GemPharmatech™

Tbcd Cas9-KO Strategy

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Overview

Target Gene Name

- Pabpc4

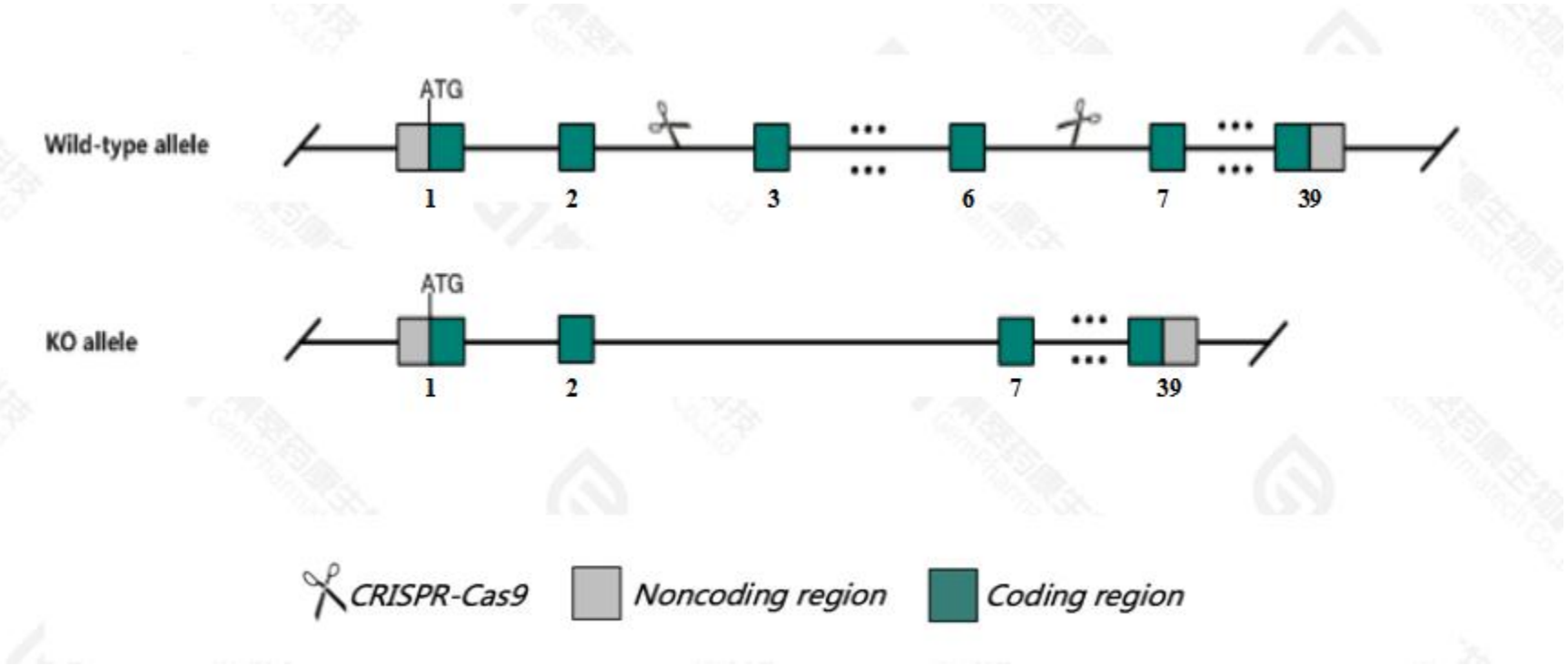
Project Type

- Cas9-KO

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*(ENSMUST00000103013.10) 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: gRNAs were transcribed in vitro. Cas9 and gRNAs 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.

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 byMGI
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	human all

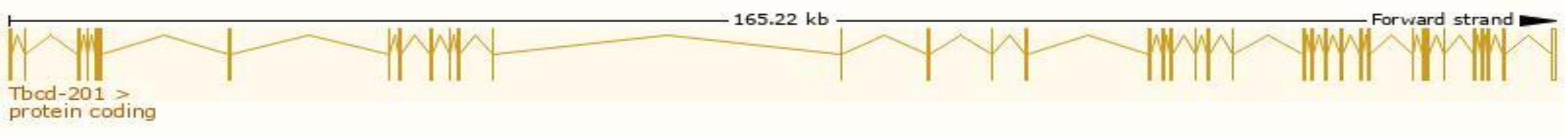
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

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

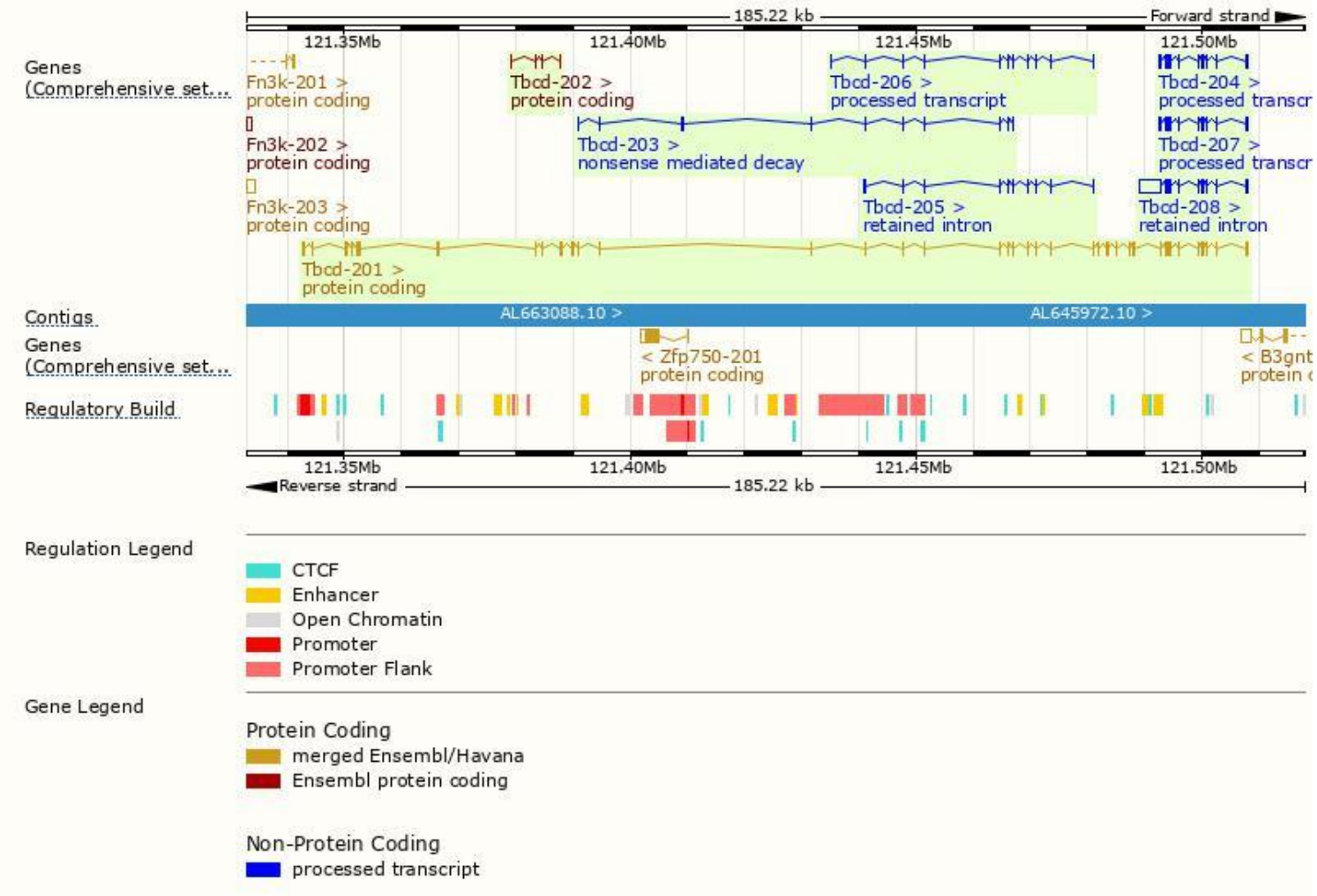
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tbcd-201	ENSMUST00000103013.10	3906	1196aa	Protein coding	CCDS25778		TSL:1 , GENCODE basic , APPRIS P1 ,
Tbcd-202	ENSMUST00000106093.2	368	84aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Tbcd-203	ENSMUST00000125167.8	944	72aa	Nonsense mediated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

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



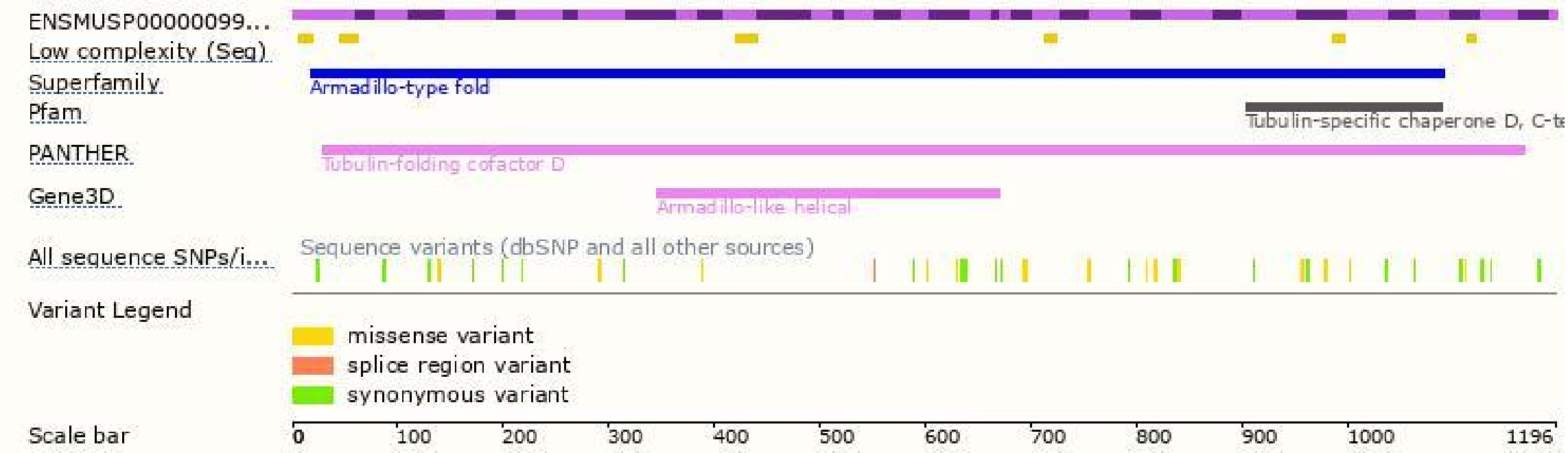
Source: https://www.ensembl.org

Genomic Information



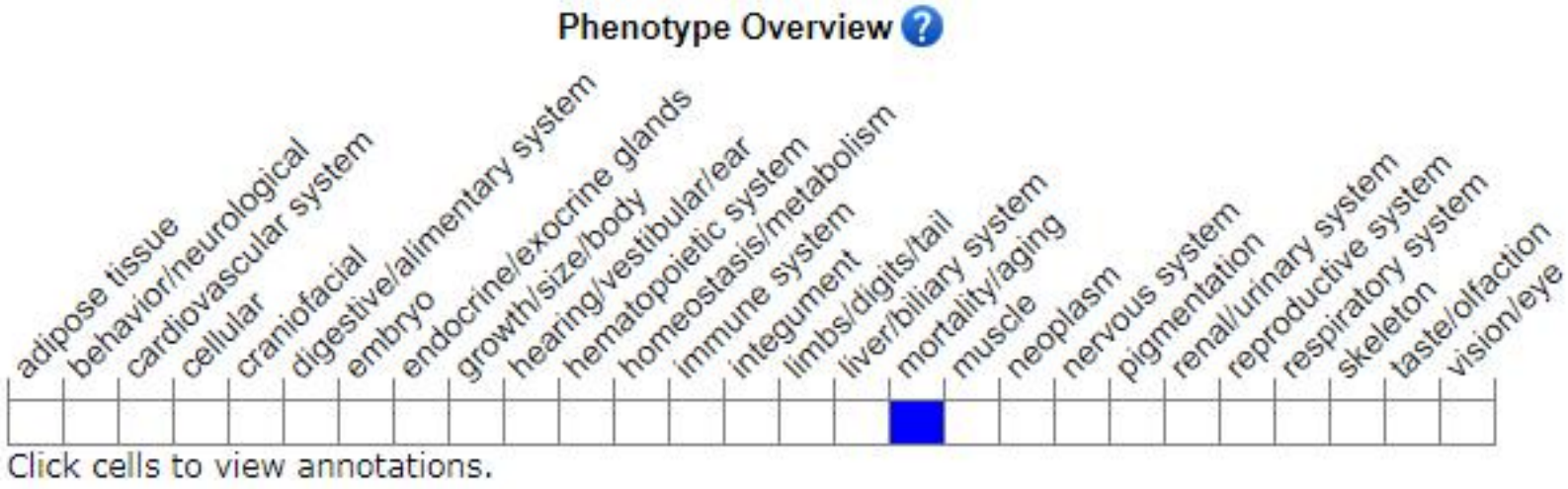
Source: : https://www.ensembl.org

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

- 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.