

# Ctsb Cas9-KO Strategy

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Reviewer: Shuang Zhang

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## **Project Overview**



Project Name Ctsb

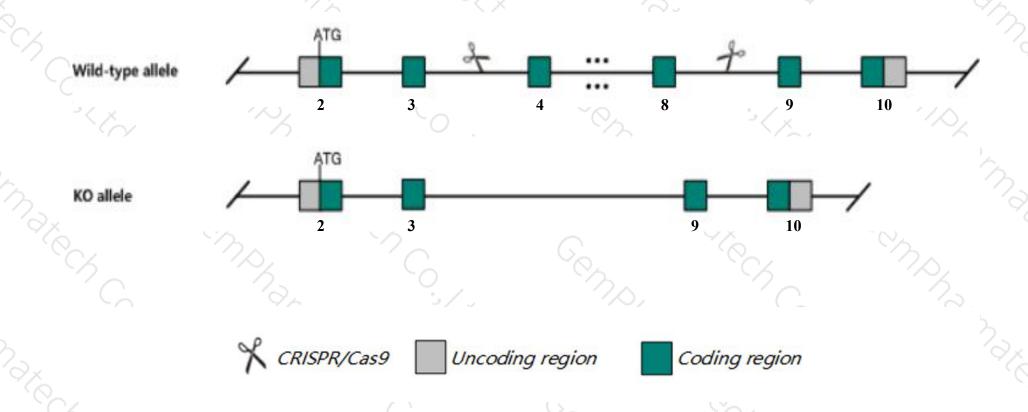
Project type Cas9-KO

Strain background C57BL/6JGpt

## **Knockout strategy**



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



### **Technical routes**



- The *Ctsb* gene has 3 transcripts. According to the structure of *Ctsb* gene, exon4-exon8 of *Ctsb*201(ENSMUST00000006235.8) transcript is recommended as the knockout region. The region contains 581bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Ctsb* gene. The brief process is as follows: CRISPR/Cas9 system 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.

### **Notice**



- ➤ According to the existing MGI data, homozygotes for targeted null mutations are born normal without gross abnormalities. Homozygous mutant has resistance to induced pancreatitis. In combination with Ctsl
- The *Ctsb* gene is located on the Chr14. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

## Gene information (NCBI)



#### Ctsb cathepsin B [Mus musculus (house mouse)]

Gene ID: 13030, updated on 13-Mar-2020

#### Summary

☆ ?

Official Symbol Ctsb provided by MGI

Official Full Name cathepsin B provided by MGI

Primary source MGI:MGI:88561

See related Ensembl:ENSMUSG00000021939

Gene type protein coding
RefSeq status REVIEWED

Organism Mus musculus

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;

Muroidea; Muridae; Murinae; Mus; Mus

Also known as CB

Summary This gene encodes a member of the peptidase C1 family and preproprotein that is proteolytically processed to generate multiple protein

products. These products include the cathepsin B light and heavy chains, which can dimerize to generate the double chain form of the enzyme. This enzyme is a lysosomal cysteine protease with both endopeptidase and exopeptidase activity that may play a role in protein turnover. Homozygous knockout mice for this gene exhibit reduced pancreatic damage following induced pancreatitis and reduced hepatocyte apoptosis in a model of liver injury. Pseudogenes of this gene have been identified in the genome. [provided by

RefSeq, Aug 2015]

Expression Ubiquitous expression in adrenal adult (RPKM 374.9), subcutaneous fat pad adult (RPKM 322.2) and 26 other tissuesSee more

Orthologs <u>human</u> all

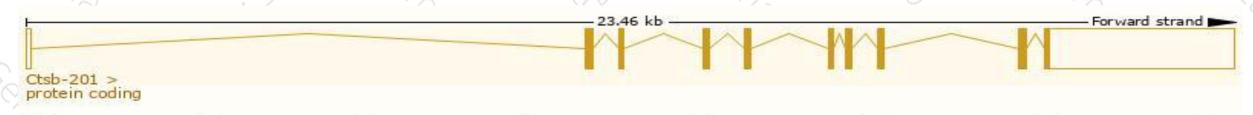
## Transcript information (Ensembl)



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

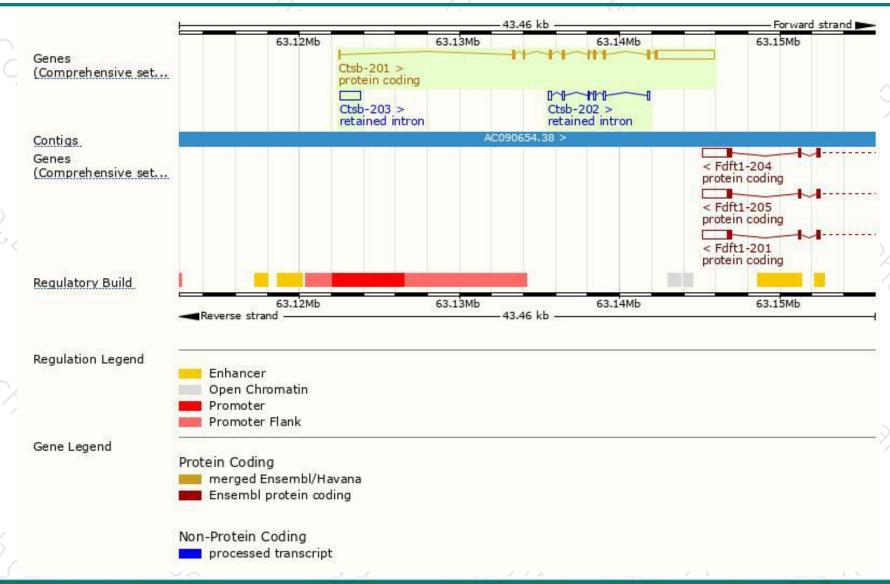
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ctsb-201	ENSMUST00000006235.8	4743	<u>339aa</u>	Protein coding	CCDS27197	P10605	TSL:1 GENCODE basic APPRIS P1
Ctsb-203	ENSMUST00000225639.1	1269	No protein	Retained intron	14-1	15-	
Ctsb-202	ENSMUST00000225540.1	801	No protein	Retained intron	0.27	) <del>-</del>	

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



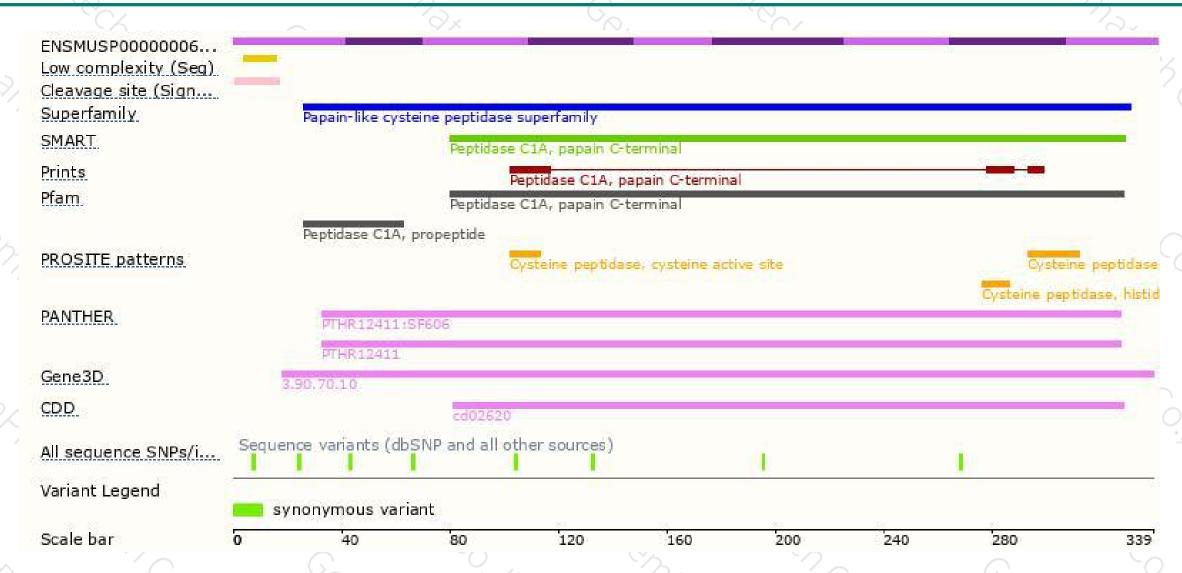
## Genomic location distribution





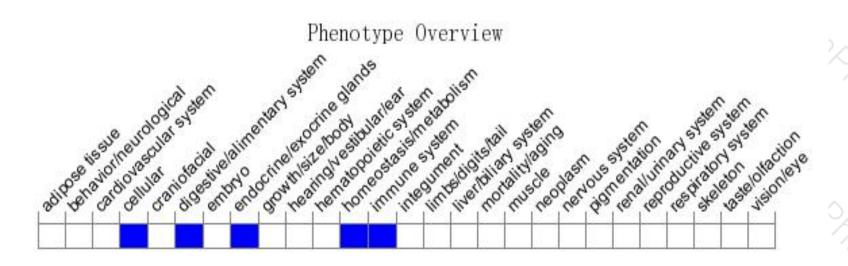
### Protein domain





## 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, homozygotes for targeted null mutations are born normal without gross abnormalities. Homozygous mutant has resistance to induced pancreatitis. In combination with Ctsl



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





