Rpl32 Cas9-CKO Strategy

Designer: Xueting Zhang
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Project Overview

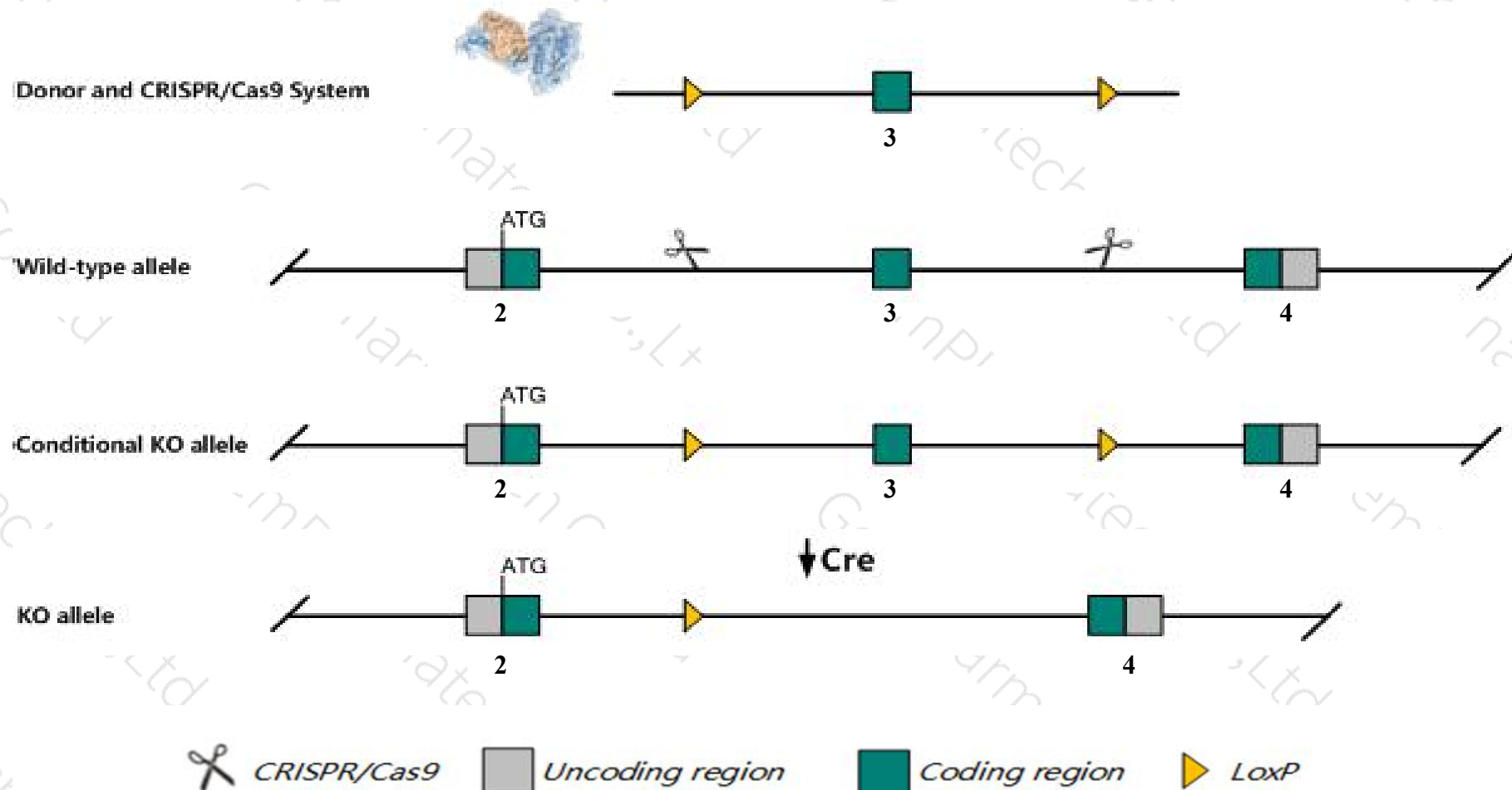|	
Project Name	*Rpl32*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rpl32* gene. The schematic diagram is as follows:

➤ The *Rpl32* gene has 2 transcripts. According to the structure of *Rpl32* gene, exon3 of *Rpl32-201* (ENSMUST00000081840.5) transcript is recommended as the knockout region.The region contains Ens 182bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Rpl32* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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

➢ The *Rpl32* 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.

➢ The floxed region is near to the C-terminal of *Snora7a* and *Cand2* gene,this strategy may influence the regulatory function of the C-terminal of these gene.

➢ The intron 2-3 of *Rpl32* gene is short,the strategy may destroy the normal splicing of *Rpl32* gene.

➢ 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）

Rpl32 ribosomal protein L32 [Mus musculus (house mouse)]

Gene ID: 19951, updated on 31-Jan-2019

▲ Summary

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Official Symbol	Rpl32 provided by MGI
Official Full Name	ribosomal protein L32 provided by MGI
Primary source	MGI:MGI:98038
See related	Ensembl:ENSMUSG00000057841
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AU020185, rpL32-3A
Expression	Ubiquitous expression in liver E14 (RPKM 752.0), bladder adult (RPKM 597.3) and 25 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rpl32-202	ENSMUST00000203816.1	906	135aa	Protein coding	CCDS20443	P62911	TSL:2 GENCODE basic APPRIS P1
Rpl32-201	ENSMUST00000081840.5	517	135aa	Protein coding	CCDS20443	P62911	TSL:1 GENCODE basic APPRIS P1

The strategy is based on the design of *Rpl32-201* transcript,The transcription is shown below

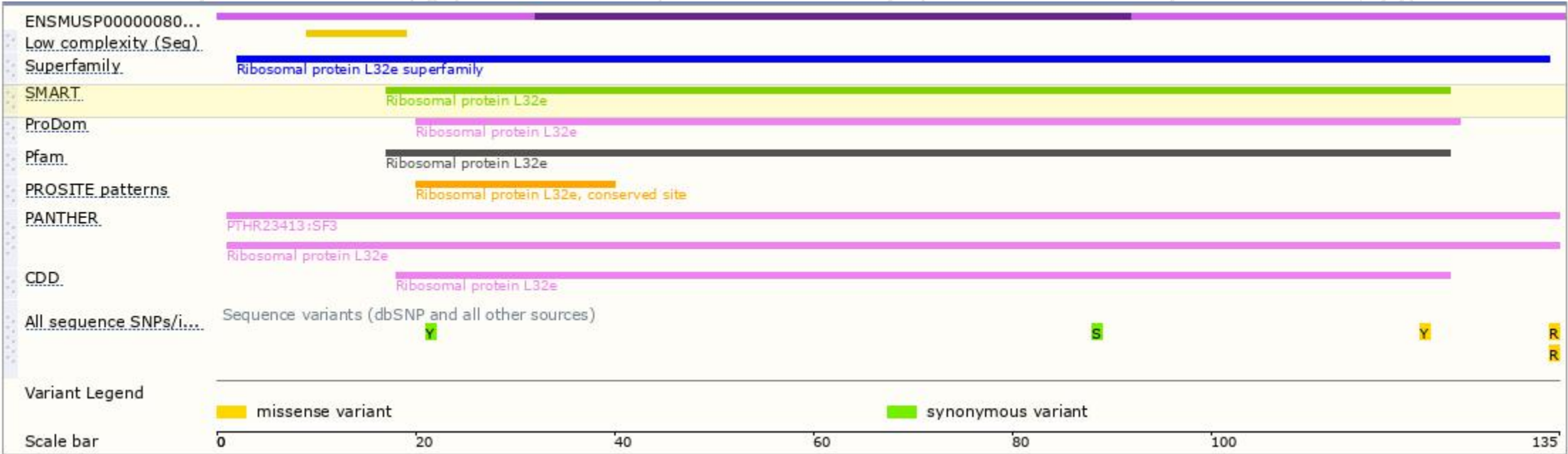< Rpl32-201
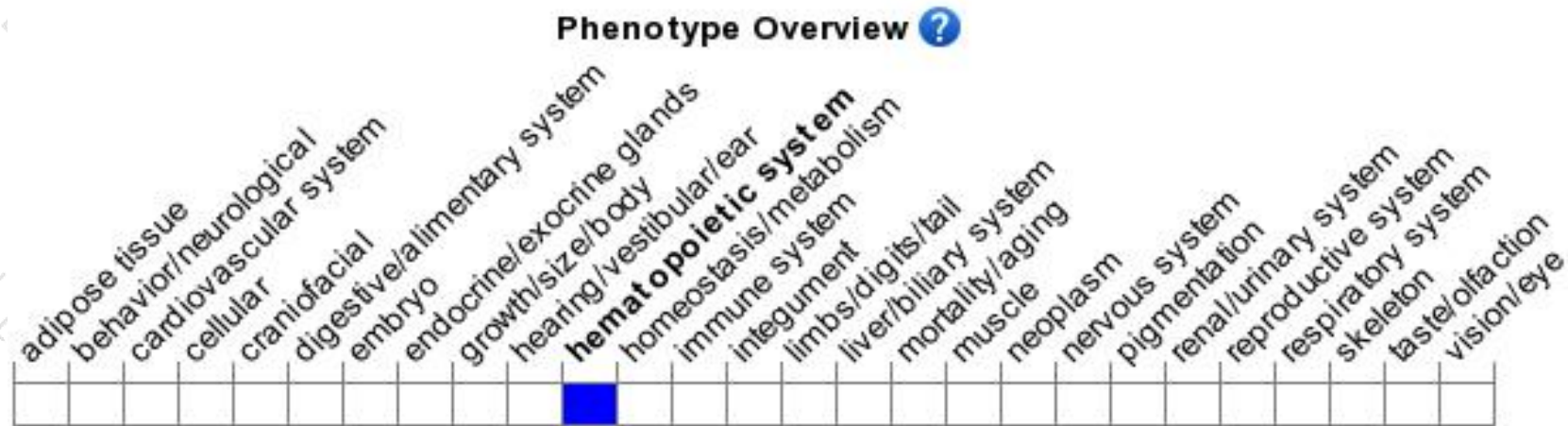protein coding
◀ Reverse strand

3.24 kb
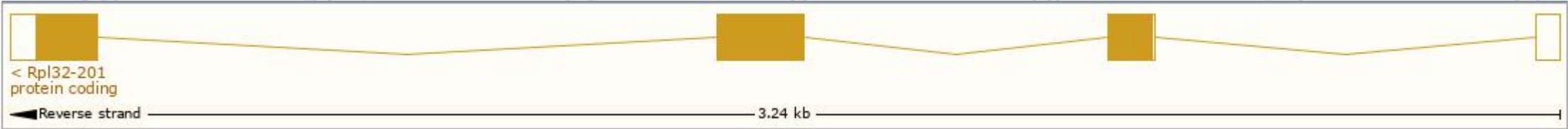
Genomic location distribution
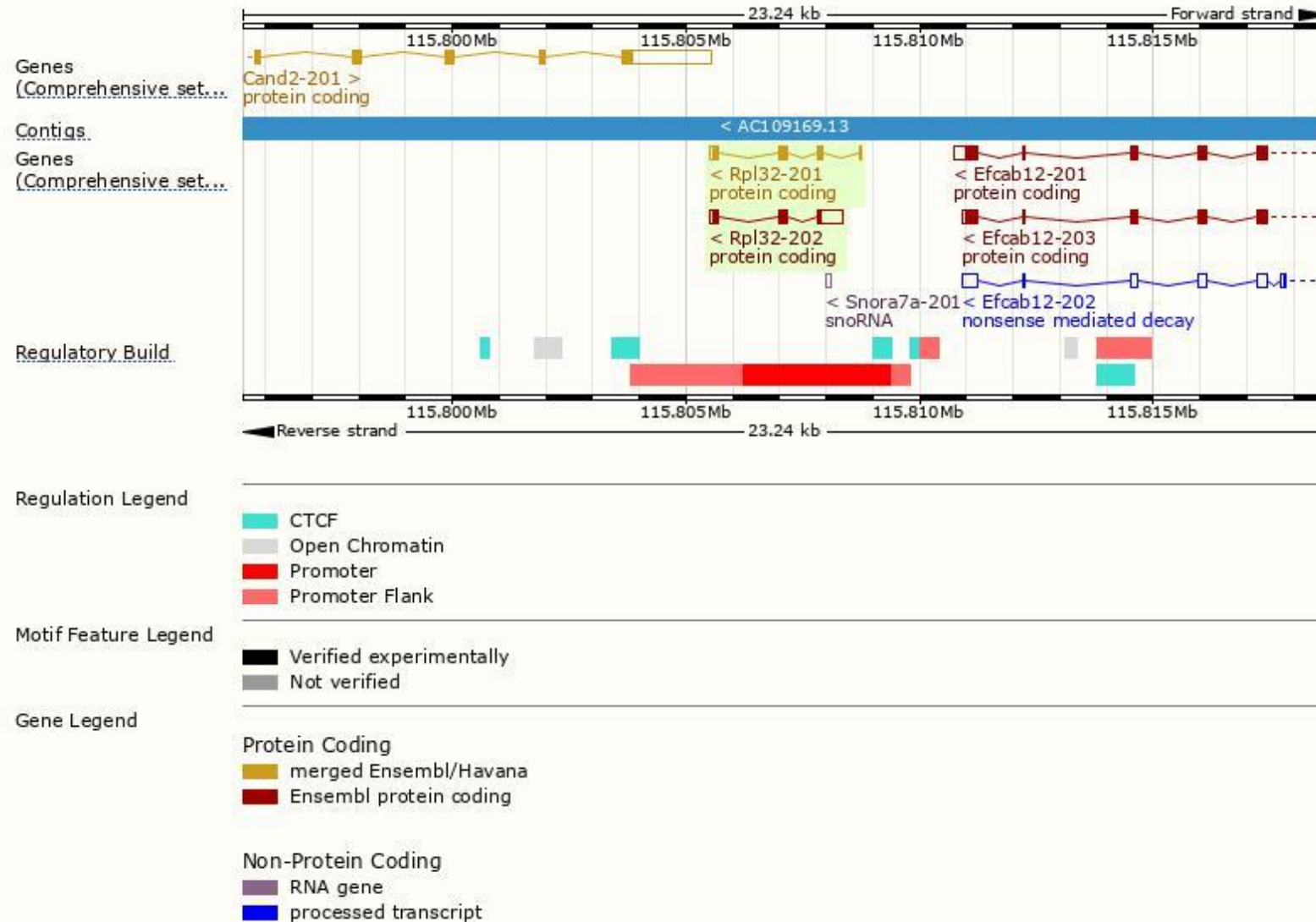
Protein domain

Mouse phenotype description(MGI)

Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

If you have any questions, you are welcome to inquire.
Tel: 400-9660890