

Tbc1d8b Cas9-CKO Strategy

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Project Overview



Project Name Tbc1d8b

Project type Cas9-CKO

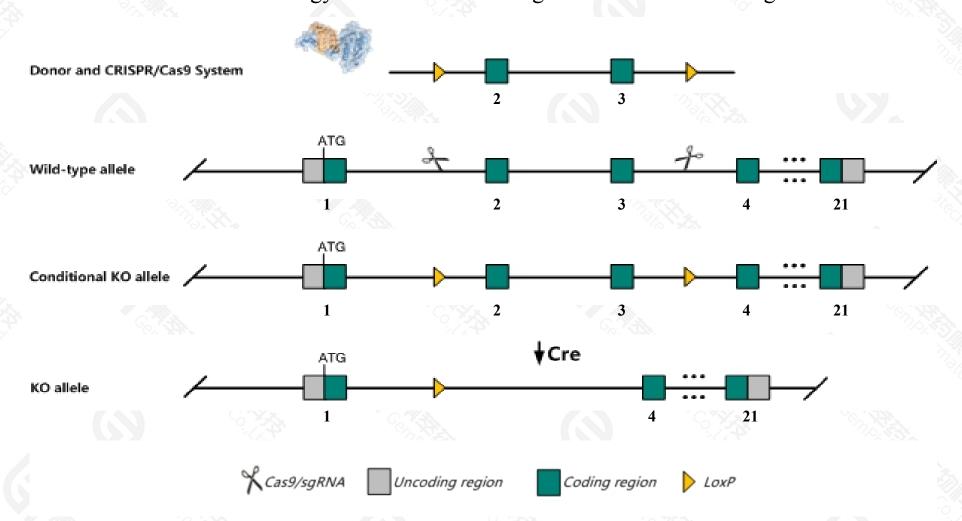
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Tbc1d8b* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Tbc1d8b* gene has 2 transcripts. According to the structure of *Tbc1d8b* gene, exon2-exon3 of *Tbc1d8b*-201(ENSMUST00000096313.3) transcript is recommended as the knockout region. The region contains 230bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Tbc1d8b* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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 *Tbc1d8b* gene is located on the ChrX. 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)



Tbc1d8b TBC1 domain family, member 8B [Mus musculus (house mouse)]

Gene ID: 245638, updated on 13-Mar-2020

Summary



Official Symbol Tbc1d8b provided by MGI

Official Full Name TBC1 domain family, member 8B provided by MGI

Primary source MGI:MGI:1918101

See related Ensembl:ENSMUSG00000042473

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 4921505D17Rik, 9030605E16Rik

Expression Broad expression in bladder adult (RPKM 3.2), limb E14.5 (RPKM 3.1) and 24 other tissuesSee more

Orthologs <u>human</u> <u>all</u>

Transcript information (Ensembl)



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

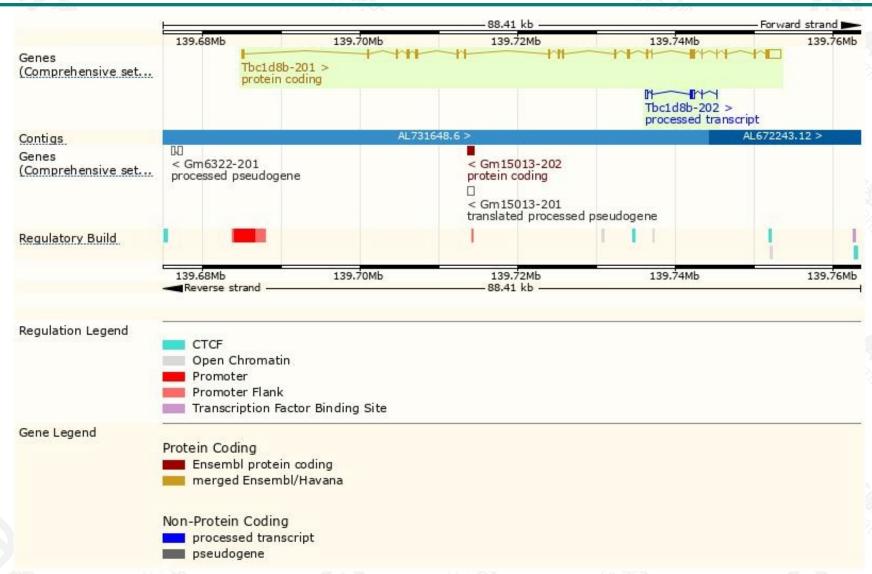
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tbc1d8b-201	ENSMUST00000096313.3	5094	<u>1114aa</u>	Protein coding	CCDS41146	A3KGB4	TSL:1 GENCODE basic APPRIS P1
Tbc1d8b-202	ENSMUST00000123430.1	758	No protein	Processed transcript	-	-	TSL:3

The strategy is based on the design of *Tbc1d8b-201* transcript, the transcription is shown below:



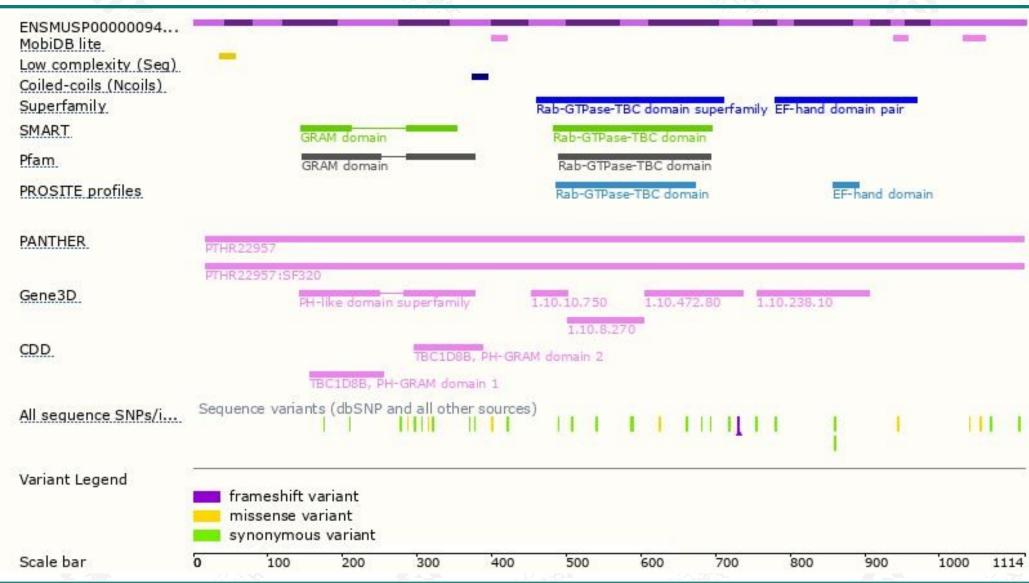
Genomic location distribution





Protein domain







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

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