# Ttl Cas9-KO Strategy matech Co. Ltd Companyated Control

**Designer:** Enphamaten C. Lt

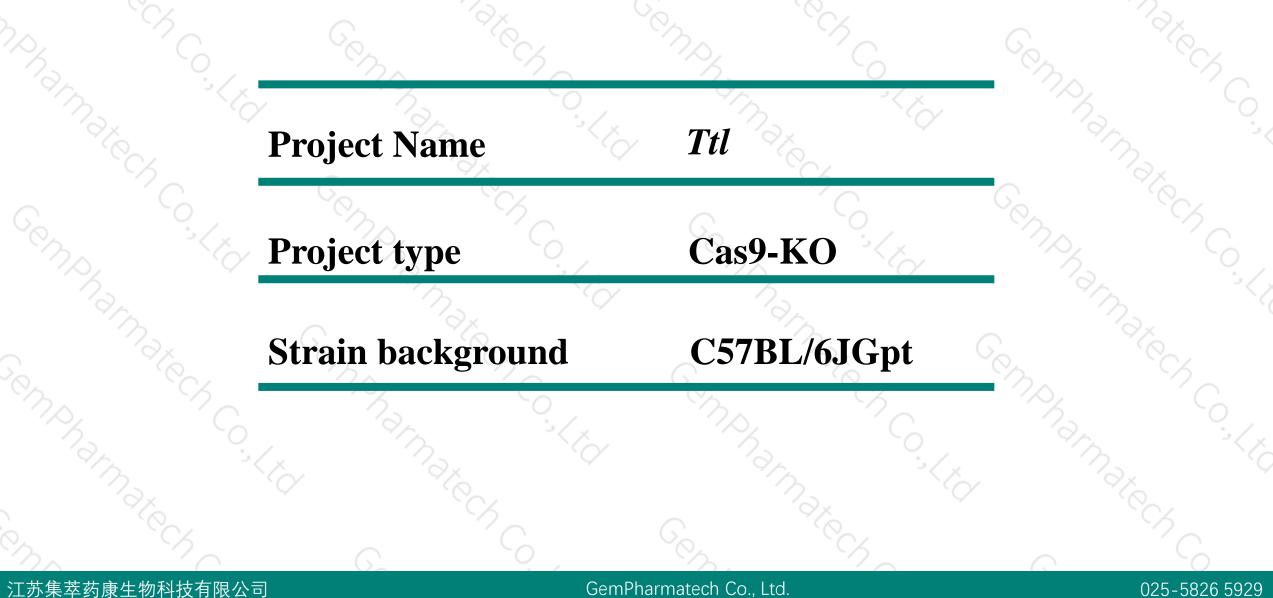
Daohua Xu AU ARA

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# **Project Overview**





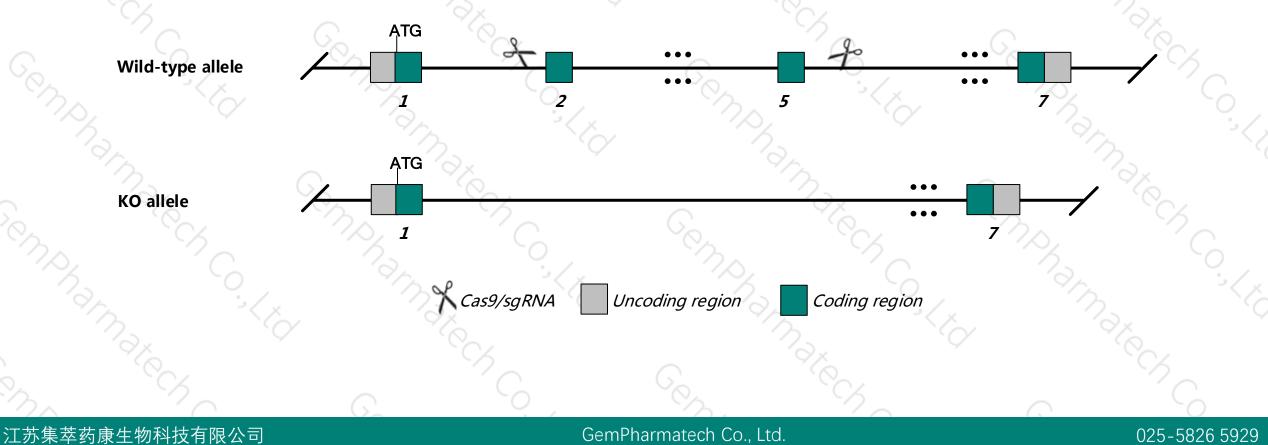
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# **Knockout strategy**



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



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- The Ttl gene has 3 transcripts. According to the structure of *Ttl* gene, exon2-exon5 of *Ttl*-201
  (ENSMUST00000035812.13) transcript is recommended as the knockout region. The region contains 718bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ttl* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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, Homozygous null mice display neonatal lethality, impaired breathing, and fail to form the internal capsule in the brain.
- The KO region contains the functional region of the *Gm14022* gene.Knockout the region may affect its function of *Gm14022* gene.
- The *Ttl* 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Notice

# Gene information (NCBI)



# Ttl tubulin tyrosine ligase [ Mus musculus (house mouse) ]

Gene ID: 69737	, updated on	28-Aug-2018
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## Summary

Ttl provided by MGI	
tubulin tyrosine ligase provided by MGI	
MGI:MGI:1916987	
Ensembl:ENSMUSG00000027394 Vega:OTTMUSG00000015410	
protein coding	
VALIDATED	
Mus musculus	
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;	
Muroidea; Muridae; Murinae; Mus; Mus	
Al848570; 2410003M22Rik; 2700049H19Rik	
Ubiquitous expression in CNS E18 (RPKM 12.5), cortex adult (RPKM 10.7) and 27 other tissues See more	
human all	
	tubulin tyrosine ligase provided by <u>MGI</u> <u>MGI:MGI:1916987</u> <u>Ensembl:ENSMUSG00000027394</u> <u>Vega:OTTMUSG00000015410</u> protein coding VALIDATED <u>Mus musculus</u> Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Murinae; Murinae; Mus; Mus Al848570; 2410003M22Rik; 2700049H19Rik Ubiquitous expression in CNS E18 (RPKM 12.5), cortex adult (RPKM 10.7) and 27 other tissues <u>See more</u>

# **Transcript information (Ensembl)**

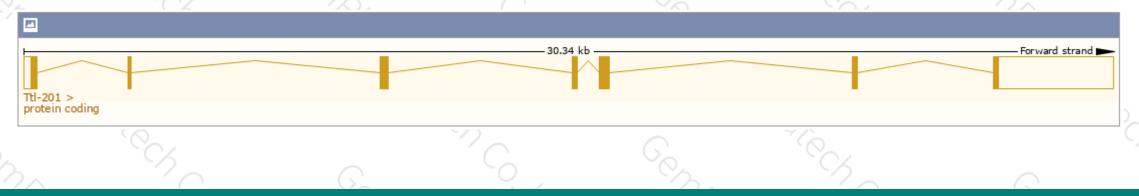


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The gene has 3 transcripts, and all transcripts are shown below :

Show/hide columns (1 hidden)									
Name 🍦	Transcript ID	bp 🍦	Protein 🖕	Biotype	CCDS 🖕	UniProt 🖕	RefSeq 🍦	Flags	
Ttl-201	ENSMUST0000035812.13	4567	<u>377aa</u>	Protein coding	<u>CCDS16719</u> ജ	<u>P38585</u> 🖗	<u>NM_027192</u> ଜ <u>NP_081468</u> ଜ	TSL:1 GENCODE basic	APPRIS P1
Ttl-202	ENSMUST00000144120.1	1238	<u>199aa</u>	Protein coding	-	<u>F6ZXS5</u> @	-	CDS 5' incomplete	TSL:1
Ttl-203	ENSMUST00000144730.1	1053	No protein	Processed transcript	-	-	-	TSL:1	

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



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# **Genomic location distribution**



#### < 🕀 🖬 🇞 🕏 Drag/Select: • Forward strand 50.34 kb 129.07Mb 129.06Mb 129.08Mb 129.09Mb 129.10Mb Π-+-Genes Ttl-201 > Polr1b-202 > (Comprehensive set... protein coding protein coding Ttl-202 > protein coding Polr1b-201 > Ttl-203 > processed transcript protein coding AL833780.6 > Contigs -0 Genes < Gm14022-201 processed transcript (Comprehensive set... Regulatory Build 129.07Mb 129.08Mb 129.09Mb 129.10Mb 129.06Mb 50.34 kb Reverse strand

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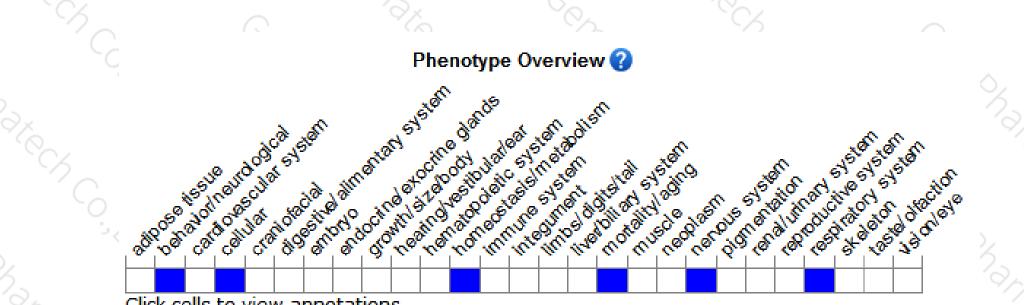
# **Protein domain**



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1	✿<≅%5										
	ENSMUSP00000046 Low complexity (Seg)										
	hmmpanther		PTHR12241:SF	6							
			PTHR12241							_	
	Superfamily domains Pfam domain			Tubulin-tyrosine ligase/Tubul	SSF56059 in polyglutamylase	_					
5,	PROSITE profiles	Tubulin-tyrosine liga	se/Tubulin polyglutamy								
$\sim$	Gene3D	3.40.50.11480									/
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# **Mouse phenotype description(MGI)**





Click cells to view annotations.

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 neonatal lethality, impaired breathing, and fail to form the internal capsule in the brain.

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If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



