

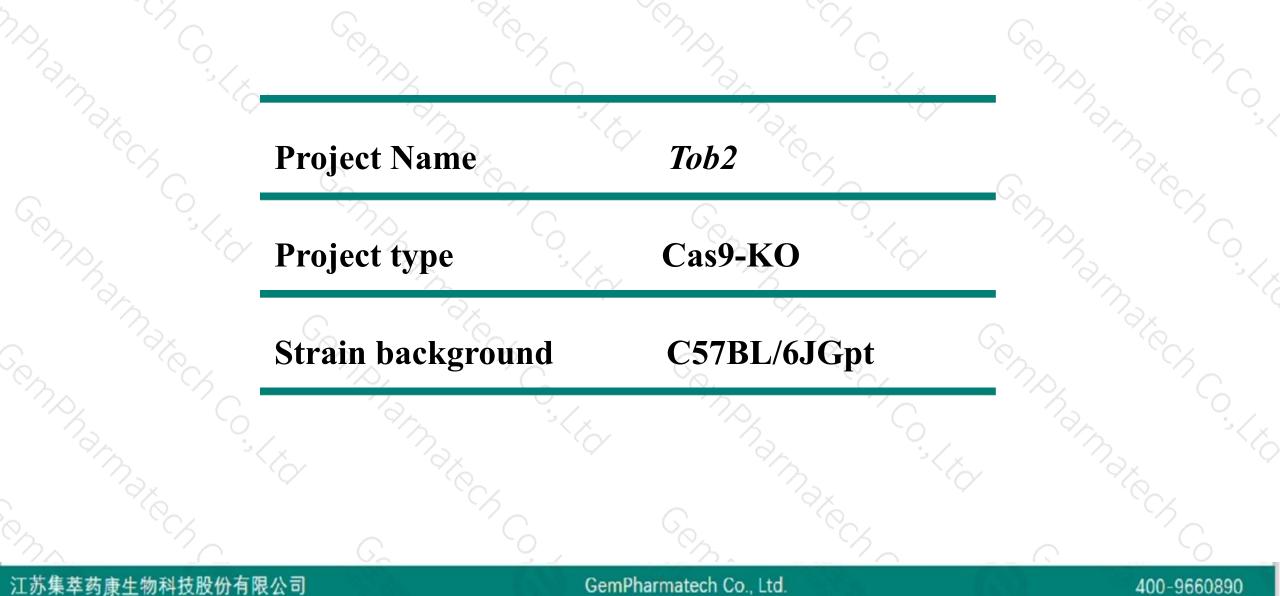
# **Tob2** Cas9-KO Strategy

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## **Project Overview**

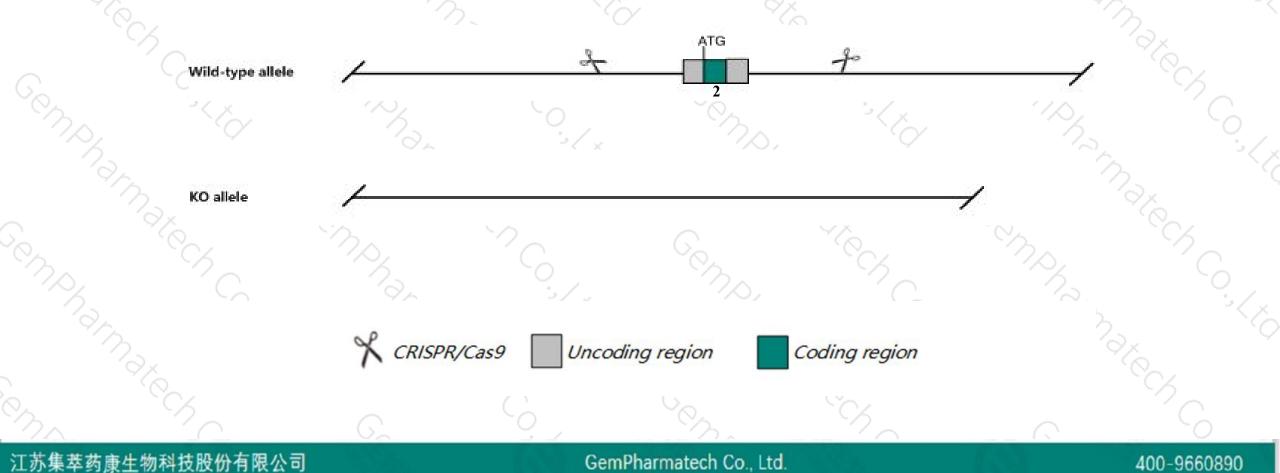




# **Knockout** strategy



This model will use CRISPR/Cas9 technology to edit the *Tob2* gene. The schematic diagram is as follows:





- The Tob2 gene has 4 transcripts. According to the structure of Tob2 gene, exon2 of Tob2-201 (ENSMUST00000050467.8) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.
- > In this project we use CRISPR/Cas9 technology to modify Tob2 gene. The brief process is as follows: CRISPR/Cas9 system v



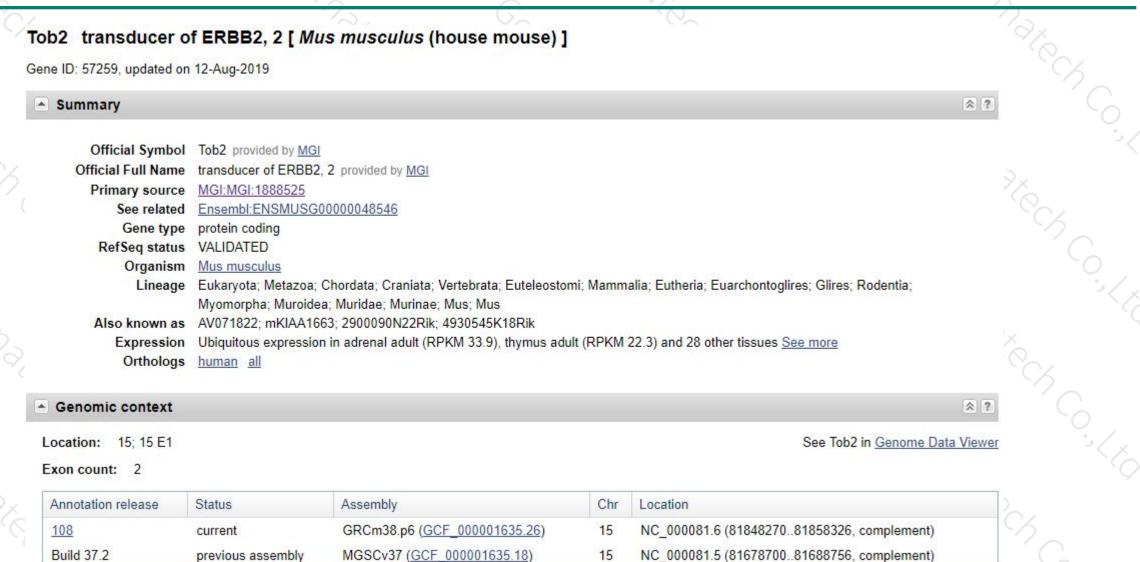
- According to the existing MGI data, Mice homozygous for a targeted mutation display reduced bone mass due to increased osteoclast numbers and acceleration of the bone resorption rate.
- The knockout region is near to the N-terminal of *Gm49476* and *Gm8444* gene, this strategy may influence the regulatory function of the N-terminal of these genes.
- ➤ Transcript *Tob2*-202&203 may not be affected.
- The *Tob2* gene is located on the Chr15. 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)**

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# **Transcript information (Ensembl)**



### The gene has 4 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tob2-201	ENSMUST0000050467.8	4151	<u>345aa</u>	Protein coding	CCDS27673	Q543X9 Q9JM55	TSL:1 GENCODE basic APPRIS P1
Tob2-204	ENSMUST00000231000.1	615	<u>44aa</u>	Protein coding	2 <del>.</del>	A0A2R8W6W6	CDS 3' incomplete
Tob2-202	ENSMUST00000229500.1	2590	No protein	Retained intron	-	ц.	
Tob2-203	ENSMUST00000230448.1	858	No protein	Retained intron	12	4	

The strategy is based on the design of *Tob2-201* transcript, The transcription is shown below

#### < Tob2-201 protein coding

Reverse strand

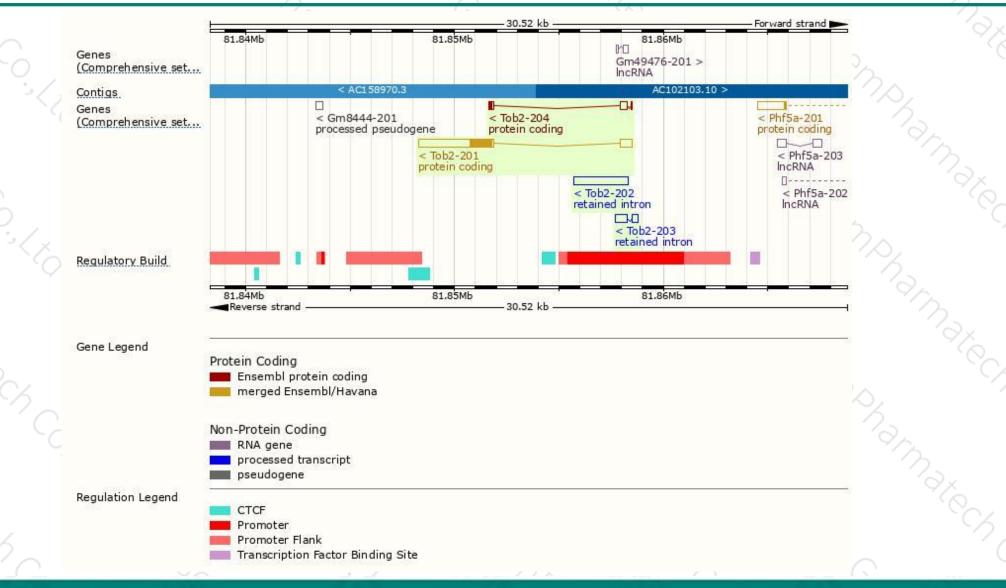
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10.21 kb

### **Genomic location distribution**



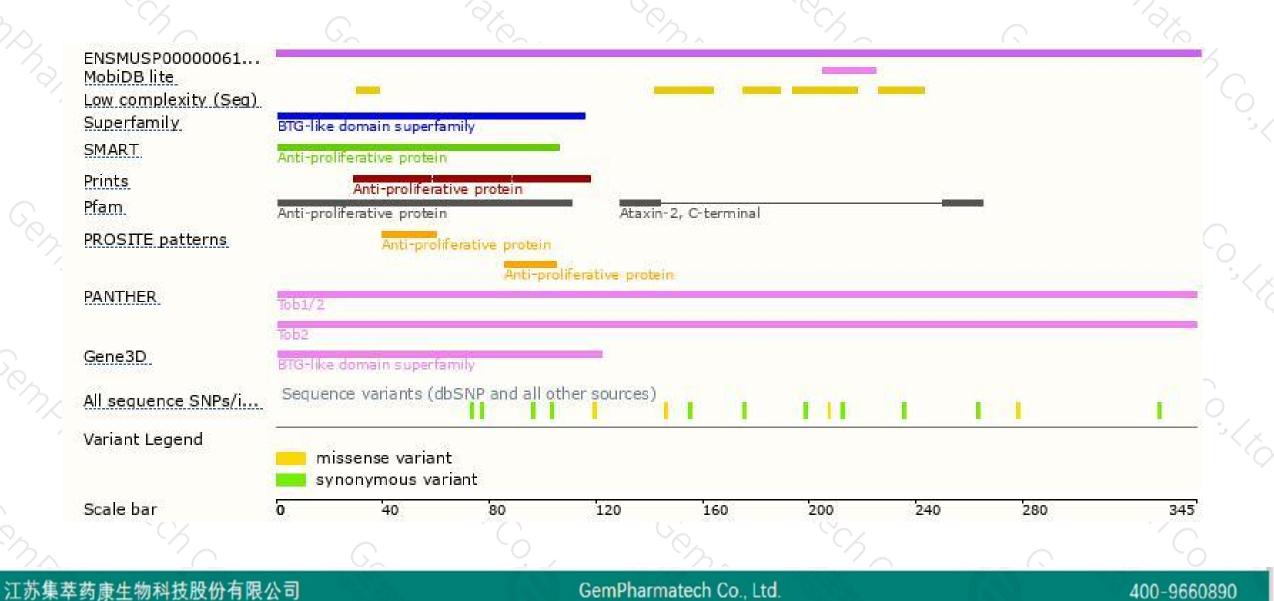


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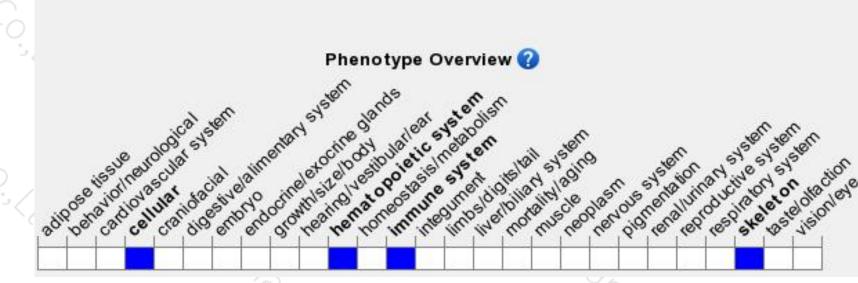
### **Protein domain**





### 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 targeted mutation display reduced bone mass due to increased osteoclast numbers and acceleration of the bone resorption rate.

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



