

# Dolar Day College Tbk1 Cas9-CKO Strategy To hall alto color color

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# **Project Overview**



**Project Name** 

Tbk1

**Project type** 

Cas9-CKO

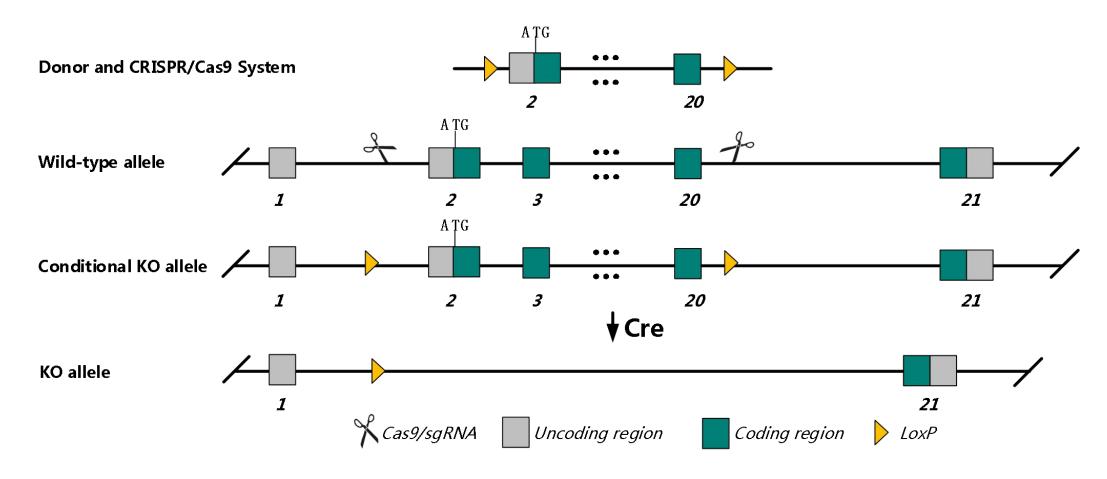
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Tbk1* gene. The schematic diagram is as follows:



## Technical routes



- The *Tbk1* gene has 7 transcripts. According to the structure of *Tbk1* gene, exon2-exon20 of *Tbk1-201* (ENSMUST00000020316.3) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Tbk1* 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.
- 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.

## **Notice**



- ➤ According to the existing MGI data, Homozygous null mice display embryonic lethality. Mice homozygous for a different knock-out allele exhibit increased prenatal lethality, increased infiltration of mononuclear and/or granulomatous cells in multiple organs and tissues at 3 months of age, and increased lethality in response to LPS.
- $\gt$  The *Tbk1* gene is located on the Chr10. 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.

## Gene information (NCBI)



#### Tbk1 TANK-binding kinase 1 [Mus musculus (house mouse)]

Gene ID: 56480, updated on 31-Jan-2019

#### Summary

☆ ?

Official Symbol Tbk1 provided by MGI

Official Full Name TANK-binding kinase 1 provided by MGI

Primary source MGI:MGI:1929658

See related Ensembl: ENSMUSG00000020115

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 1200008B05Rik, Al462036, AW048562

Expression Ubiquitous expression in bladder adult (RPKM 7.5), CNS E11.5 (RPKM 7.4) and 28 other tissuesSee more

Orthologs <u>human</u> all

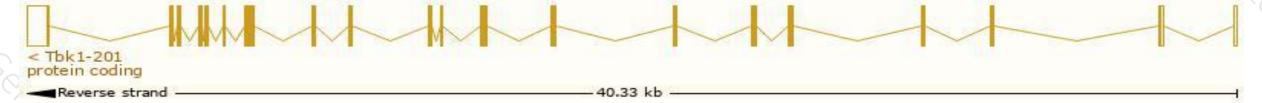
# Transcript information (Ensembl)



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

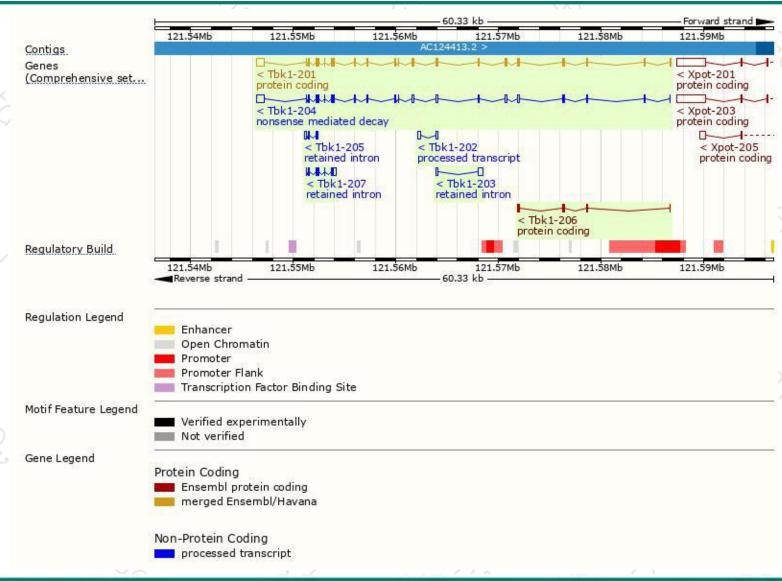
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tbk1-201	ENSMUST00000020316.3	3024	729aa	Protein coding	CCDS24212	A1L361 Q9WUN2	TSL:1 GENCODE basic APPRIS P1
Tbk1-206	ENSMUST00000219493.1	442	<u>108aa</u>	Protein coding		A0A1W2P835	CDS 3' incomplete TSL:3
Tbk1-204	ENSMUST00000219400.1	3005	<u>121aa</u>	Nonsense mediated decay	(120)	A0A1W2P6W7	TSL:2
Tbk1-202	ENSMUST00000218460.1	406	No protein	Processed transcript	121	3/20	TSL:3
Tbk1-207	ENSMUST00000220386.1	747	No protein	Retained intron	15.	(5)	TSL:3
Tbk1-203	ENSMUST00000219244.1	634	No protein	Retained intron	(#K	680	TSL:3
Tbk1-205	ENSMUST00000219439.1	485	No protein	Retained intron	(44)	140	TSL:3

The strategy is based on the design of *Tbk1-201* transcript, The transcription is shown below



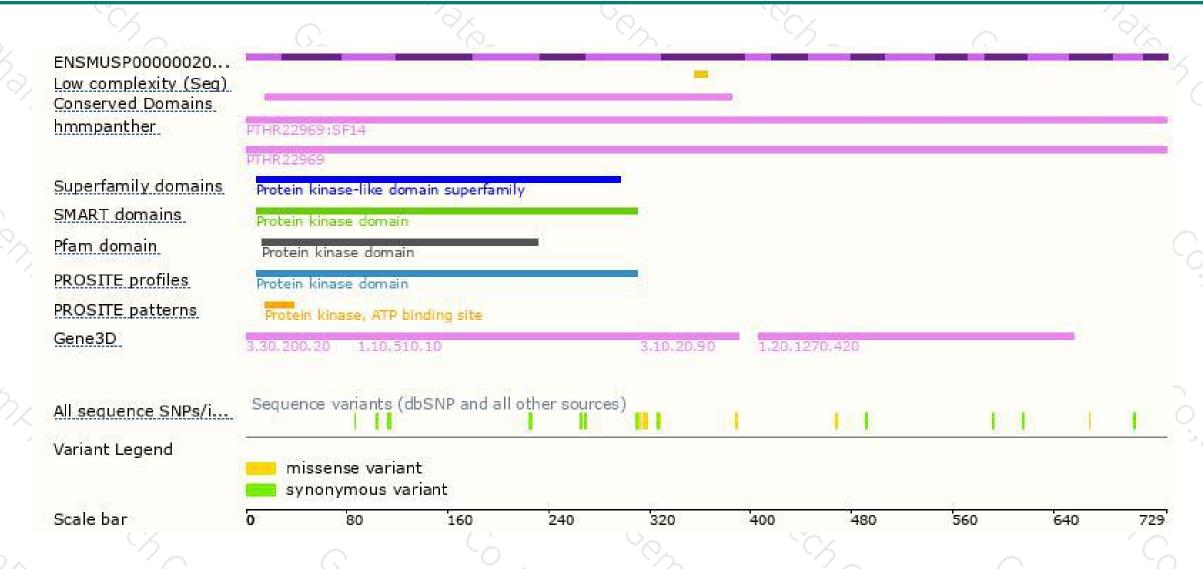
## Genomic location distribution





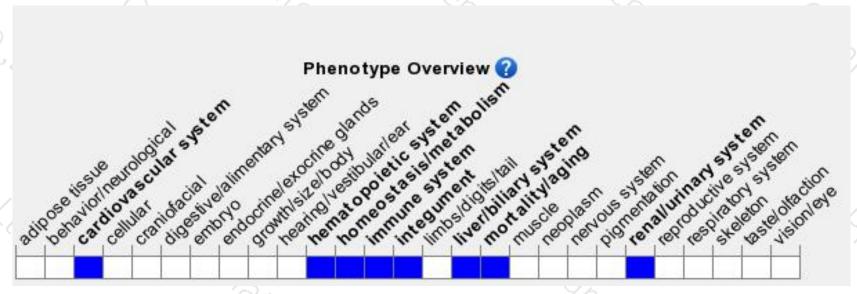
### Protein domain





## 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, Homozygous null mice display embryonic lethality. Mice homozygous for a different knock-out allele exhibit increased prenatal lethality, increased infiltration of mononuclear and/or granulomatous cells in multiple organs and tissues at 3 months of age, and increased lethality in response to LPS.



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





