Lmx1b Cas9-CKO Strategy

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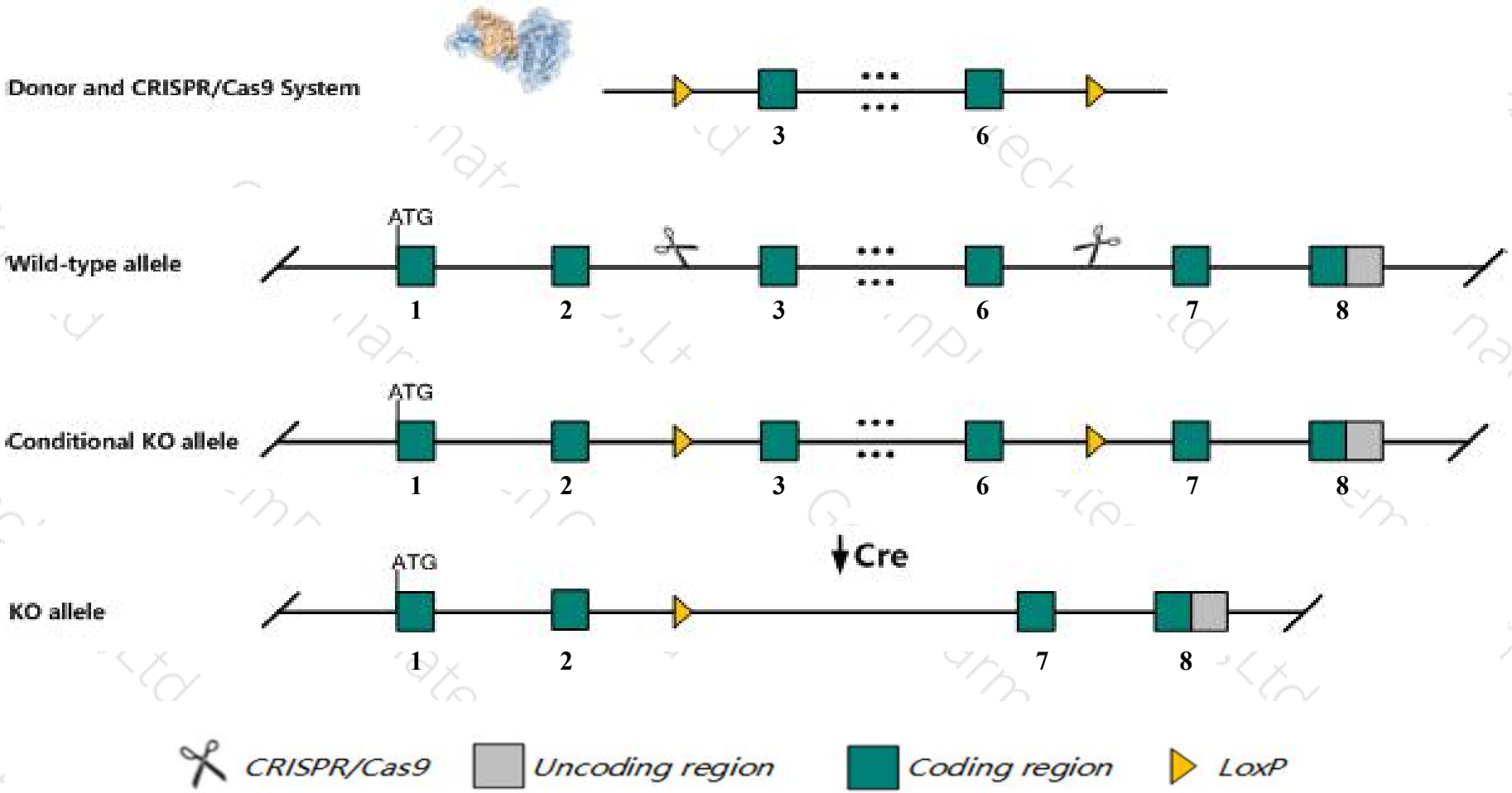
Project Overview

Project Name	***Lmx1b***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Lmx1b* gene. The schematic diagram is as follows:



Technical routes

- The *Lmx1b* gene has 3 transcripts. According to the structure of *Lmx1b* gene, exon3-exon6 of *Lmx1b-201* (ENSMUST00000041730.10) transcript is recommended as the knockout region.The region contains 560bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Lmx1b* 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 various skeletal, kidney, and eye defects. Pups also fail to suckle. Heterozygous mice with a homeodomain V265D mutation exhibit a variety of eye defects.

➢ The *Lmx1b* 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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Lmx1b LIM homeobox transcription factor 1 beta [Mus musculus (house mouse)]

Gene ID: 16917, updated on 19-Mar-2019

Summary

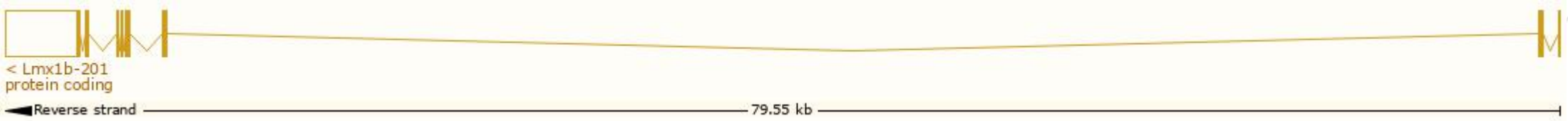
Official Symbol	Lmx1b provided by MGI
Official Full Name	LIM homeobox transcription factor 1 beta provided by MGI
Primary source	MGI:MGI:1100513
See related	Ensembl:ENSMUSG00000038765
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Icst, LMX1.1, LMX1.2
Expression	Biased expression in limb E14.5 (RPKM 9.6), mammary gland adult (RPKM 5.0) and 4 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

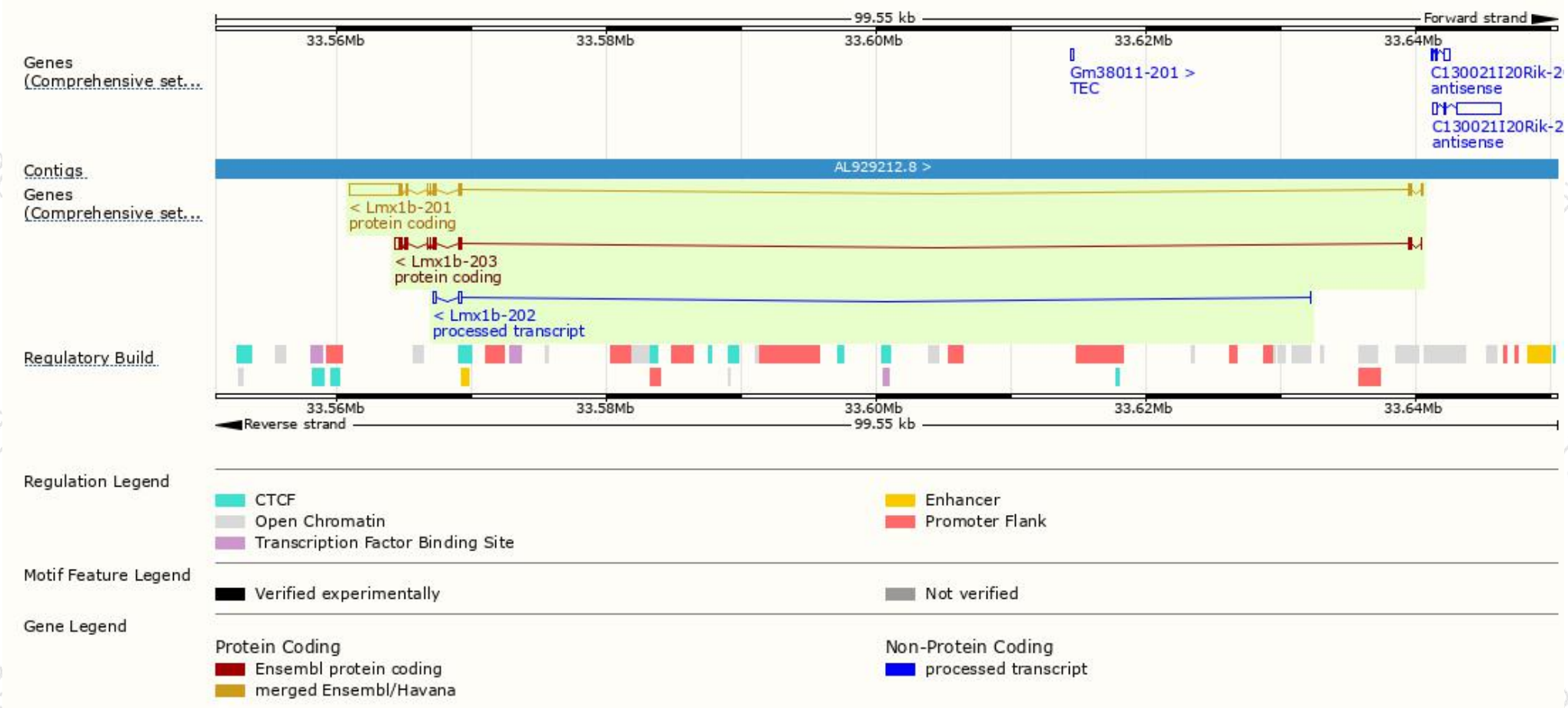
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags
Lmx1b-201	ENSMUST00000041730.10	4790	372aa	Protein coding	CCDS15944	A0A0R4J0I7	NM_010725 NP_034855	TSL:1 GENCODE basic APPRIS P1
Lmx1b-203	ENSMUST00000176067.1	1420	369aa	Protein coding	-	H3BJD7	-	CDS 5' incomplete TSL:5
Lmx1b-202	ENSMUST00000137559.2	476	No protein	Processed transcript	-	-	-	TSL:2

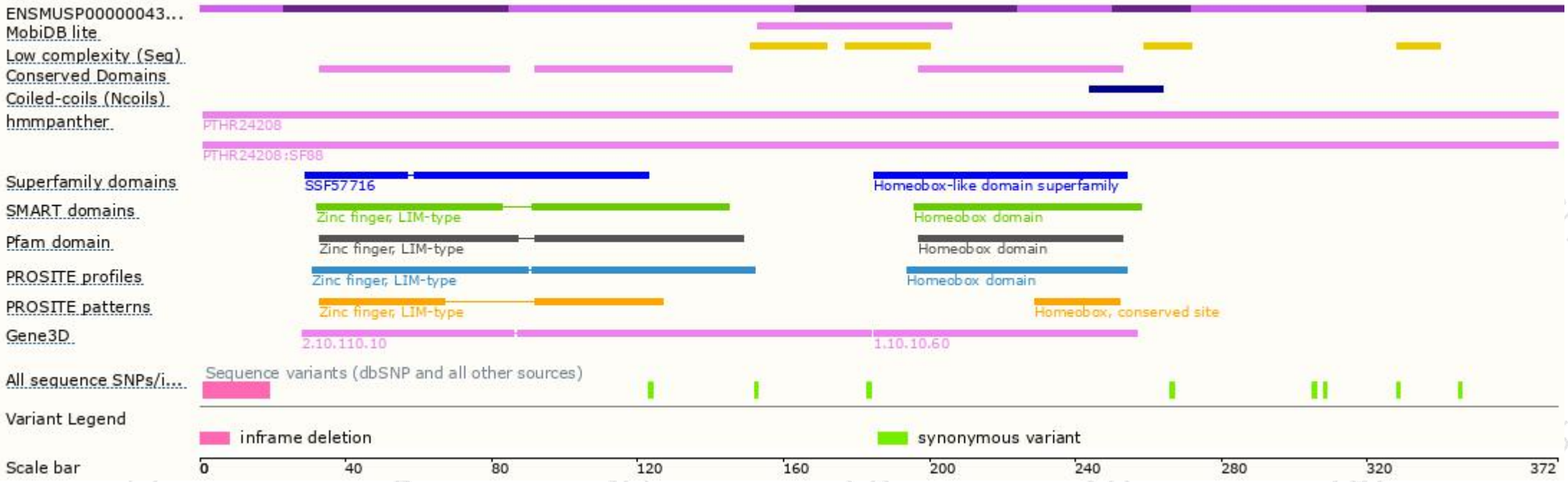
The strategy is based on the design of *Lmx1b-201* transcript,The transcription is shown below



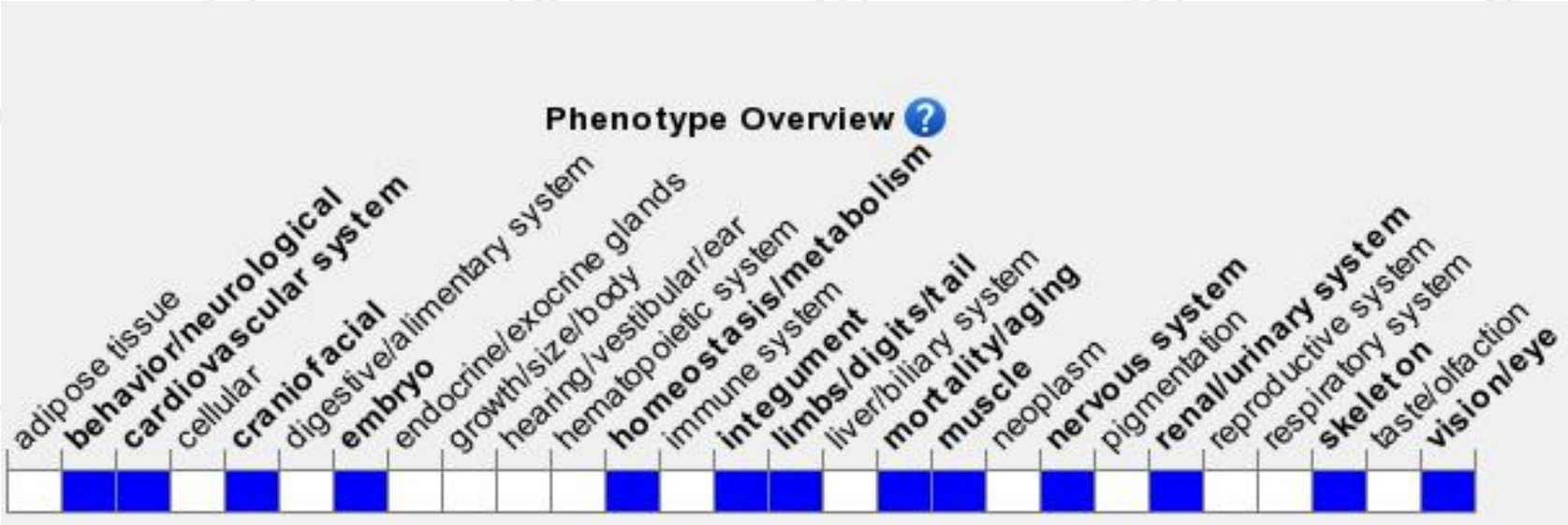
Genomic location distribution



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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