

Tbcd Cas9-CKO Strategy

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Overview

Target Gene Name

• Tbcd

Project Type

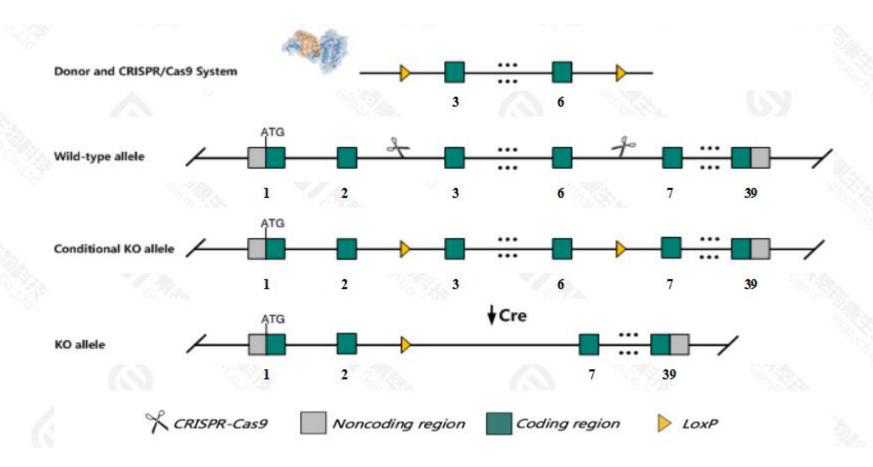
• Cas9-CKO

Genetic Background

• C57BL/6JGpt



Strain Strategy

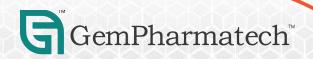


Schematic representation of CRISPR-Cas9 engineering used to edit the *Tbcd* gene.



Technical Information

- The *Tbcd* gene has 8 transcripts. According to the structure of *Tbcd* gene, exon3-exon6 of *Tbcd-201*(ENSMUST0000103013.10) transcript is recommended as the knockout region. The region contains 403bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Tbcd*gene. The brief process is as follows: CRISPR-Cas9 system 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- 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.



Gene Information

Tbcd tubulin-specific chaperone d [Mus musculus (house mouse)]

Gene ID: 108903, updated on 24-Apr-2022

Summary



Official Symbol Tbcd provided by MGI

Official Full Name tubulin-specific chaperone d provided by MGI

Primary source MGI:MGI:1919686

See related Ensembl:ENSMUSG00000039230

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 2310057L06Rik, A030005L14Rik, mKIAA0988

Expression Ubiquitous expression in CNS E18 (RPKM 8.3), whole brain E14.5 (RPKM 7.8) and 28 other tissuesSee more

Orthologs <u>human all</u>

Source: https://www.ncbi.nlm.nih.gov/

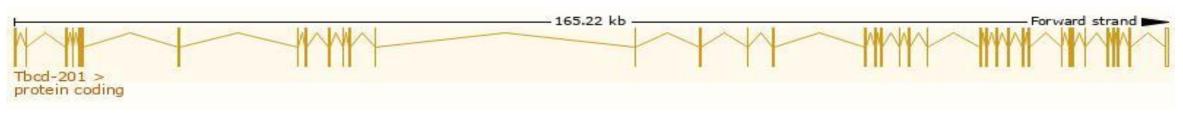


Transcript Information

The gene has 8 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tbcd-201	ENSMUST00000103013.10	3906	1196aa	Protein coding	CCDS25778		TSL:1 , GENCODE basic , APPRIS P1
Tbcd-202	ENSMUST00000106093.2	368	<u>84aa</u>	Protein coding	-3		CDS 3' incomplete , TSL:3 ,
Tbcd-203	ENSMUST00000125167.8	944	<u>72aa</u>	Nonsense mediated decay	-		CDS 5' incomplete , TSL:5 ,
Tbcd-207	ENSMUST00000151666.2	1324	No protein	Processed transcript	-		TSL:1,
Tbcd-204	ENSMUST00000139414.8	1292	No protein	Processed transcript	20		TSL:1,
Tbcd-206	ENSMUST00000147560.8	699	No protein	Processed transcript	-		TSL:5,
Tbcd-208	ENSMUST00000155666.8	4832	No protein	Retained intron	-2		TSL:1,
Tbcd-205	ENSMUST00000147470.2	716	No protein	Retained intron	20		TSL:5,

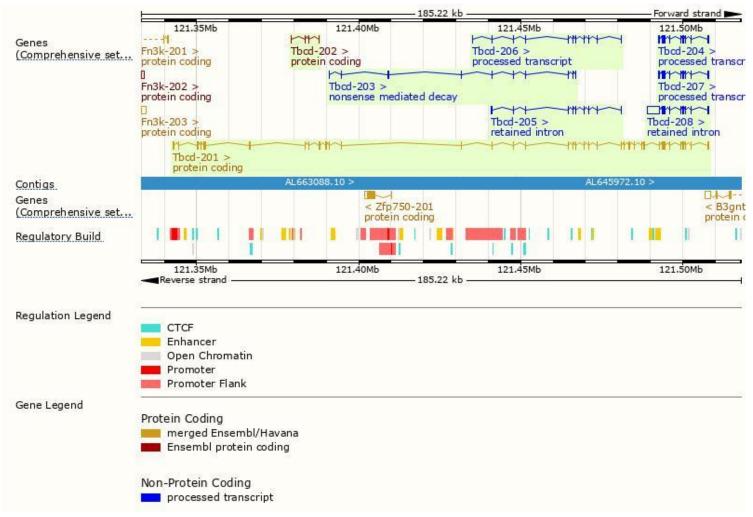
The strategy is based on the design of Tbcd-201 transcript, the transcription is shown below:

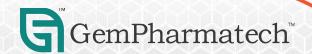


Source: https://www.ensembl.org



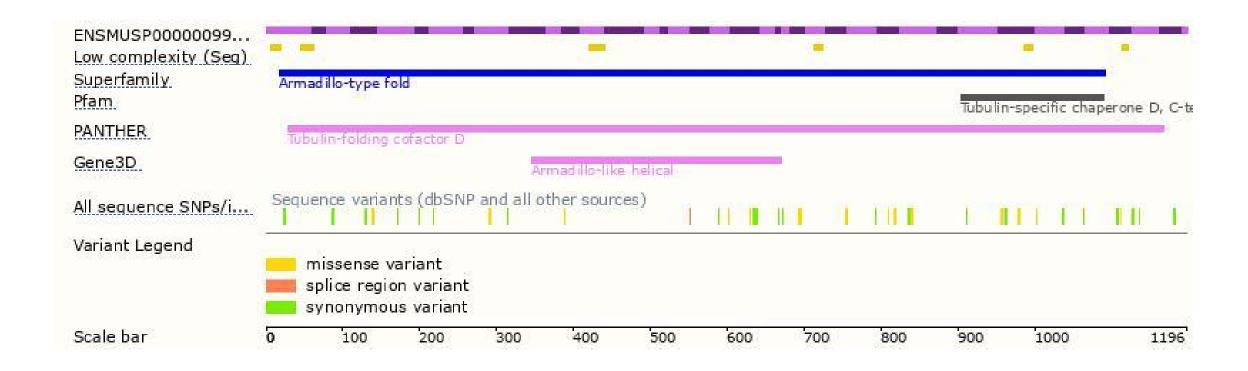
Genomic Information





Source: : https://www.ensembl.org

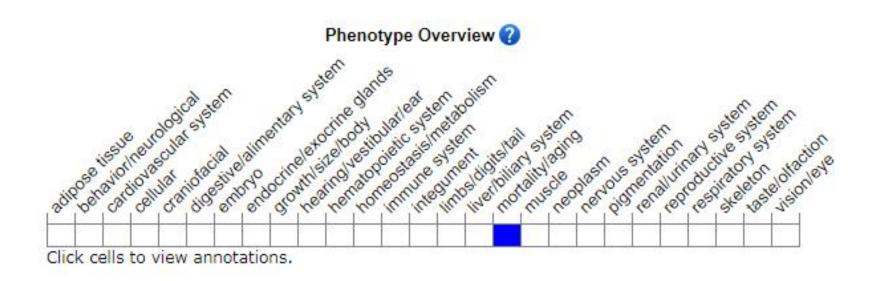
Protein Information





Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



• Phenotypes affected by the mutations of Tbcd gene are marked in blue.



Source: https://www.informatics.jax.org

Important Information

- Transcript Tbcd-202 may not be affected.
- The *Tbcd* gene is located on the Chr11. 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.

