Lactb Cas9-CKO Strategy

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Overview

Target Gene Name
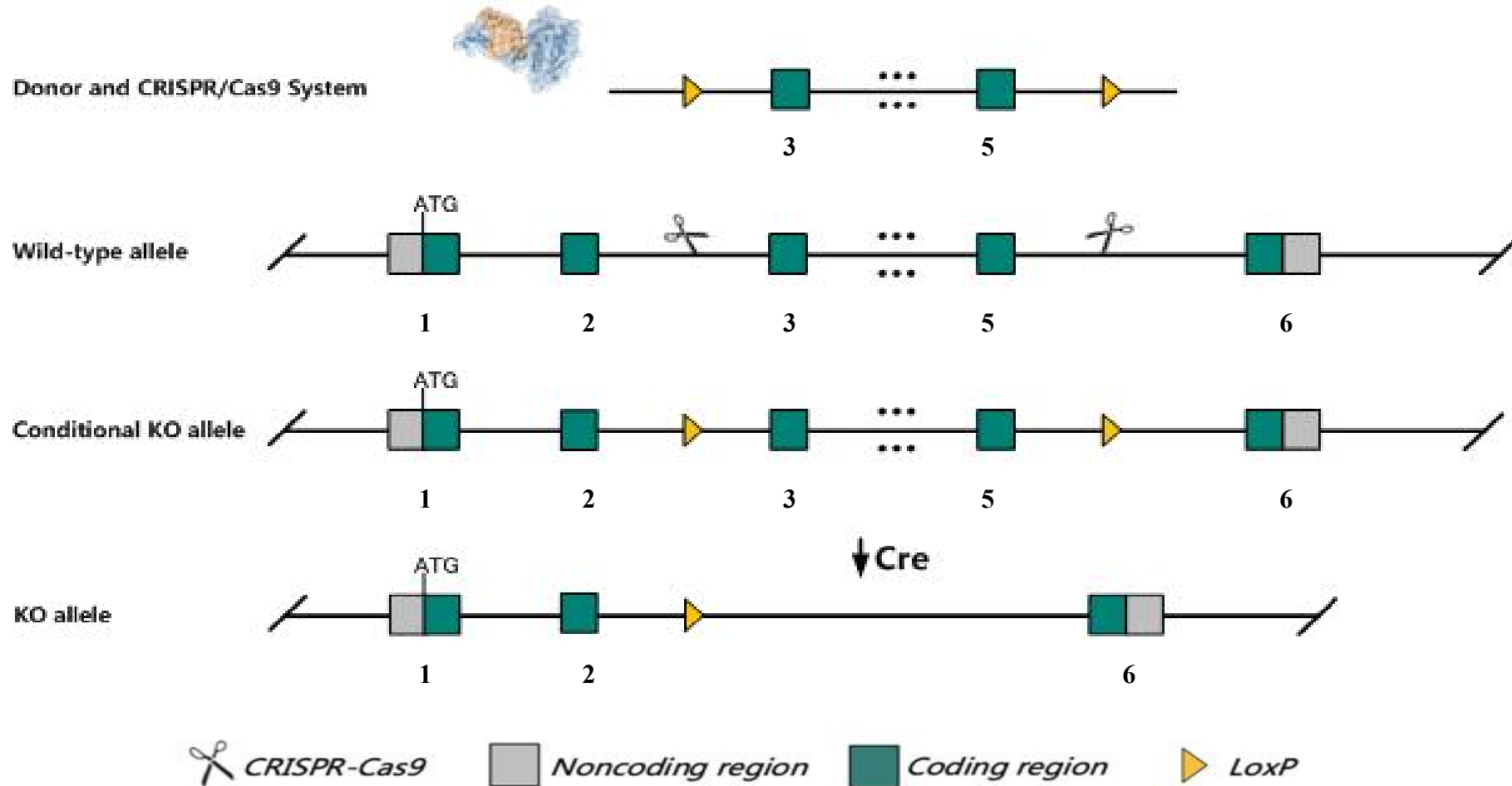
- Lactb

Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy

Schematic representation of CRISPR-Cas9 engineering used to edit the *Lactb* gene.

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Technical Information

- The *Lactb* gene has 2 transcripts. According to the structure of *Lactb* gene, exon3-exon5 of *Lactb*-201 (ENSMUST00000034929.7) transcript is recommended as the knockout region. The region contains 712bp coding sequence. Knocking out the region will result in disruption of protein function.

- In this project we use CRISPR-Cas9 technology to modify *Lactb* 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.

- 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.
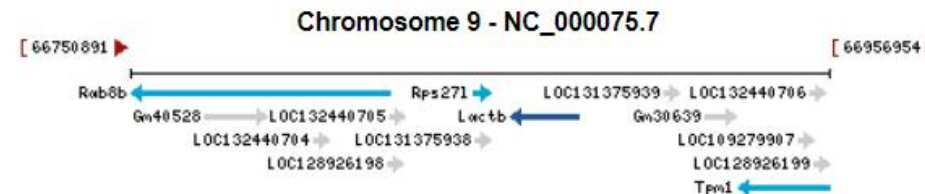
Gene Information

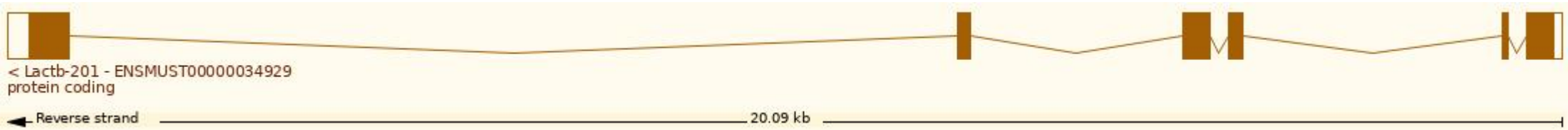Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

The gene has 2 transcripts,all transcripts are shown below:

Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000034929.7	Lactb-201	2031	551aa	Protein coding	CCDS40674 ⧉	B2RWI2 ⧉ Q9EP89 ⧉	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1
ENSMUST00000215172.2	Lactb-202	1806	356aa	Protein coding		A0A1L1SVF9 ⧉	GENCODE basic TSL:1

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

< Lactb-201 - ENSMUST00000034929
protein coding

◄ Reverse strand   20.09 kb

Source: https://www.ensembl.org

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Genomic Information

Source: : https://www.ensembl.org

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Protein Information

Source: : https://www.ensembl.org
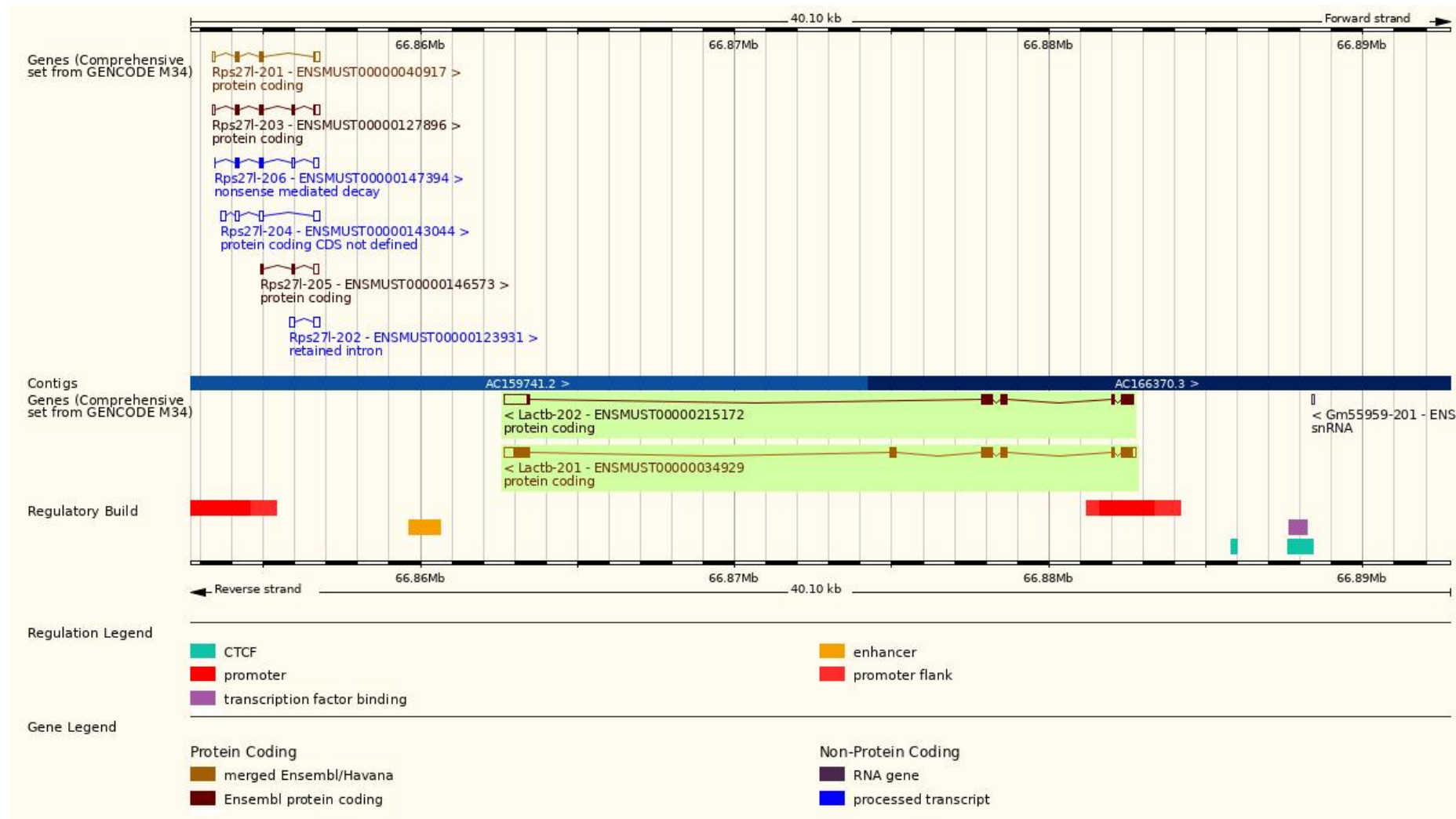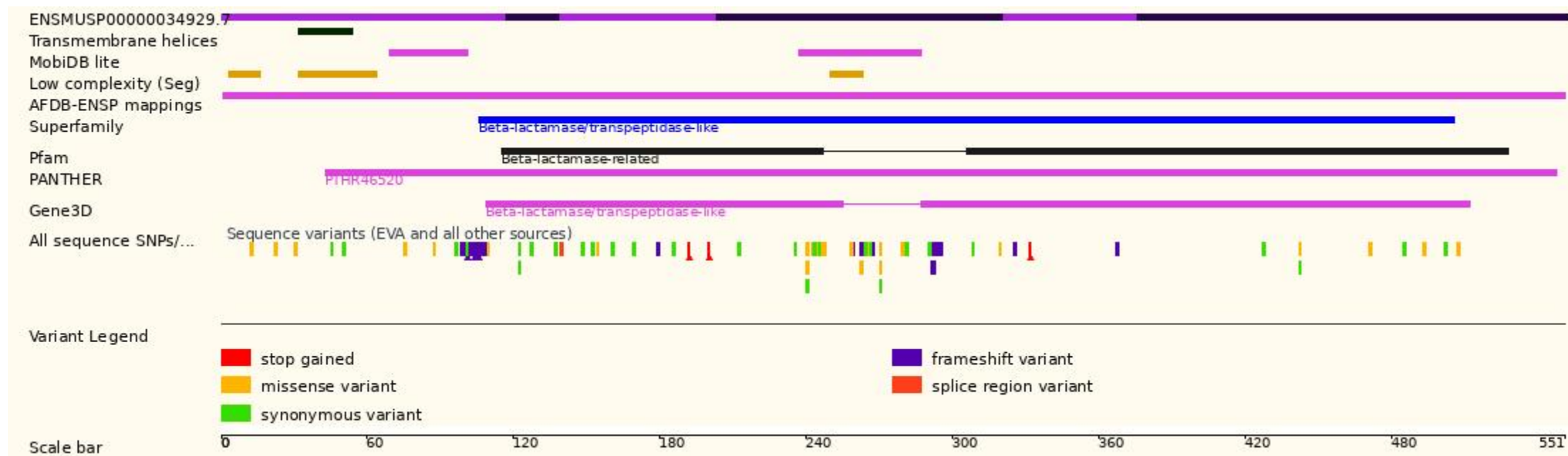
Mouse Phenotype Information (MGI)

Phenotype Overview

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Source: https://www.informatics.jax.org
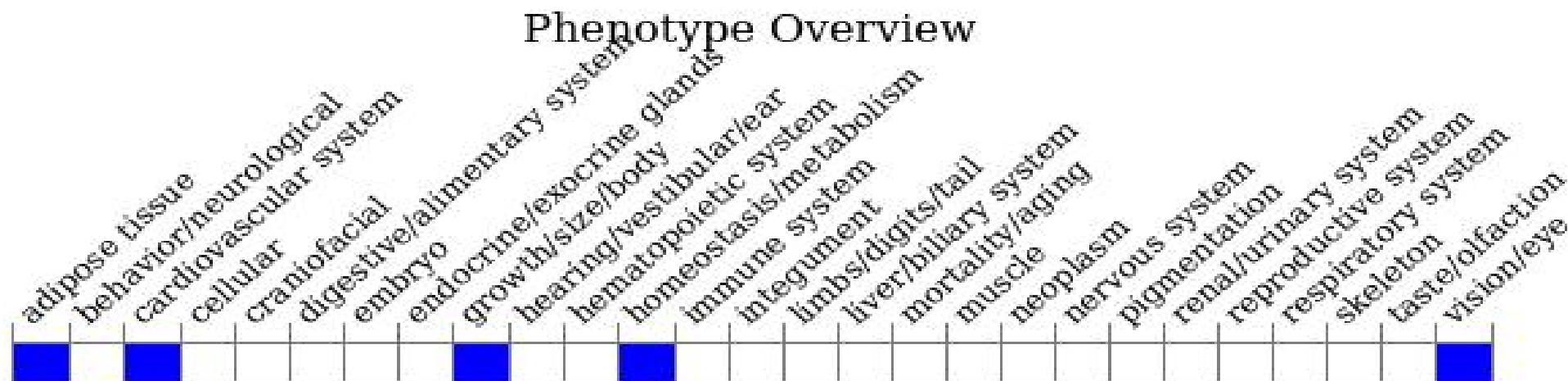
Important Information

- This strategy does not cause frameshift mutation for *Lactb*-202, and it may leave some protein structures, the retained function is unknown.

- *Lactb* is located on Chr9. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is 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 the existing technology level.

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