

Ttl Cas9-CKO Strategy

Designer:

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Project Overview

Project Name

Ttl

Project type

Cas9-CKO

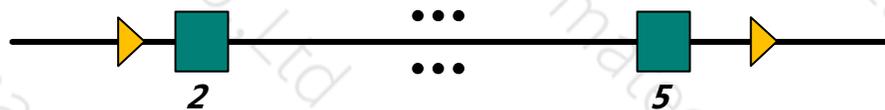
Strain background

C57BL/6JGpt

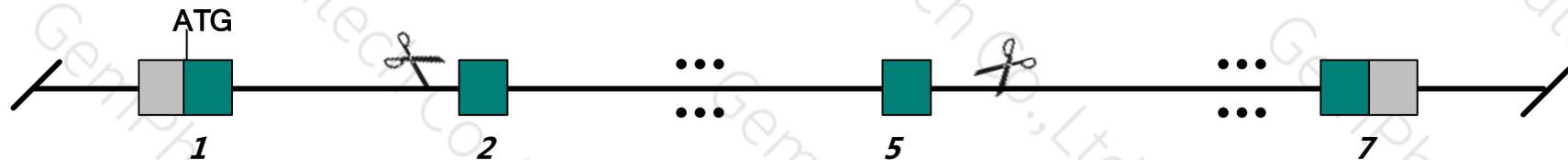
Conditional Knockout strategy

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

Donor and CRISPR/Cas9 System



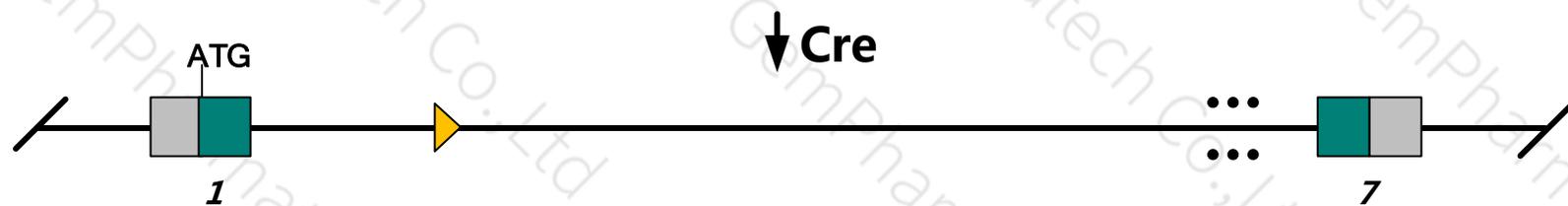
Wild-type allele



Conditional KO allele



KO allele



- 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, donor vector was constructed. Cas9, sgRNA 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.
- The flox mice was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues or cell types.

- 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.

Gene information (NCBI)



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

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

Summary

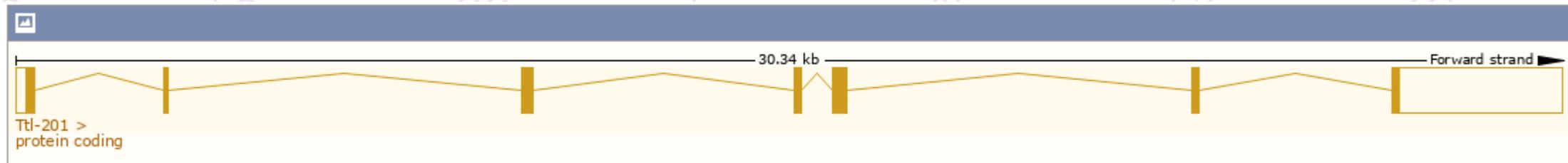
| | |
|---------------------------|---|
| Official Symbol | Ttl provided by MGI |
| Official Full Name | tubulin tyrosine ligase provided by MGI |
| Primary source | MGI:MGI:1916987 |
| See related | Ensembl:ENSMUSG00000027394 ; Vega:OTTMUSG00000015410 |
| Gene type | protein coding |
| RefSeq status | 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 | AI848570; 2410003M22Rik; 2700049H19Rik |
| Expression | Ubiquitous expression in CNS E18 (RPKM 12.5), cortex adult (RPKM 10.7) and 27 other tissues See more |
| Orthologs | human all |

Transcript information (Ensembl)

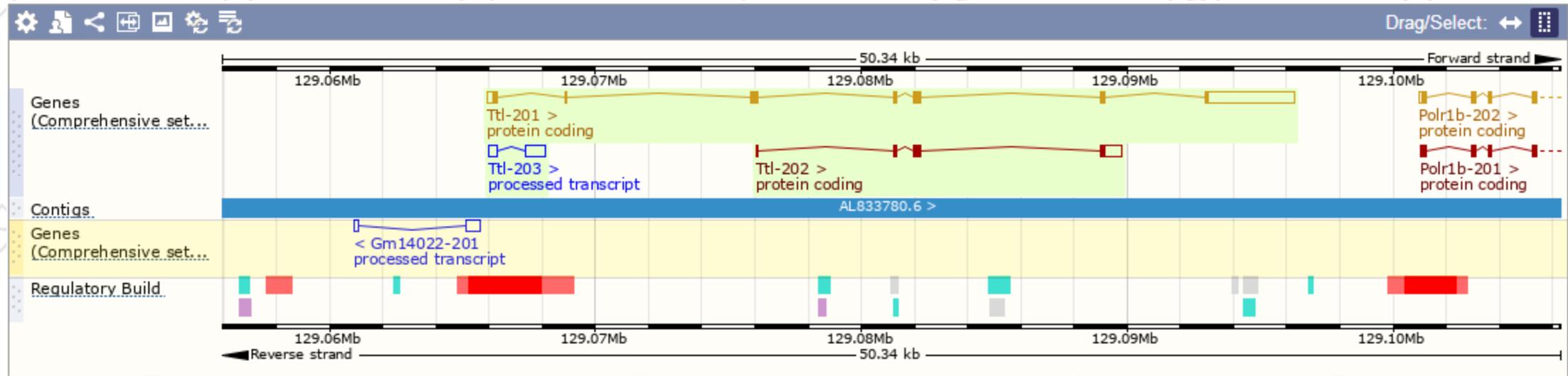
The gene has 3 transcripts, and all transcripts are shown below :

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | RefSeq | Flags |
|---------|---------------------------------------|------|-----------------------|----------------------|---------------------------|------------------------|--|-------------------------------|
| Ttl-201 | ENSMUST00000035812.13 | 4567 | 377aa | Protein coding | CCDS16719 | P38585 | NM_027192 NP_081468 | TSL:1 GENCODE basic APPRIS P1 |
| Ttl-202 | ENSMUST00000144120.1 | 1238 | 199aa | Protein coding | - | F6ZXS5 | - | CDS 5' incomplete TSL:1 |
| Ttl-203 | ENSMUST00000144730.1 | 1053 | No protein | Processed transcript | - | - | - | TSL:1 |

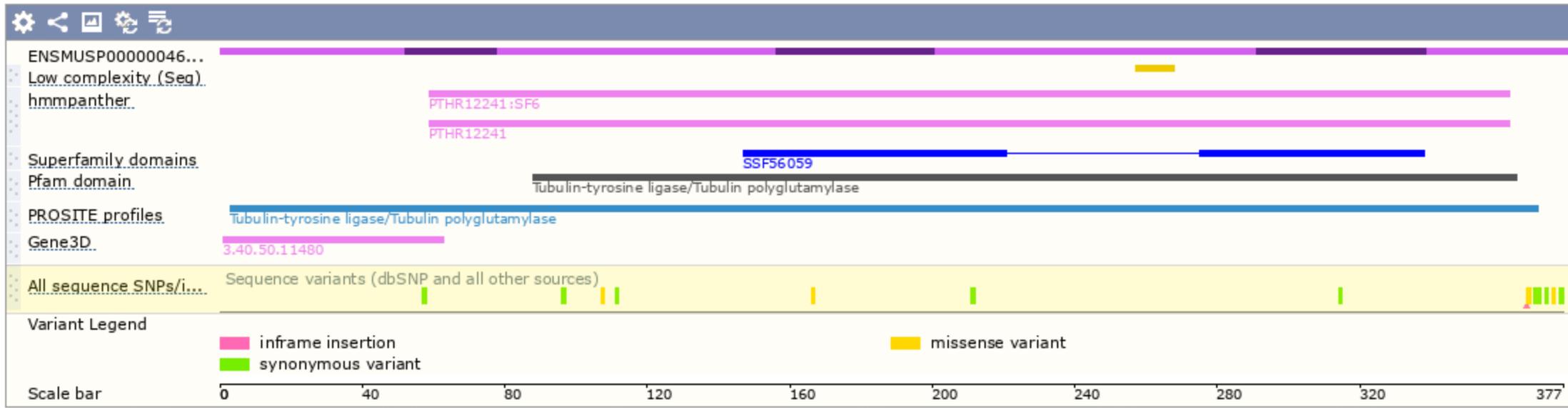
The strategy is based on the design of *Ttl-201* transcript, The transcription is shown below



Genomic location distribution

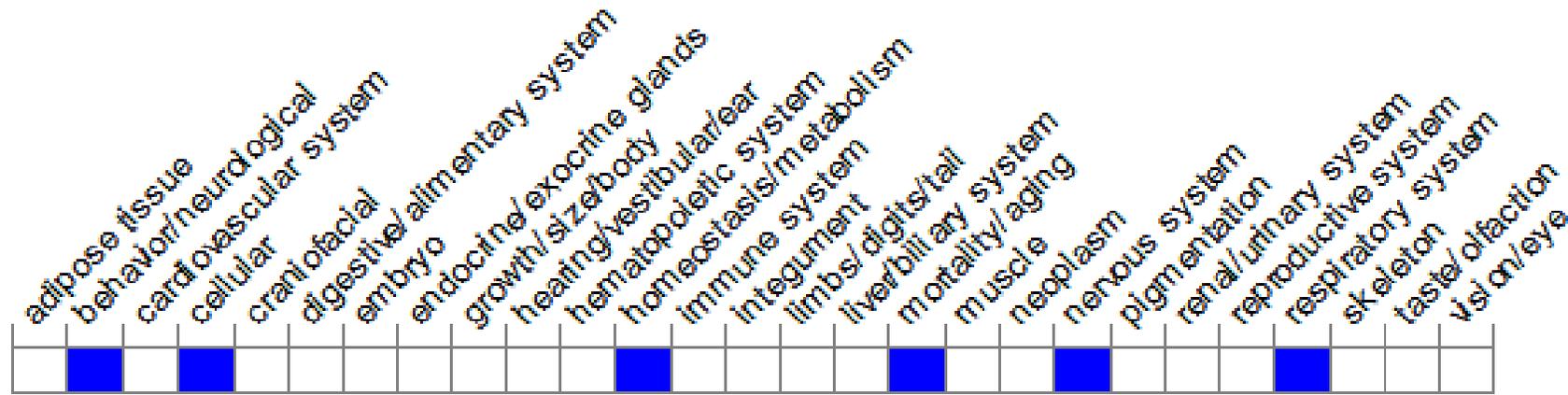


Protein domain



Mouse phenotype description(MGI)

Phenotype Overview ?



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.

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