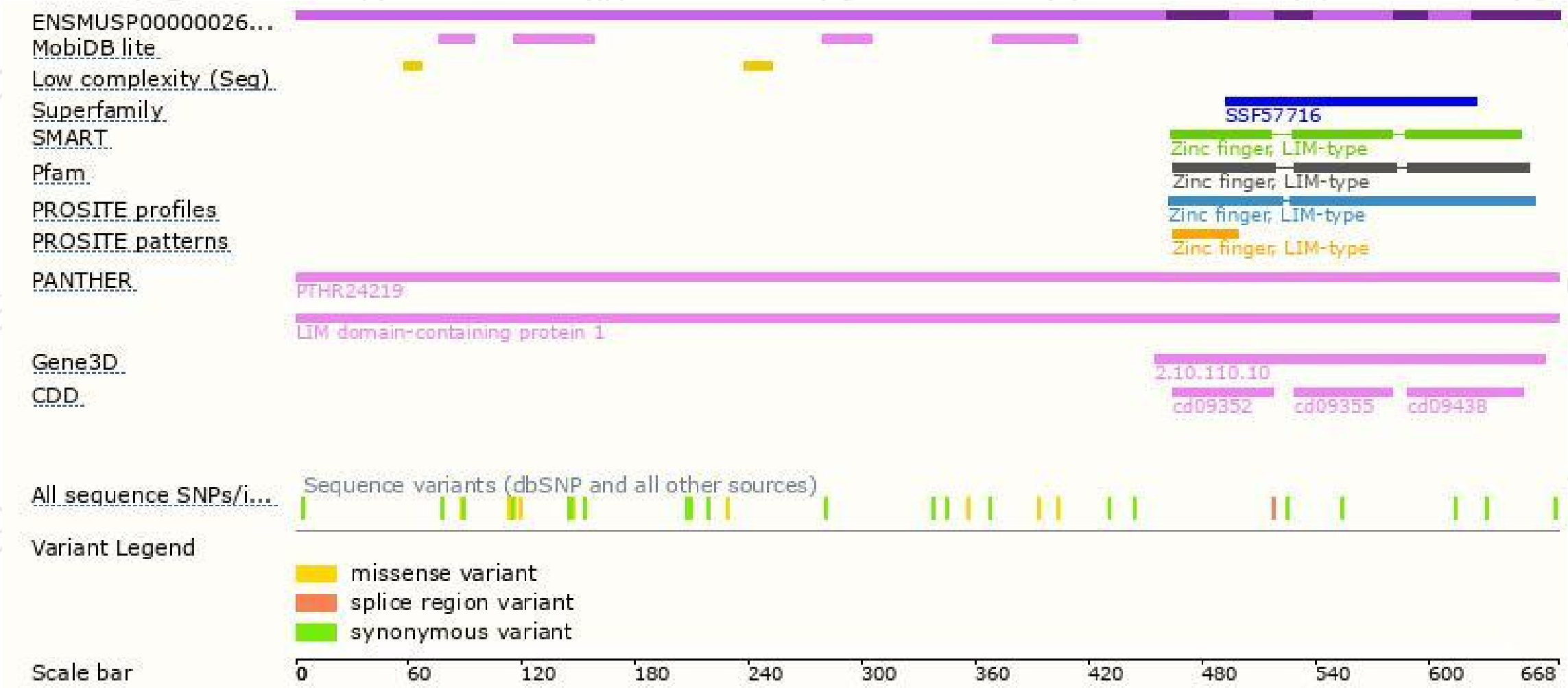
Gene Legend

Protein Coding
- Ensembl protein coding
- merged Ensembl/Havana

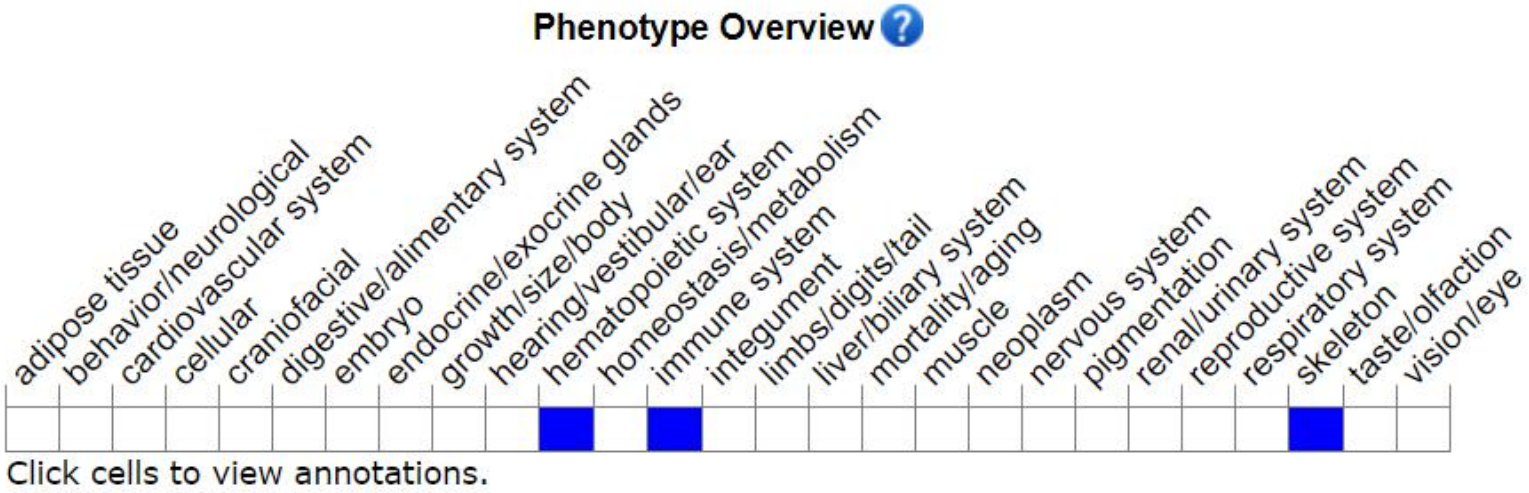
Non-Protein Coding
- RNA gene
- processed transcript

Regulation Legend
- CTCF
- Enhancer
- Promoter
- Promoter Flank
- Transcription Factor Binding Site

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 homozygous for a knock-out allele exhibit normal basal bone osteoclast numbers and bone density but are resistant to physiological and pathologic osteoclastogenic stimuli.

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

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