

Ttll5 Cas9-CKO Strategy

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Overview

Target Gene Name

• Ttll5

Project Type

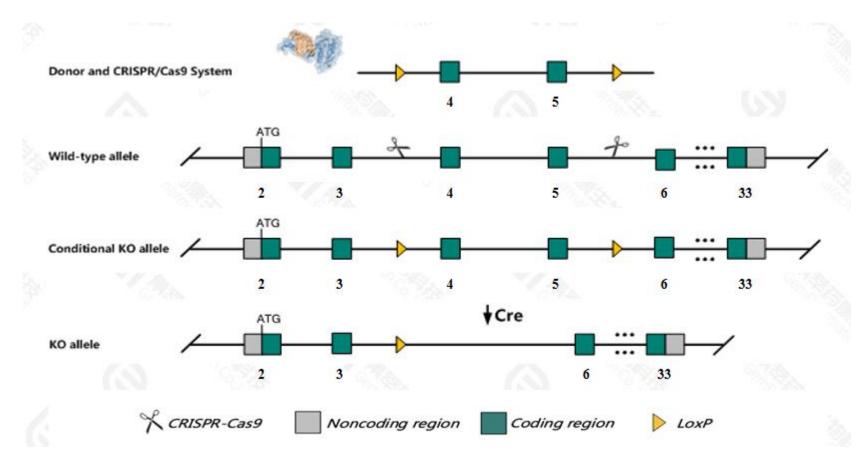
• Cas9-CKO

Genetic Background

• C57BL/6JGpt



Strain Strategy

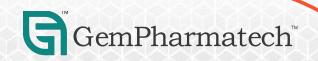


Schematic representation of CRISPR-Cas9 engineering used to edit the Ttll5 gene.



Technical Information

- The *Ttll5* gene has 20 transcripts. According to the structure of *Ttll5* gene, exon4-exon5 of *Ttll5*-201 (ENSMUST00000040179.14) 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: 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.

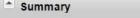


Gene Information

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

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



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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
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 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 <u>human</u> all

NEW Try the new Gene table

Try the new Transcript table

Genomic context

Location: 12; 12 D2

See Ttll5 in Genome Data Viewer

Exon count: 39

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

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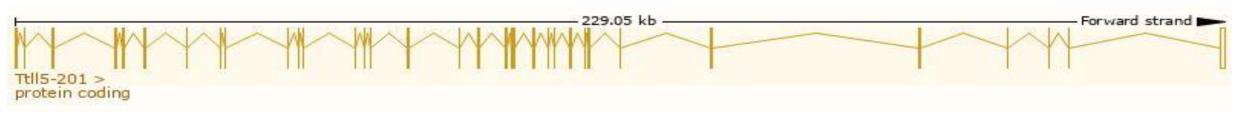


Transcript Information

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

Transcript ID	Name 🍦	bp 🍦	Protein	Biotype	CCDS 🍦	UniProt Match	Flags
ENSMUST00000040179.14	Ttll5-201	5081	1328aa	Protein coding	CCDS36499 ₽	Q8CHB8-1 ₽	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1
ENSMUST00000110224.8	Ttll5-205	4647	<u>1281aa</u>	Protein coding		D3YVZ5 €	GENCODE basic TSL:5
ENSMUST00000095536.10	Ttll5-203	4150	<u>438aa</u>	Protein coding		Q8C125₽	GENCODE basic TSL:2
ENSMUST00000040273.14	Ttll5-202	4147	1295aa	Protein coding	CCDS83991 ₽	E9Q425₽	GENCODE basic TSL:5
ENSMUST00000177168.8	Ttll5-217	3005	795aa	Protein coding		H3BJ73₽	TSL:1 CDS 5' incomplete
ENSMUST00000110220.9	Ttll5-204	2655	348aa	Protein coding		Q8BUF0函	GENCODE basic TSL:1
ENSMUST00000177525.8	Ttll5-219	1788	395aa	Protein coding		H3BLA3 ₽	TSL:1 CDS 5' incomplete
ENSMUST00000176937.8	Ttll5-215	1223	408aa	Protein coding		H3BLC0 €	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	176aa	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	563aa	Nonsense mediated decay		Q8CHB8-2₽	TSL:2
ENSMUST00000176695.8	Ttll5-214	4168	432aa	Nonsense mediated decay		H3BLN3 ₢₣	TSL:5
ENSMUST00000177114.2	Ttll5-216	3999	432aa	Nonsense mediated decay		H3BLN3 €	TSL:5
ENSMUST00000175844.2	Ttll5-210	1297	297aa	Nonsense mediated decay		H3BJC7₽	TSL:1 CDS 5' incomplete
ENSMUST00000153570.9	Ttll5-208	655	91aa	Nonsense mediated decay		H3BJV4₽	TSL:3
ENSMUST00000176008.8	Ttll5-212	5482	No protein	Protein coding CDS not defined		2	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		2	TSL:2
ENSMUST00000175899.2	Ttll5-211	636	No protein	Retained intron		2	TSL:3

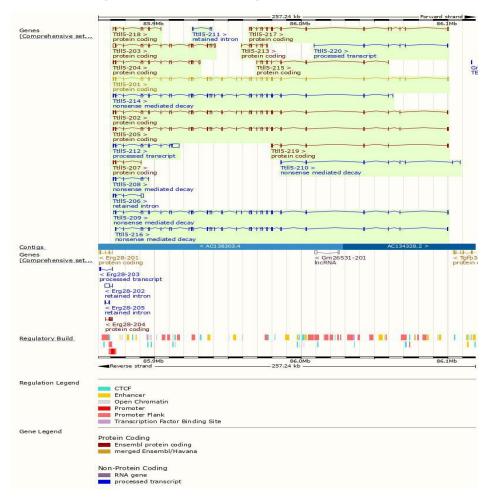
The strategy is based on the design of *Ttll5*-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org



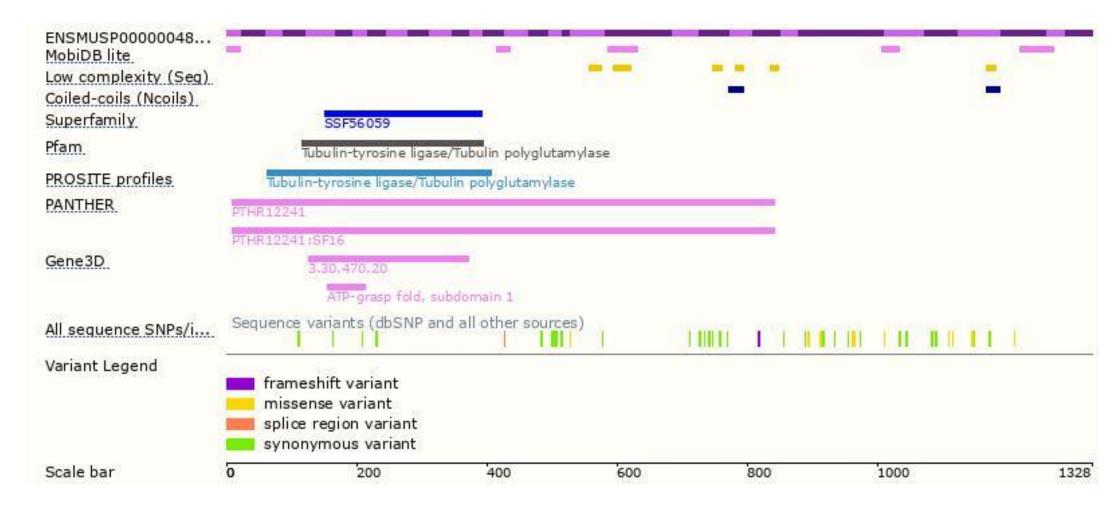
Genomic Information





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

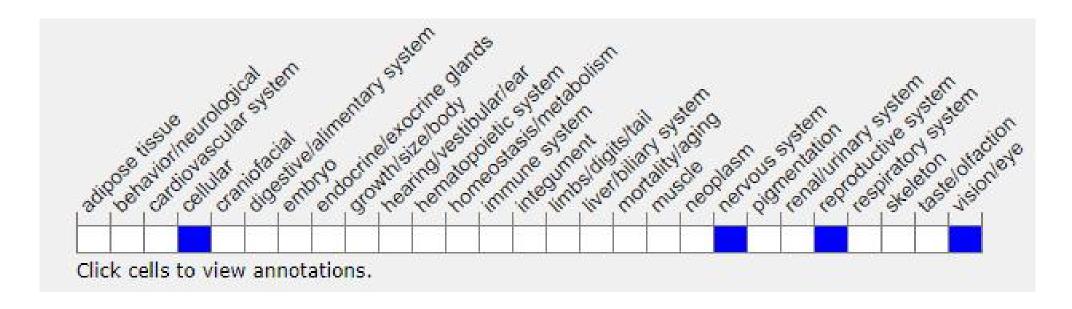
Protein Information





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

Mouse Phenotype Information (MGI)



• Phenotypes affected by the mutations of *Ttll5* gene are marked in blue. 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.



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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

