

# Ttll5 Cas9-KO Strategy

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Design Date: 2023-02-06

### Overview

### Target Gene Name

• Ttll5

Project Type

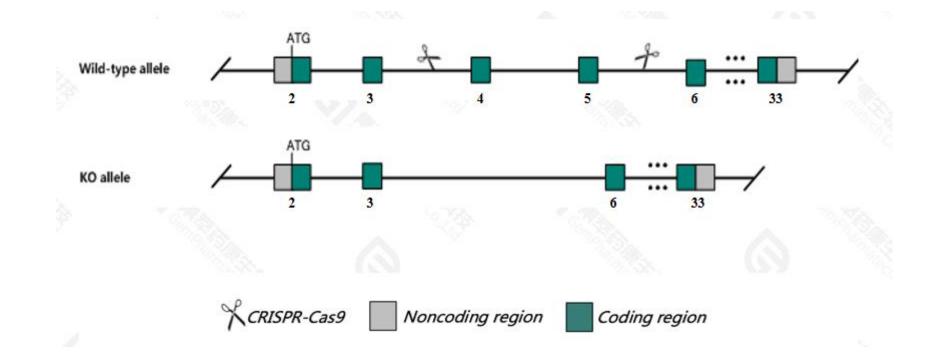
• Cas9-KO

Genetic Background

• C57BL/6JGpt



## Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the Ttll5 gene.

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### Technical Information

- The *Ttll5* gene has 20 transcripts. According to the structure of *Ttll5* gene, exon4exon5 of *Ttll5*-201 (ENSMUST0000040179.14) transcript is recommended as the knockout region. The region contains 190bp of coding sequences. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Ttll5* 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

#### Ttll5 tubulin tyrosine ligase-like family, member 5 [ Mus musculus (house mouse) ]

Gene ID: 320244, updated on 29-Oct-2022

#### Summary

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Official Symbol Ttll5 provided by MGI Official Full Name tubulin tyrosine ligase-like family, member 5 provided by MGI Primary source MGI:MGI:2443657 See related Ensembl:ENSMUSG00000012609 AllianceGenome:MGI:2443657 Gene type protein coding RefSeg 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 Stamp; mKIAA0998; 1700048H13Rik; 2310009M18Rik; 4930556H18Rik; D630041K24Rik Summary Predicted to enable tubulin binding activity and tubulin-glutamic acid ligase activity. Acts upstream of or within fertilization; protein polyglutamylation; and sperm axoneme assembly. Located in 9+0 non-motile cilium and ciliary basal body. Is expressed in choroid invagination; diencephalon roof plate; medulla oblongata part of 4th ventricle choroid plexus; and metencephalon part of 4th ventricle choroid plexus. Human ortholog(s) of this gene implicated in cone-rod dystrophy 19. Orthologous to human TTLL5 (tubulin tyrosine ligase like 5). [provided by Alliance of Genome Resources, Apr 2022] Expression Biased expression in testis adult (RPKM 34.3), cerebellum adult (RPKM 11.1) and 14 other tissues See more Orthologs human all Try the new Gene table NEW Try the new Transcript table Genomic context < ?

Location: 12; 12 D2

Exon count: 39

See Ttll5 in Genome Data Viewer

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



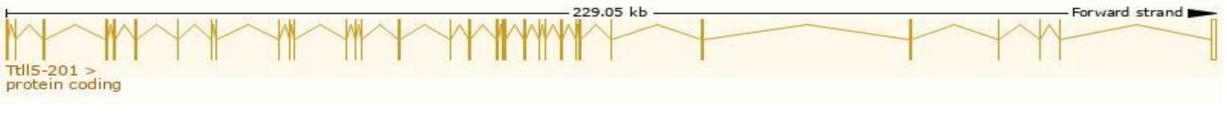
### Transcript Information

### The gene has 20 transcripts, all transcripts are shown below:

Transcript ID	Name 🖕	bp 🖕	Protein 🖕	Biotype .	CCDS 🖕	UniProt Match	Flags
ENSMUST0000040179.14	Ttll5-201	5081	<u>1328aa</u>	Protein coding	<u>CCDS36499</u> &	<u>Q8CHB8-1</u> ₽	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1
ENSMUST00000110224.8	Ttll5-205	4647	<u>1281aa</u>	Protein coding		<u>D3YVZ5</u> &	GENCODE basic TSL:5
ENSMUST0000095536.10	Ttll5-203	4150	<u>438aa</u>	Protein coding		Q8C125dP	GENCODE basic TSL:2
ENSMUST0000040273.14	Ttll5-202	4147	<u>1295aa</u>	Protein coding	<u>CCDS83991</u> ഒ	E9Q425@	GENCODE basic TSL:5
ENSMUST00000177168.8	Ttll5-217	3005	<u>795aa</u>	Protein coding		H3BJ73	TSL:1 CDS 5' incomplete
ENSMUST00000110220.9	Ttll5-204	2655	<u>348aa</u>	Protein coding		Q8BUF0⊮	GENCODE basic TSL:1
ENSMUST00000177525.8	Ttll5-219	1788	<u>395aa</u>	Protein coding		H3BLA3	TSL:1 CDS 5' incomplete
ENSMUST00000176937.8	Ttll5-215	1223	<u>408aa</u>	Protein coding		<u>H3BLC0</u> &	TSL:5 CDS 5' and 3' incomplete
ENSMUST00000176460.8	Ttll5-213	902	<u>301aa</u>	Protein coding		H3BK74@	TSL:5 CDS 5' and 3' incomplete
ENSMUST00000177188.8	Ttll5-218	699	<u>176aa</u>	Protein coding		H3BJC2	TSL:5 CDS 3' incomplete
ENSMUST00000142411.9	Ttll5-207	650	<u>63aa</u>	Protein coding		D3Z5H9@	TSL:5 CDS 3' incomplete
ENSMUST00000155448.9	Ttll5-209	4534	<u>563aa</u>	Nonsense mediated decay		Q8CHB8-2	TSL:2
ENSMUST00000176695.8	Ttll5-214	4168	<u>432aa</u>	Nonsense mediated decay		<u>H3BLN3</u> ៤	TSL:5
ENSMUST00000177114.2	Ttll5-216	3999	<u>432aa</u>	Nonsense mediated decay		H3BLN3 &	TSL:5
ENSMUST00000175844.2	Ttll5-210	1297	<u>297aa</u>	Nonsense mediated decay		H3BJC7@	TSL:1 CDS 5' incomplete
ENSMUST00000153570.9	Ttll5-208	655	<u>91aa</u>	Nonsense mediated decay		H3BJV4	TSL:3
ENSMUST00000176008.8	Ttll5-212	5482	No protein	Protein coding CDS not defined		12	TSL:5
ENSMUST00000220906.2	Ttll5-220	1039	No protein	Protein coding CDS not defined		2	TSL:1
ENSMUST00000124701.8	Ttll5-206	2165	No protein	Retained intron		-	TSL:2
ENSMUST00000175899.2	Ttll5-211	636	No protein	Retained intron		<u>.</u>	TSL:3

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The strategy is based on the design of *Ttll5*-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org

### Genomic Information

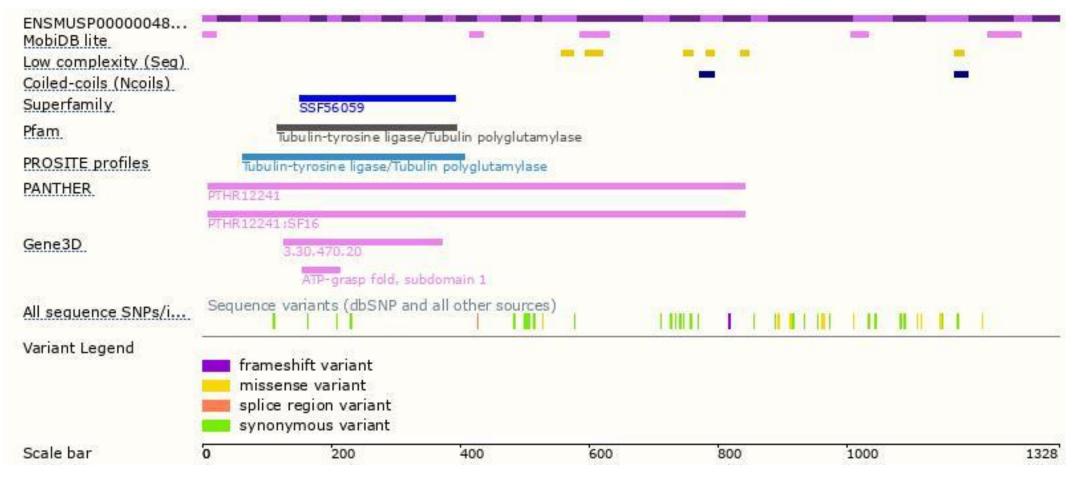
nd 🛌 85.9M 86.0M 86.1M Ttll5-211 > Ttll5-217 > protein coding Ttll5-218 > protein coding + mm Genes (Comprehensive set... Ttll5-203 > protein coding -111 -1110 Ttll5-213 > protein coding Ttll5-220 > processed transcript N Ttll5-204 > protein coding Ttll5-215 > protein coding Gr Ttll5-201 > protein cod HI HANNEL m - manan Ttll5-219 > protein coding Ttll5-210 > ponsense m nediated decay -----m - HAMAN Contigs Genes (Comprehensive set... < Erg28-201 protein coding < Gm26531-201 IncRNA < Tgfb3 protein < Erg28-203 processed transcript ⊂4 < Erg28-202 retained intron H < Erg28-205 retained intron < Erg28-204 protein coding **Regulatory Build** 85.9M 86.0Mb - 257.24 kb 86.1Mb Reverse strand -Regulation Legend CTCF Enhancer Open Chromatin Promoter Promoter Flank Transcription Factor Binding Site Gene Legend Protein Coding Ensembl protein coding merged Ensembl/Havana Non-Protein Coding RNA gene

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

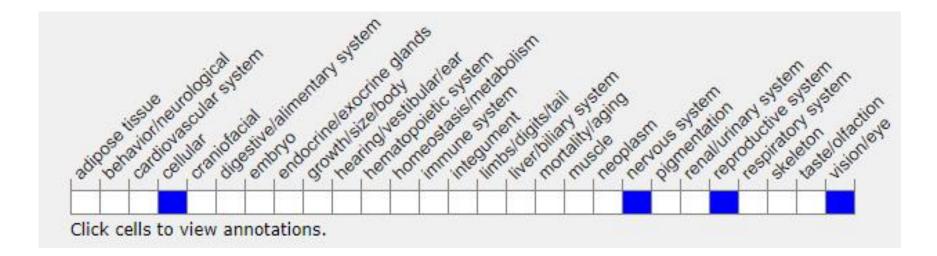
### Protein Information

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

### Mouse Phenotype Information (MGI)



• Phenotypes affected by the mutations of *Ttll5* gene are marked in blue. Mice homozygous for a hypomorphic allele exhibit male infertility associated with abnormal sperm morphology and reduced tubulin polyglutamylation in the spermatozoa.

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

### Important Information

- According to the existing MGI data,mice homozygous for a hypomorphic allele exhibit male infertility associated with abnormal sperm morphology and reduced tubulin polyglutamylation in the spermatozoa.
- Transcripts *Ttll5*-213、215、217、219 may not be affected.
- The *Ttll5* gene is located on the Chr12. 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.

