Lta Cas9-KO *Strategy*

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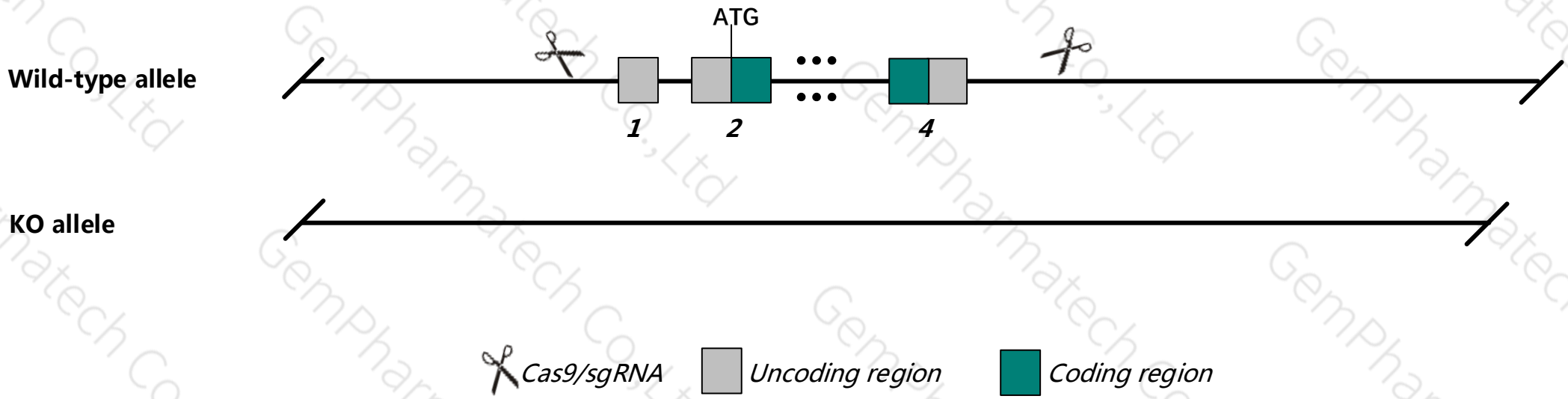
Project Overview

Project Name	***Lta***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

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



Technical routes

- The *Lta* gene has 1 transcript.According to the structure of *Lta* gene, exon1-exon4 of *Lta*-201 (ENSMUST00000025266.5) transcript is recommended as the knockout region.The region contains all coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Lta* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9, 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.

Notice

- According to the existing MGI data，Homozygotes for targeted null mutations exhibit absence of lymph nodes, Peyer's patches, and splenic germinal centers, impaired class switching and NK cell recruitment, and greater susceptibility to Mycobacterium bovis, influenza A, and Toxoplasma gondii.
- The KO region contains functional region of the *Mir6974* gene.Knockout the region may affect the function of *Mir6974* gene.
- This strategy may affect the 5-terminal regulation of the *Tnf* gene.
- The *Lta* gene is located on the Chr17. 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）

Lta lymphotoxin A [*Mus musculus* (house mouse)]

Gene ID: 16992, updated on 11-Sep-2019

Summary

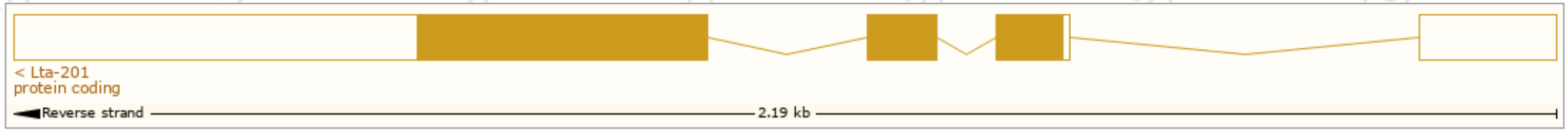
Official Symbol	Lta provided by MGI
Official Full Name	lymphotoxin A provided by MGI
Primary source	MGI:MGI:104797
See related	Ensembl:ENSMUSG00000024402
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	LT; Ltx; Tnfb; LT[a]; LT-[a]; TNFSF1; Tnlg1e; hlb382; LTalpha; Tnfsf1b; LT-alpha; TNF-beta
Expression	Biased expression in spleen adult (RPKM 15.0), mammary gland adult (RPKM 7.3) and 2 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

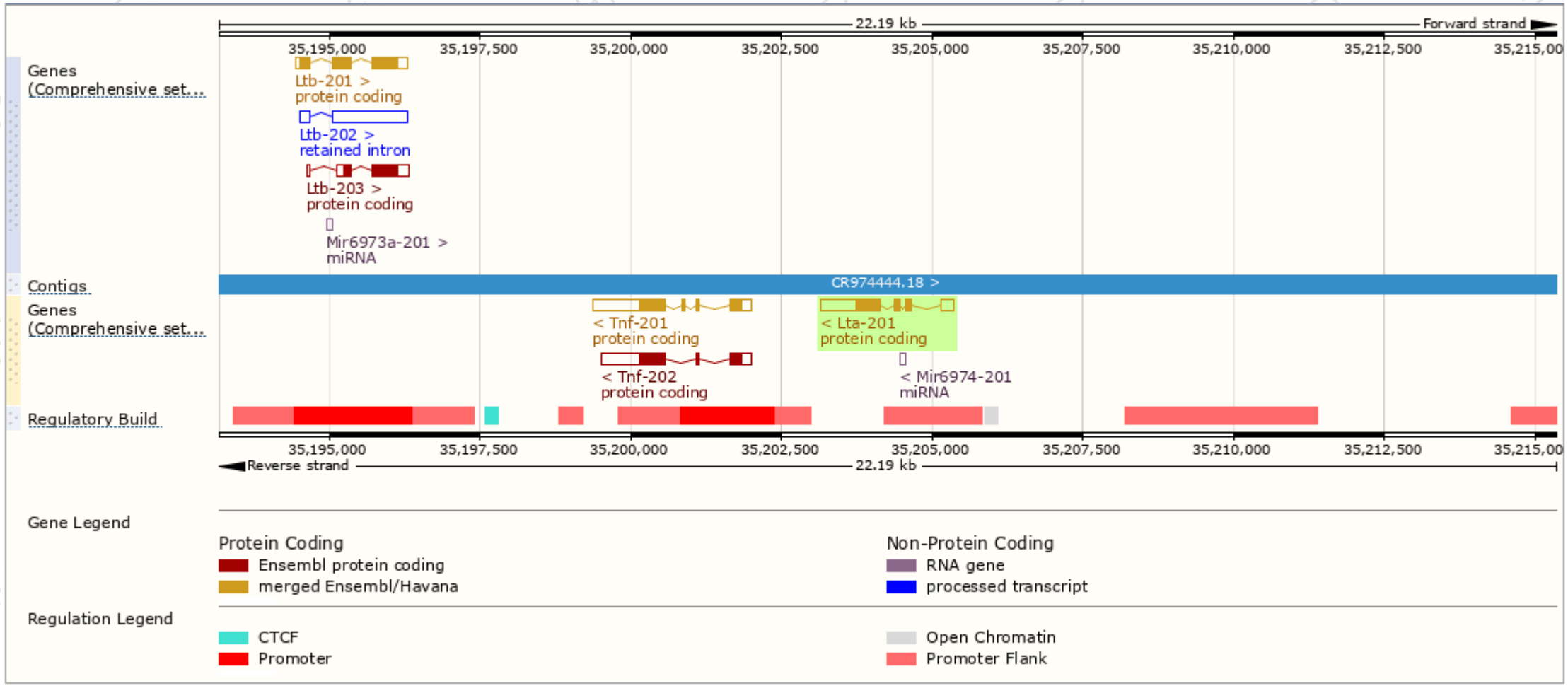
The gene has 1 transcript, and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lta-201	ENSMUST00000025266.5	1383	202aa	Protein coding	CCDS28692	P09225 Q542S2	TSL:1 GENCODE basic APPRIS P1

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

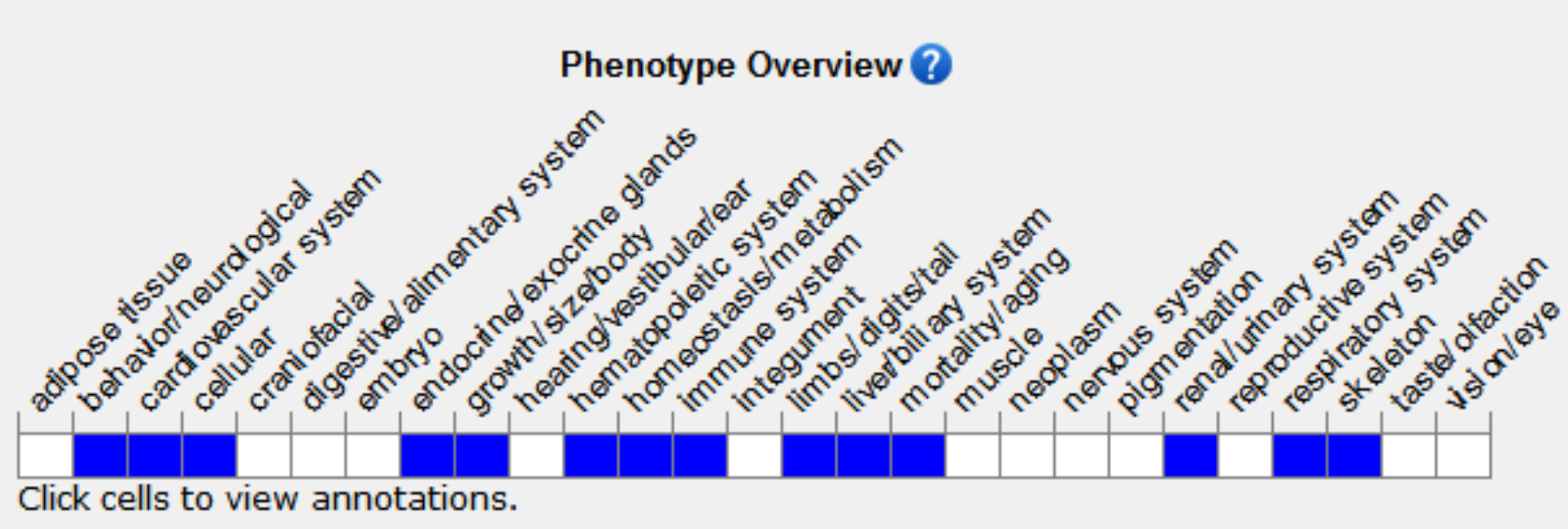


Genomic location distribution



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/) .

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

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