

# Tbc1d31 Cas9-KO Strategy

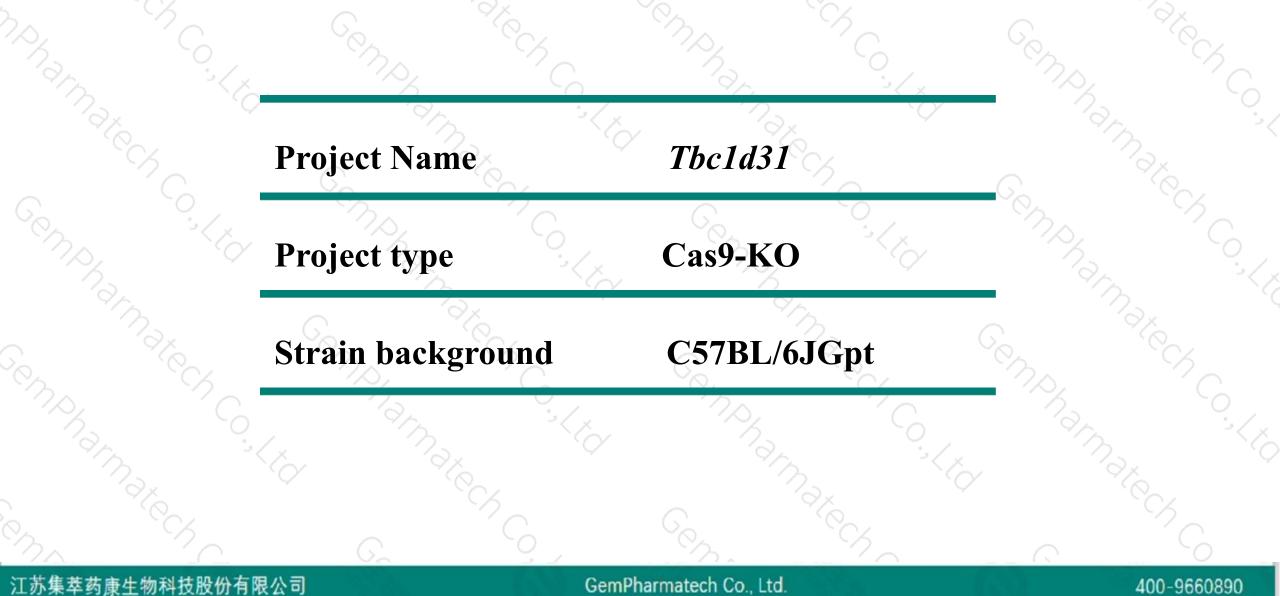
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**Reviewer: Yanhua Shen** 

Design Date: 2020-10-12

### **Project Overview**

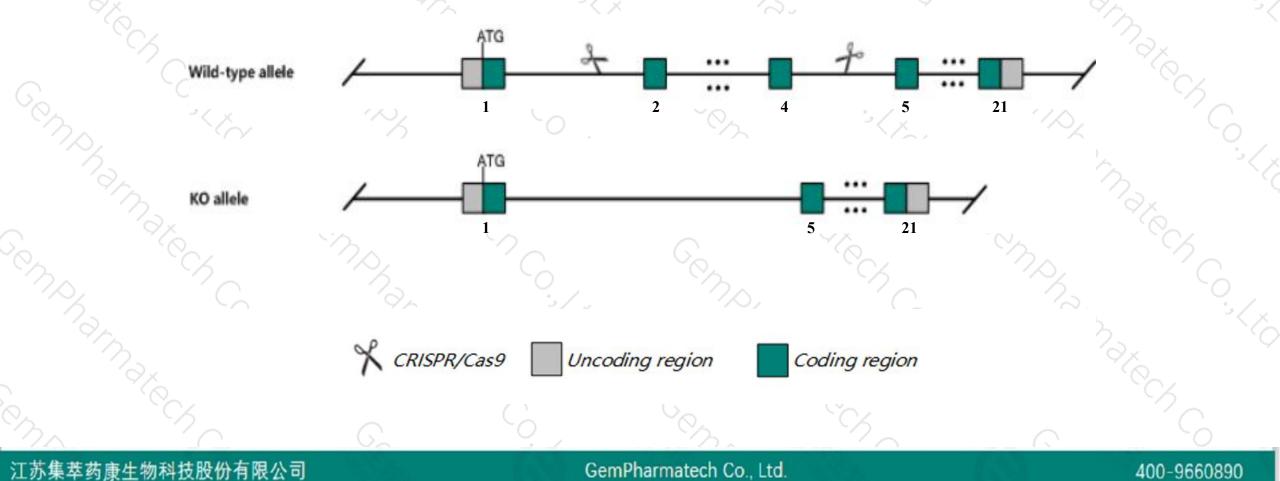




# **Knockout** strategy



This model will use CRISPR/Cas9 technology to edit the *Tbc1d31* gene. The schematic diagram is as follows:





> The *Tbc1d31* gene has 7 transcripts. According to the structure of *Tbc1d31* gene, exon2-exon4 of *Tbc1d31*-201(ENSMUST00000022992.12) transcript is recommended as the knockout region. The region contains 442bp coding sequence. Knock out the region will result in disruption of protein function.

> In this project we use CRISPR/Cas9 technology to modify *Tbc1d31* 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.



➤ Transcript *Tbc1d31*-206 may not be affected.

> The *Tbc1d31* 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.

Notice

### **Gene information** (NCBI)



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### Tbc1d31 TBC1 domain family, member 31 [ Mus musculus (house mouse) ]

Gene ID: 210544, updated on 25-Sep-2020

#### Summary

Official Symbol Tbc1d31 provided by MGI TBC1 domain family, member 31 provided by MGI Official Full Name MGI:MGI:2684931 Primary source Ensembl:ENSMUSG0000022364 See related Gene type protein coding **RefSeq status** VALIDATED Organism Mus musculus Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Lineage Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus Also known as 4B3; Gm85; Wdr6; Wdr67; D330013L20Rik Expression Broad expression in testis adult (RPKM 7.4), CNS E11.5 (RPKM 4.6) and 25 other tissues See more Orthologs human all

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## **Transcript information (Ensembl)**



### The gene has 7 transcripts, all transcripts are shown below:

						1	
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tbc1d31-201	ENSMUST00000022992.12	3311	<u>996aa</u>	Protein coding	CCDS37077	Q6NXY1	TSL:1 GENCODE basic APPRIS P1
Tbc1d31-206	ENSMUST00000161329.1	914	<u>234aa</u>	Protein coding	-	<u>F6T4B0</u>	CDS 5' incomplete TSL:5
Tbc1d31-203	ENSMUST00000159775.7	434	<u>49aa</u>	Nonsense mediated decay	2	E0CYF4	TSL:5
Tbc1d31-202	ENSMUST00000110175.8	3468	No protein	Processed transcript	-	a=1	TSL:1
Tbc1d31-207	ENSMUST00000162157.1	3727	No protein	Retained intron	4	8 <b>-</b> 2	TSL:1
Tbc1d31-204	ENSMUST00000159801.7	3583	No protein	Retained intron	-	0.70	TSL:1
Tbc1d31-205	ENSMUST00000160098.7	2457	No protein	Retained intron	-	0.00	TSL:1

57.86 kb

The strategy is based on the design of *Tbc1d31-201* transcript, the transcription is shown below:



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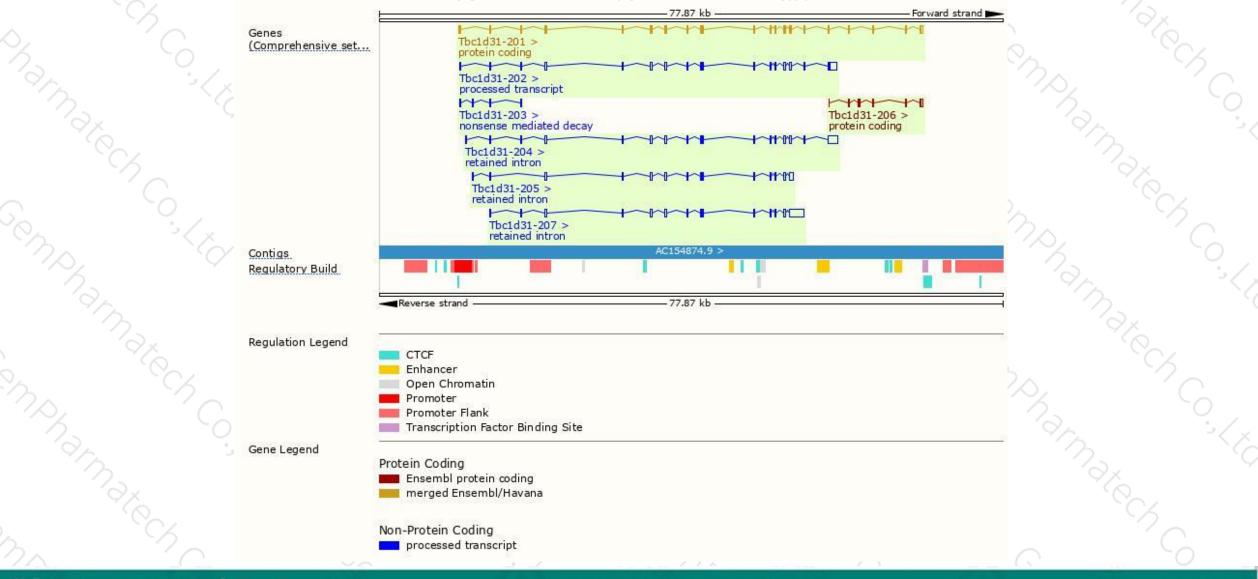
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Forward strand

### **Genomic location distribution**



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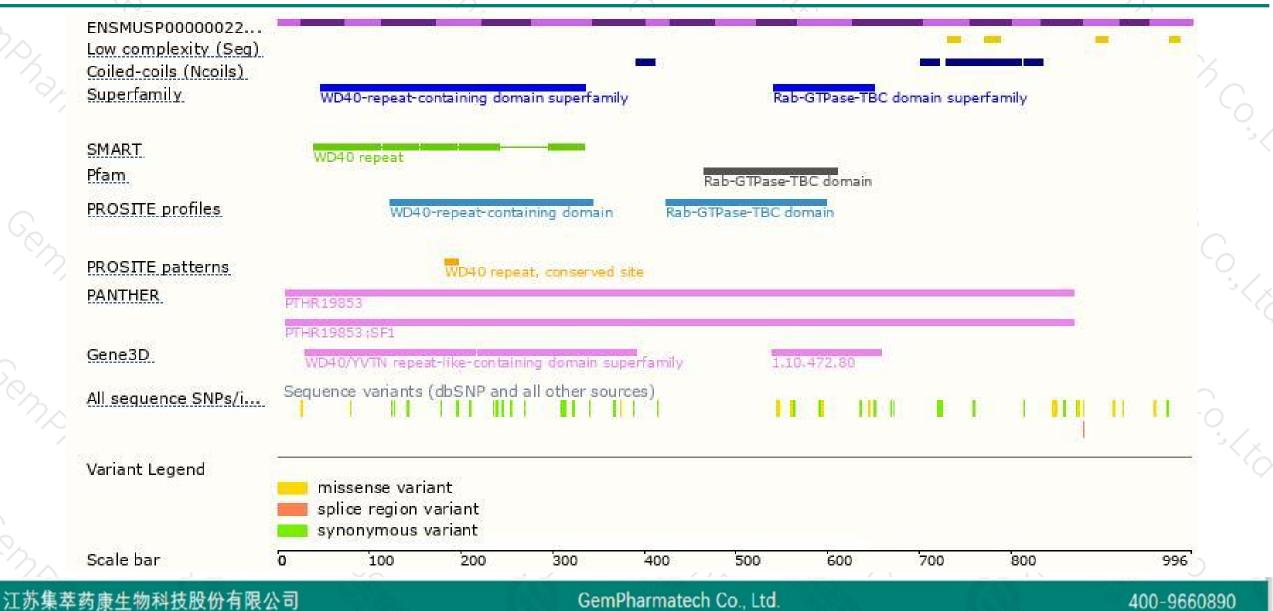


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### **Protein domain**







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



