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GemPharmatech

Loxl3 Cas9-CKO Strategy

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Project Overview

Project Name	***Loxl3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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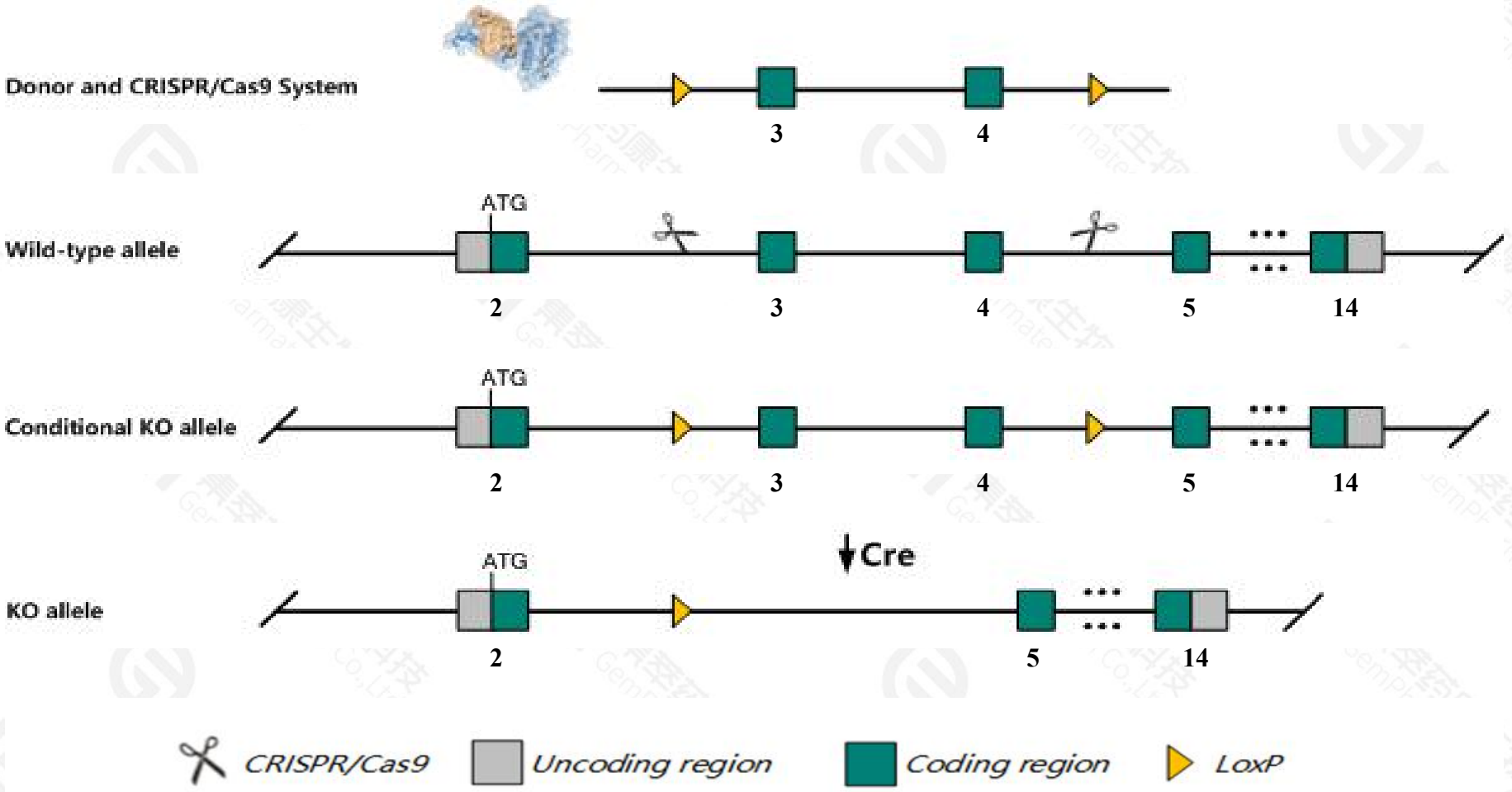
This model will use CRISPR/Cas9 technology to edit the *Loxl3* gene. The schematic diagram is as follows:



Technical routes

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➢ The *Loxl3* gene has 5 transcripts. According to the structure of *Loxl3* gene, exon3-exon4 of *Loxl3-201*(ENSMUST00000000707.9) transcript is recommended as the knockout region.The region contains 379bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Loxl3* 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 lethality shortly after birth, craniofacial and vertebral abnormalities associated with collagen deformities.

➢ The *Loxl3* gene is located on the Chr6. 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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Loxl3 lysyl oxidase-like 3 [Mus musculus (house mouse)]

Gene ID: 16950, updated on 13-Mar-2020

Summary

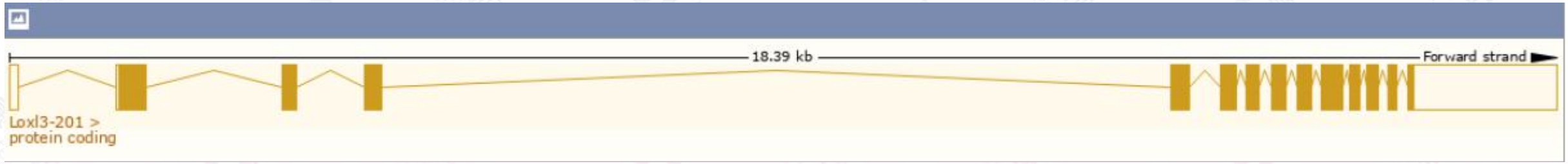
Official Symbol	Loxl3 provided by MGI
Official Full Name	lysyl oxidase-like 3 provided byMGI
Primary source	MGI:MGI:1337004
See related	Ensembl:ENSMUSG00000000693
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Lor2, Loxl2
Expression	Ubiquitous expression in limb E14.5 (RPKM 18.2), bladder adult (RPKM 8.2) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

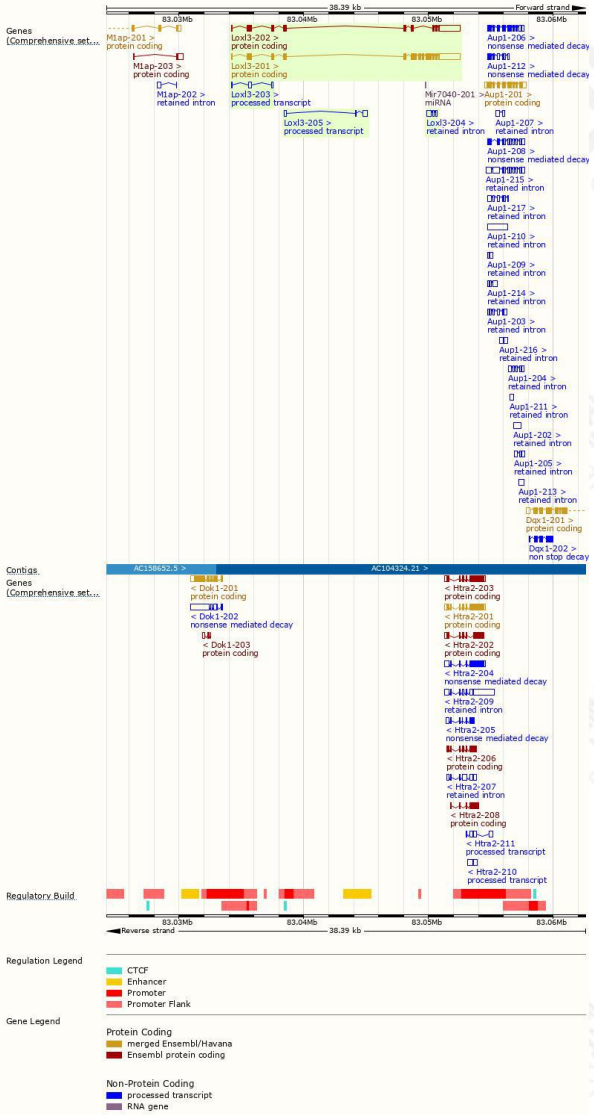
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Loxl3-201	ENSMUST00000000707.8	4098	754aa	Protein coding	CCDS20266	Q9Z175	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Loxl3-202	ENSMUST00000101257.3	3252	472aa	Protein coding	-	E9Q0X7	TSL:1 GENCODE basic
Loxl3-205	ENSMUST00000204318.1	617	No protein	Processed transcript	-	-	TSL:5
Loxl3-203	ENSMUST00000152679.7	462	No protein	Processed transcript	-	-	TSL:2
Loxl3-204	ENSMUST00000155502.1	611	No protein	Retained intron	-	-	TSL:5

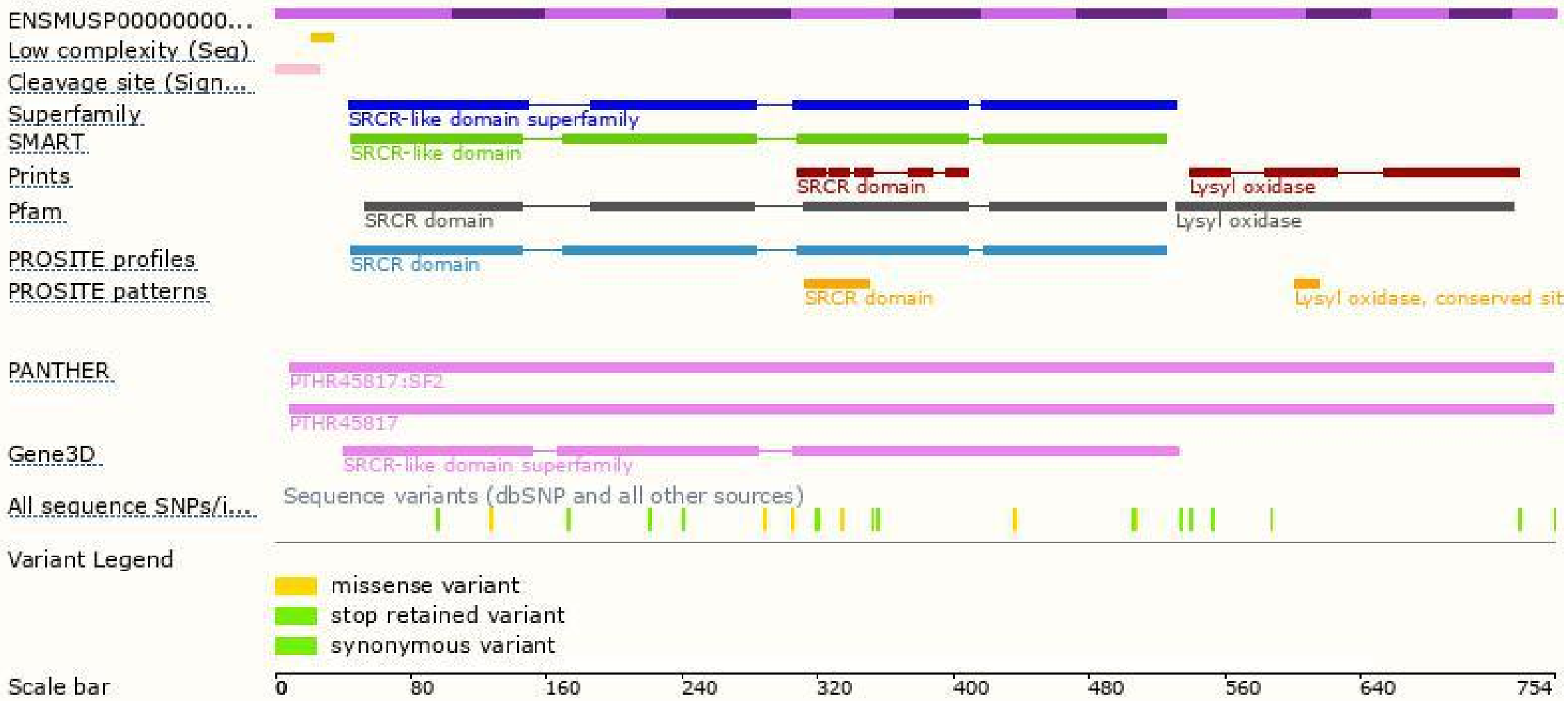
The strategy is based on the design of *Loxl3-201* transcript,the transcription is shown below:



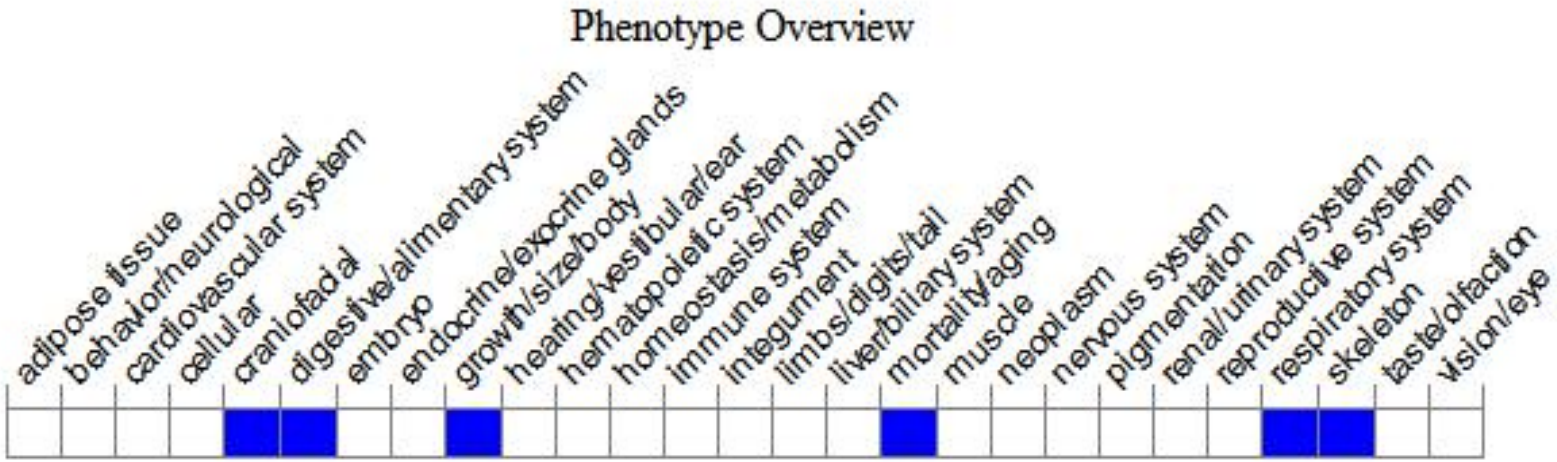
Genomic location distribution



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/).

According to the existing MGI data,mice homozygous for a knock-out allele exhibit lethality shortly after birth, craniofacial and vertebral abnormalities associated with collagen deformities.

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