

# *Ttll9* Cas9-KO Strategy

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**Reviewer: Daohua Xu**

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# Project Overview

**Project Name**

***Ttll9***

**Project type**

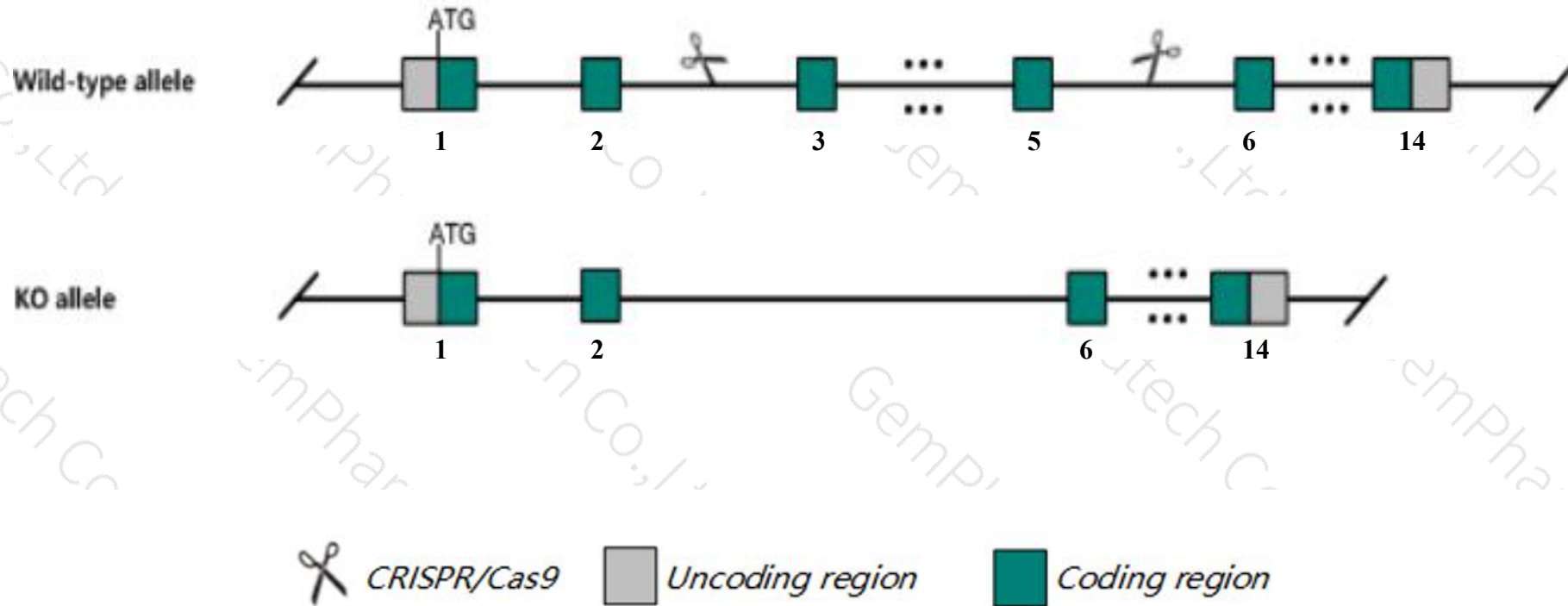
**Cas9-KO**

**Strain background**

**C57BL/6JGpt**

# Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ttll9* gene. The schematic diagram is as follows:



- The *Tll9* gene has 9 transcripts. According to the structure of *Tll9* gene, exon3-exon5 of *Tll9*-202(ENSMUST00000103155.9) transcript is recommended as the knockout region. The region contains 391bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Tll9* gene. The brief process is as follows: CRISPR/Cas9 system 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.

- According to the existing MGI data, mice homozygous for a knock-out allele exhibit male infertility associated with oligozoospermia, detached sperm flagella, abnormal sperm axonemes with loss of doublet 7 and reduced polyglutamylation of doublet 5, and asthenozoospermia with frequent stalls after anti-hook bending.
- Transcript *Ttll9*-205&206 may not be affected.
- The *Ttll9* gene is located on the Chr2. 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.



# Gene information (NCBI)

## Ttll9 tubulin tyrosine ligase-like family, member 9 [Mus musculus (house mouse)]

Gene ID: 74711, updated on 13-Mar-2020

### Summary



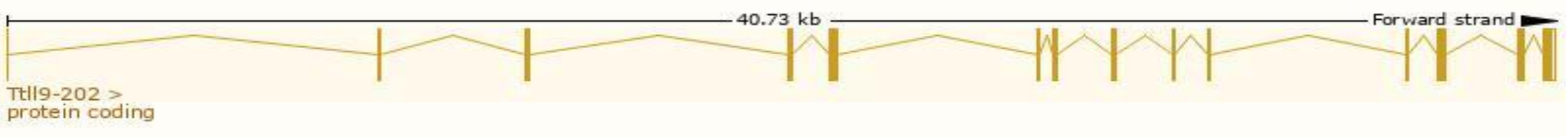
<b>Official Symbol</b>	Ttll9 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	tubulin tyrosine ligase-like family, member 9 provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:1913589</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000074673</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	VALIDATED
<b>Organism</b>	<a href="#">Mus musculus</a>
<b>Lineage</b>	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
<b>Also known as</b>	1700016F23Rik, 4930509O20Rik, AW046572
<b>Expression</b>	Biased expression in testis adult (RPKM 39.3) and genital fat pad adult (RPKM 1.2) <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

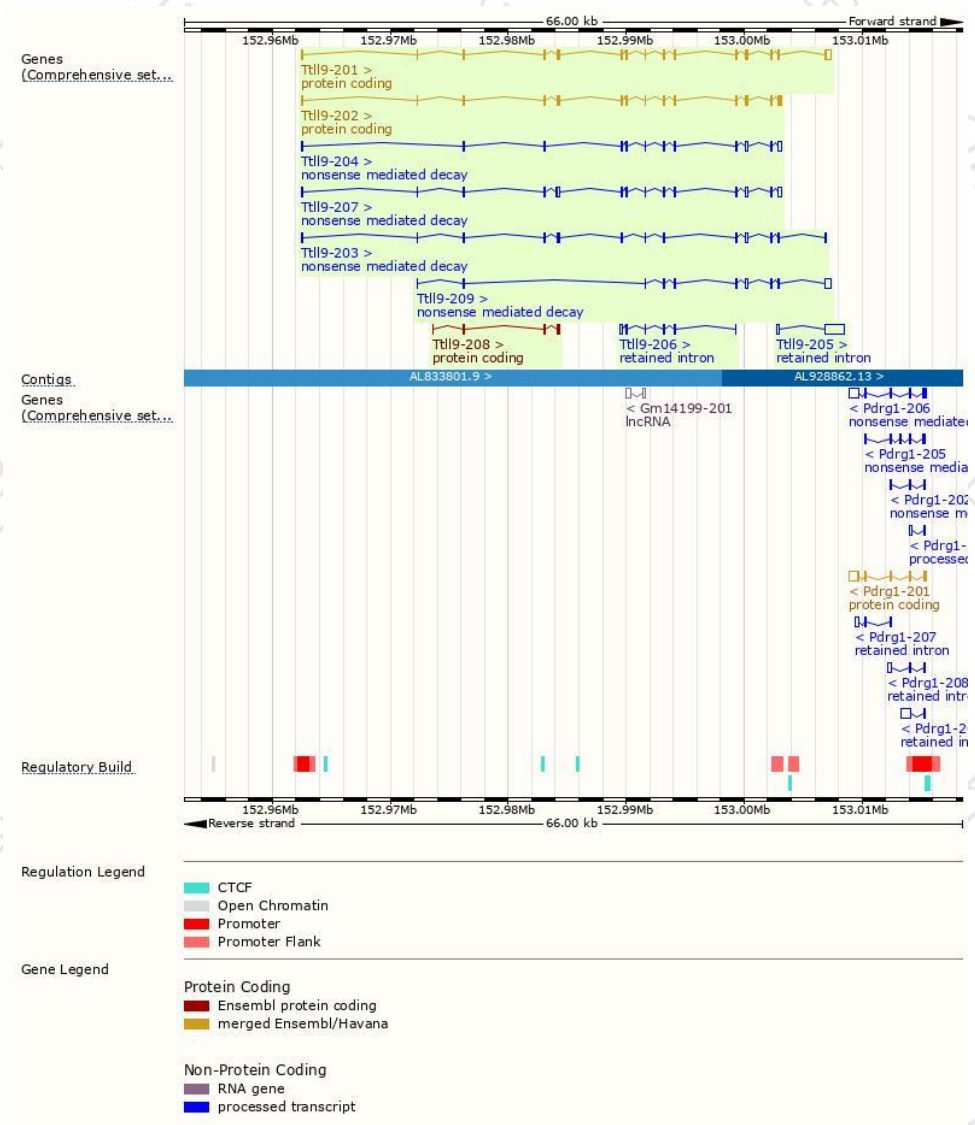
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ttll9-201	<a href="#">ENSMUST00000099197.8</a>	1797	<a href="#">461aa</a>	Protein coding	<a href="#">CCDS38283</a>	<a href="#">A2APC3</a>	TSL:1 GENCODE basic APPRIS P1
Ttll9-202	<a href="#">ENSMUST00000103155.9</a>	1552	<a href="#">464aa</a>	Protein coding	<a href="#">CCDS16903</a>	<a href="#">A2APC3</a>	TSL:1 GENCODE basic
Ttll9-208	<a href="#">ENSMUST00000155631.2</a>	428	<a href="#">139aa</a>	Protein coding	-	<a href="#">Z4YLM0</a>	CDS 3' incomplete TSL:3
Ttll9-207	<a href="#">ENSMUST00000152158.7</a>	1556	<a href="#">126aa</a>	Nonsense mediated decay	-	<a href="#">D6RG93</a>	TSL:1
Ttll9-209	<a href="#">ENSMUST00000165343.7</a>	1284	<a href="#">73aa</a>	Nonsense mediated decay	-	<a href="#">F6ZRM1</a>	CDS 5' incomplete TSL:5
Ttll9-204	<a href="#">ENSMUST00000146626.8</a>	1278	<a href="#">36aa</a>	Nonsense mediated decay	-	<a href="#">D6RCJ6</a>	TSL:1
Ttll9-203	<a href="#">ENSMUST00000109801.8</a>	1239	<a href="#">234aa</a>	Nonsense mediated decay	-	<a href="#">E9PUG4</a>	TSL:5
Ttll9-205	<a href="#">ENSMUST00000150218.1</a>	1816	No protein	Retained intron	-	-	TSL:1
Ttll9-206	<a href="#">ENSMUST00000151641.2</a>	526	No protein	Retained intron	-	-	TSL:5

The strategy is based on the design of *Ttll9-202* transcript,the transcription is shown below:

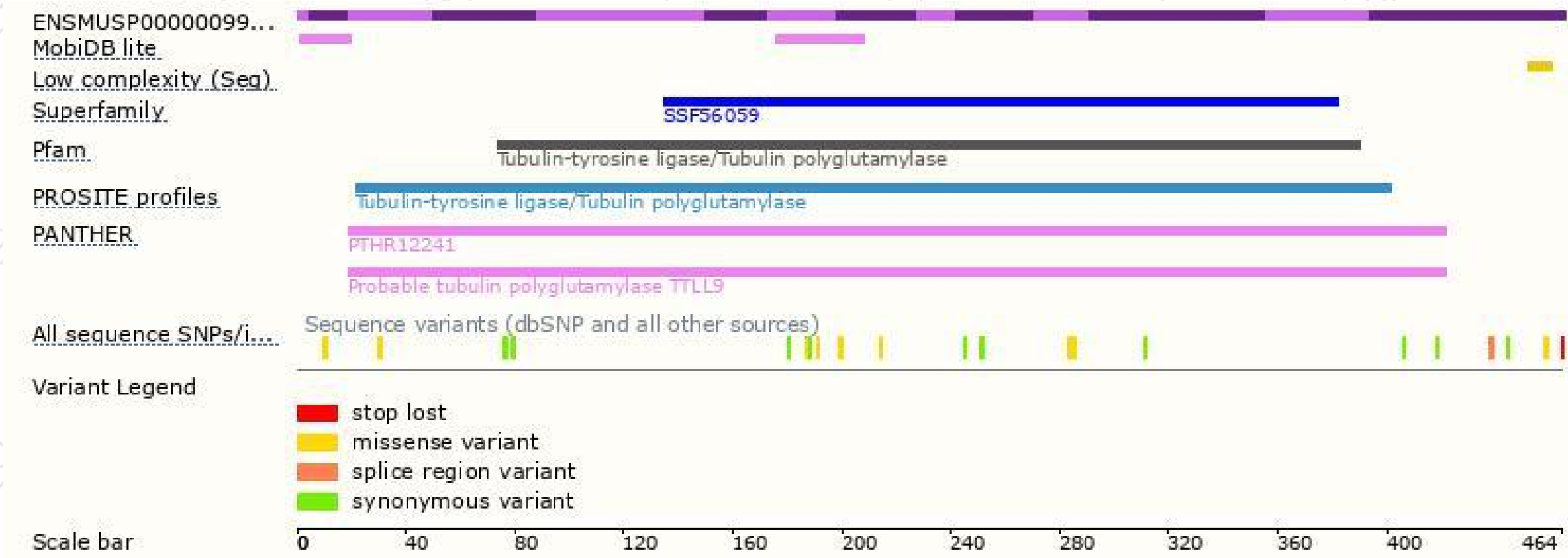


# Genomic location distribution

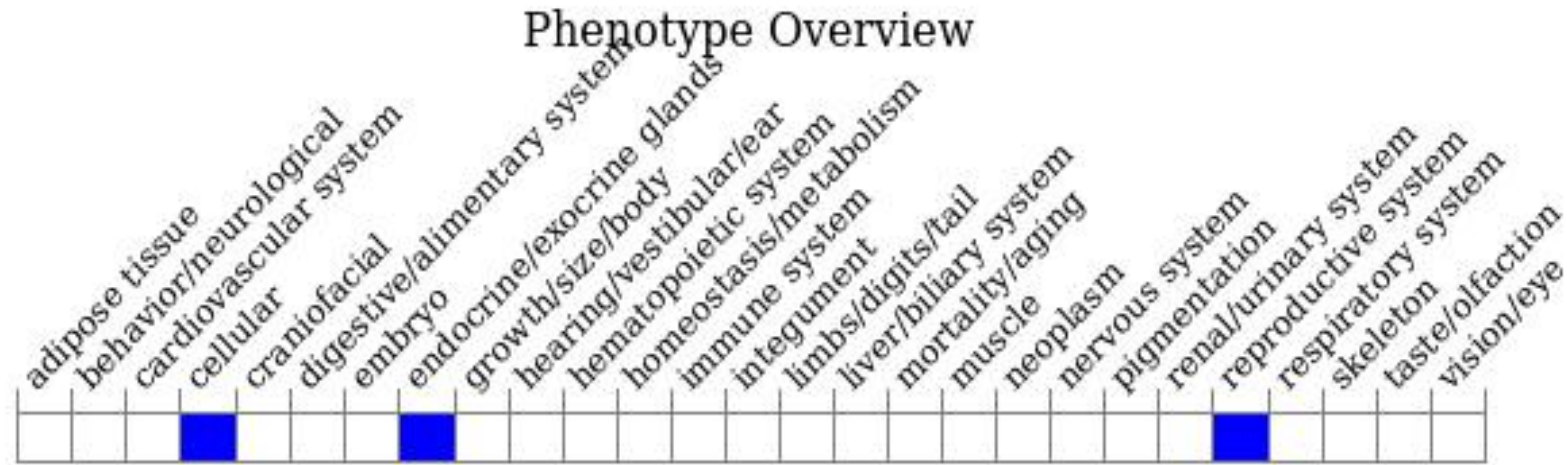




# Protein domain



# 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, mice homozygous for a knock-out allele exhibit male infertility associated with oligozoospermia, detached sperm flagella, abnormal sperm axonemes with loss of doublet 7 and reduced polyglutamylation of doublet 5, and asthenozoospermia with frequent stalls after anti-hook bending.

If you have any questions, you are welcome to inquire.

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