

# Ttll4 Cas9-KO Strategy

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#### Overview

#### Target Gene Name

• Ttll4

### Project Type

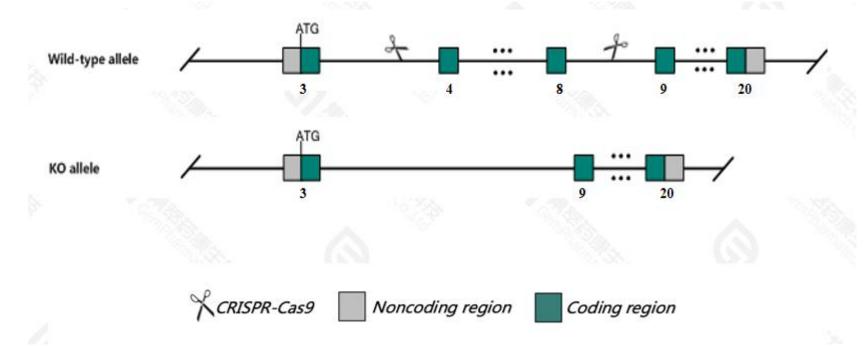
• Cas9-KO

#### Genetic Background

• C57BL/6JGpt



## Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the Ttll4 gene.



#### Technical Information

- The *Ttll4* gene has 11 transcripts. According to the structure of *Ttll4* gene, exon4-exon8 of *Ttll4*-201 (ENSMUST00000042125.15) transcript is recommended as the knockout region. The region contains 487bp of coding sequences. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Ttll4* 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

Ttll4 tubulin tyrosine ligase-like family, member 4 [ Mus musculus (house mouse) ]

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Gene ID: 67534, updated on 26-Sep-2022

Summary



Official Full Name tubulin tyrosine ligase-like family, member 4 provided by MGI

Primary source MGI:MGI:1914784

See related Ensembl: ENSMUSG00000033257 AllianceGenome: MGI: 1914784

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 mKIAA0173; 4632407P03Rik

Summary Enables protein-glutamic acid ligase activity and tubulin binding activity. Involved in peptidyl-glutamic acid modification. Acts upstream of or within protein

polyglutamylation and regulation of blastocyst development. Located in 9+0 non-motile cilium and ciliary basal body. Orthologous to human TTLL4 (tubulin tyrosine

ligase like 4). [provided by Alliance of Genome Resources, Apr 2022]

Expression Ubiquitous expression in testis adult (RPKM 16.4), CNS E11.5 (RPKM 10.3) and 27 other tissues See more

Orthologs <u>human</u> all

**NEW** 

Try the new Gene table

Try the new Transcript table

#### Genomic context

See Ttll4 in Genome Data Viewer

Location: 1; 1 C4

Exon count: 20

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

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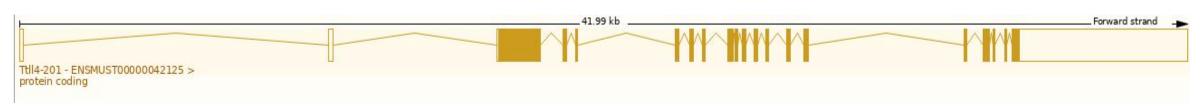


## Transcript Information

The gene has 11 transcripts, all transcripts are shown below:

Transcript ID	Name 4	bp 🍦	Protein 4	Biotype	CCDS 🍦	UniProt Match	Flags
ENSMUST00000042125.15	Ttll4-201	10019	<u>1193aa</u>	Protein coding	CCDS15053 ₪	Q80UG8@	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1
ENSMUST00000113678.2	Ttll4-202	3762	<u>1129aa</u>	Protein coding		D3YY08@	GENCODE basic TSL:5
ENSMUST00000141119.8	Ttll4-206	664	<u>113aa</u>	Protein coding		D3Z402₽	TSL:3 CDS 3' incomplete
ENSMUST00000129890.8	Ttll4-204	493	103aa	Protein coding		D3Z7H1@	TSL:3 CDS 3' incomplete
ENSMUST00000145132.8	Ttll4-209	5078	No protein	Protein coding CDS not defined		-	TSL:5
ENSMUST00000143925.8	Ttll4-208	812	No protein	Protein coding CDS not defined		-2	TSL:3
ENSMUST00000143790.2	Ttll4-207	760	No protein	Protein coding CDS not defined		-2	TSL:3
ENSMUST00000140591.2	Ttll4-205	491	No protein	Protein coding CDS not defined		-	TSL:3
ENSMUST00000128891.8	Ttll4-203	3389	No protein	Retained intron		-	TSL:1
ENSMUST00000155753.2	Ttll4-211	754	No protein	Retained intron			TSL:3
ENSMUST00000153636.8	Ttll4-210	485	No protein	Retained intron		-2	TSL:2

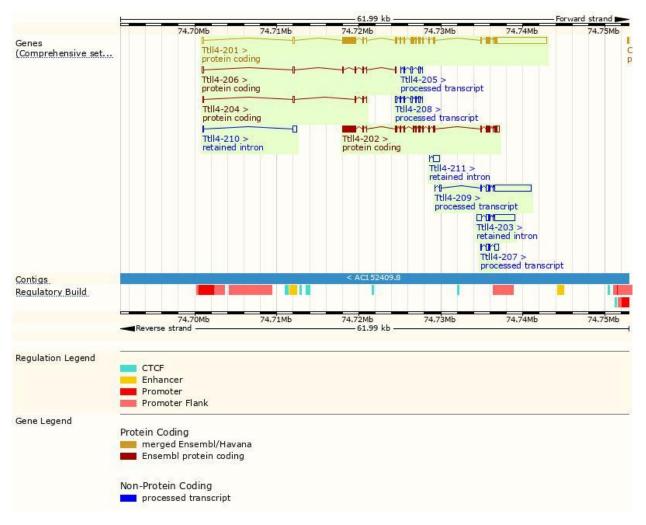
The strategy is based on the design of *Ttll4*-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org



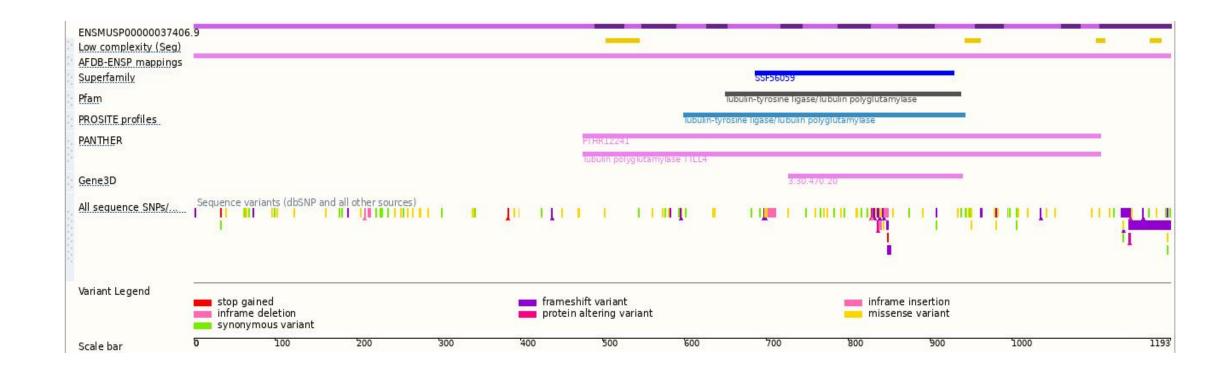
## Genomic Information





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

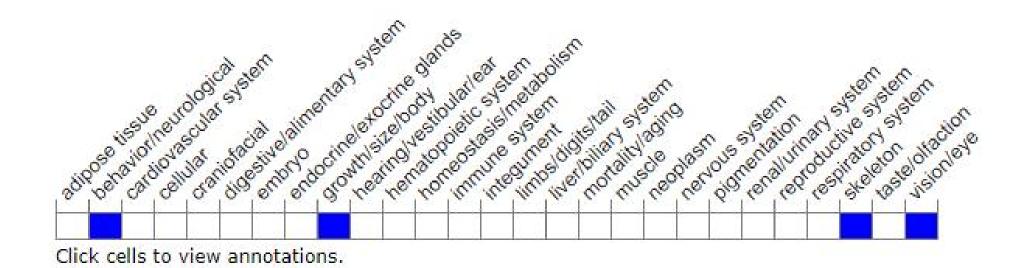
#### Protein Information





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

## Mouse Phenotype Information (MGI)



• Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(http://www.informatics.jax.org/).



## Important Information

- The *Ttll4* gene is located on the Chr1. 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.

