

Lck Cas9-CKO Strategy

Designer:

Daohua Xu

Reviewer:

Huimin Su

Design Date:

2019-12-18

Project Overview

Project Name

Lck

Project type

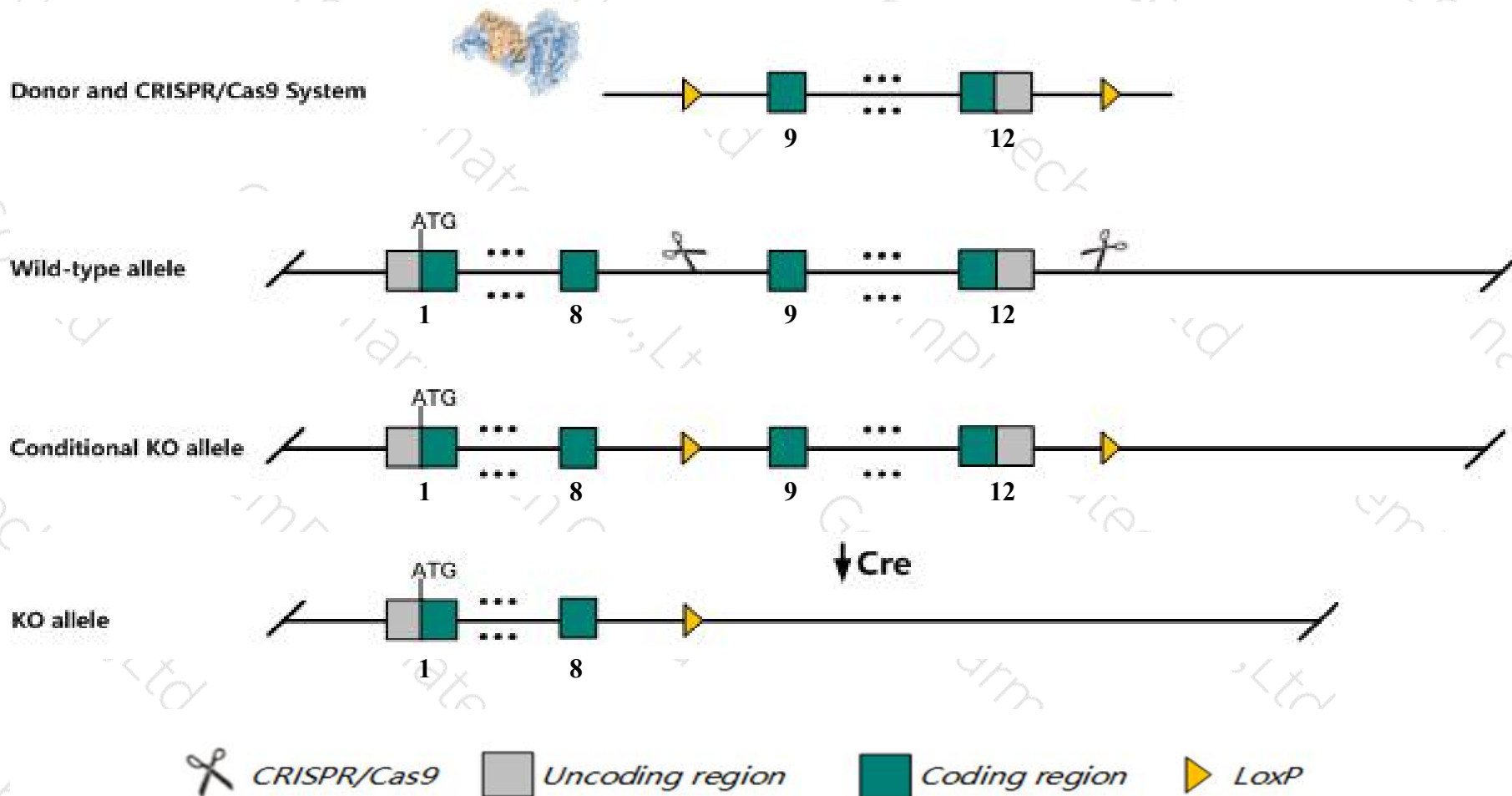
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



Technical routes

- The *Lck* gene has 8 transcripts. According to the structure of *Lck* gene, exon9-exon12 of *Lck-201* (ENSMUST00000067240.10) transcript is recommended as the knockout region. The region contains 566bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Lck* 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.
- 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.

- According to the existing MGI data, Mice homozygous for mutations of this gene exhibit thymic atrophy with reduced numbers of peripheral T cells. Null mutants have few double positive and no mature single positive (SP) thymocytes. A hypomorph has decreased expression of CD3epsilon chain on SP thymocytes, whose numbers are reduced.
- The *Lck* gene is located on the Chr4. 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.

Gene information (NCBI)

Lck lymphocyte protein tyrosine kinase [Mus musculus (house mouse)]

Gene ID: 16818, updated on 12-Mar-2019

Summary



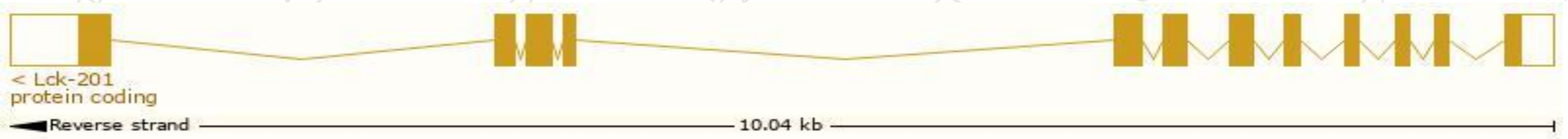
Official Symbol	Lck provided by MGI
Official Full Name	lymphocyte protein tyrosine kinase provided by MGI
Primary source	MGI:MGI:96756
See related	Ensembl:ENSMUSG00000000409
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Hck-3, Lsk, Lskt, p56, p56Lck
Expression	Biased expression in thymus adult (RPKM 430.2), spleen adult (RPKM 43.4) and 1 other tissue See more
Orthologs	human all

Transcript information (Ensembl)

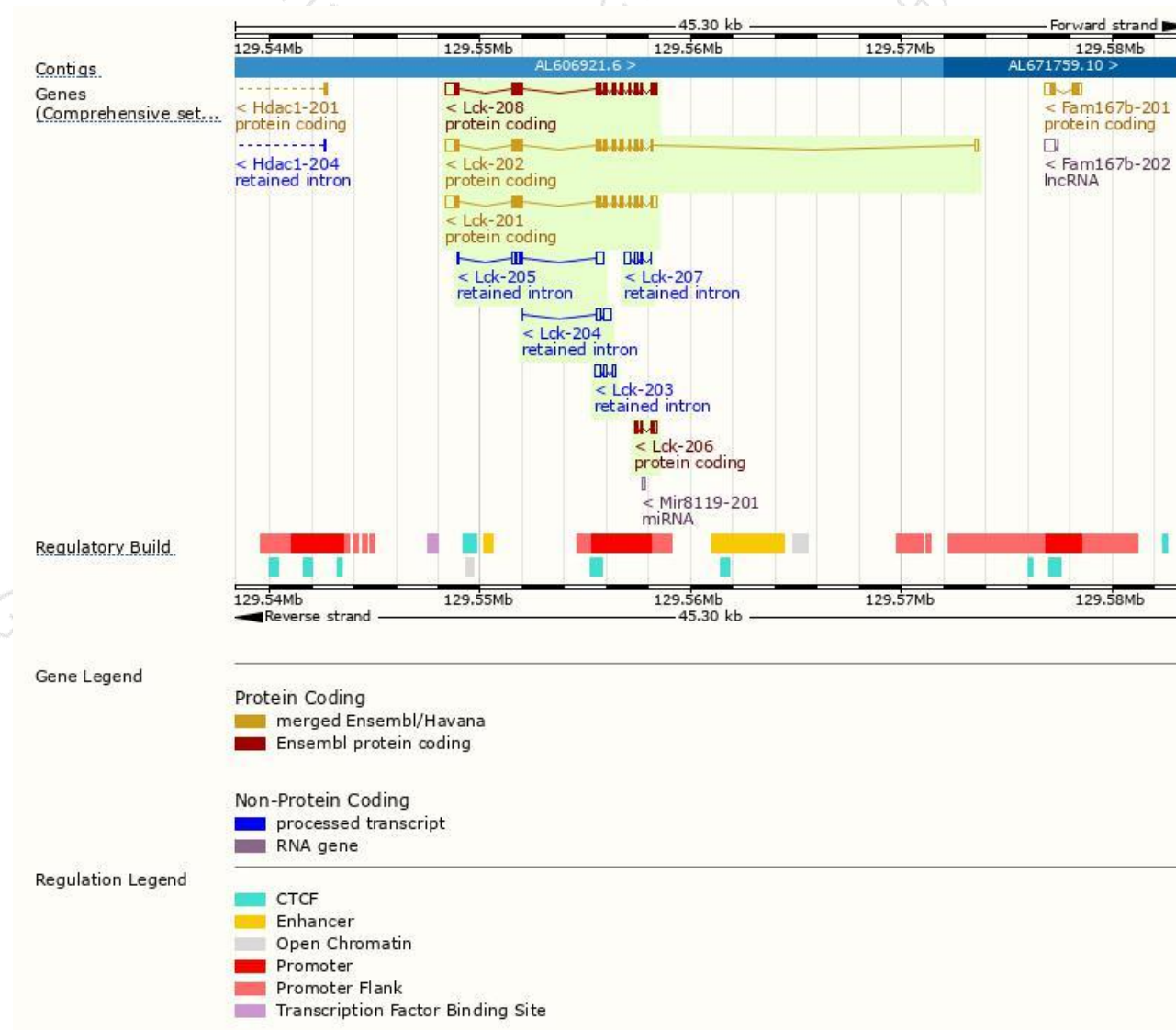
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lck-201	ENSMUST00000067240.10	2185	509aa	Protein coding	CCDS18697	P06240	TSL:1 GENCODE basic APPRIS P1
Lck-202	ENSMUST00000102596.7	2111	509aa	Protein coding	CCDS18697	P06240	TSL:1 GENCODE basic APPRIS P1
Lck-208	ENSMUST00000167288.7	2074	520aa	Protein coding	CCDS51306	E9Q696	TSL:5 GENCODE basic
Lck-206	ENSMUST00000134336.2	368	92aa	Protein coding	-	B2KG65	CDS 3' incomplete TSL:2
Lck-205	ENSMUST00000132030.1	779	No protein	Retained intron	-	-	TSL:2
Lck-204	ENSMUST00000127943.1	608	No protein	Retained intron	-	-	TSL:3
Lck-203	ENSMUST00000123640.1	568	No protein	Retained intron	-	-	TSL:2
Lck-207	ENSMUST00000139957.1	450	No protein	Retained intron	-	-	TSL:1

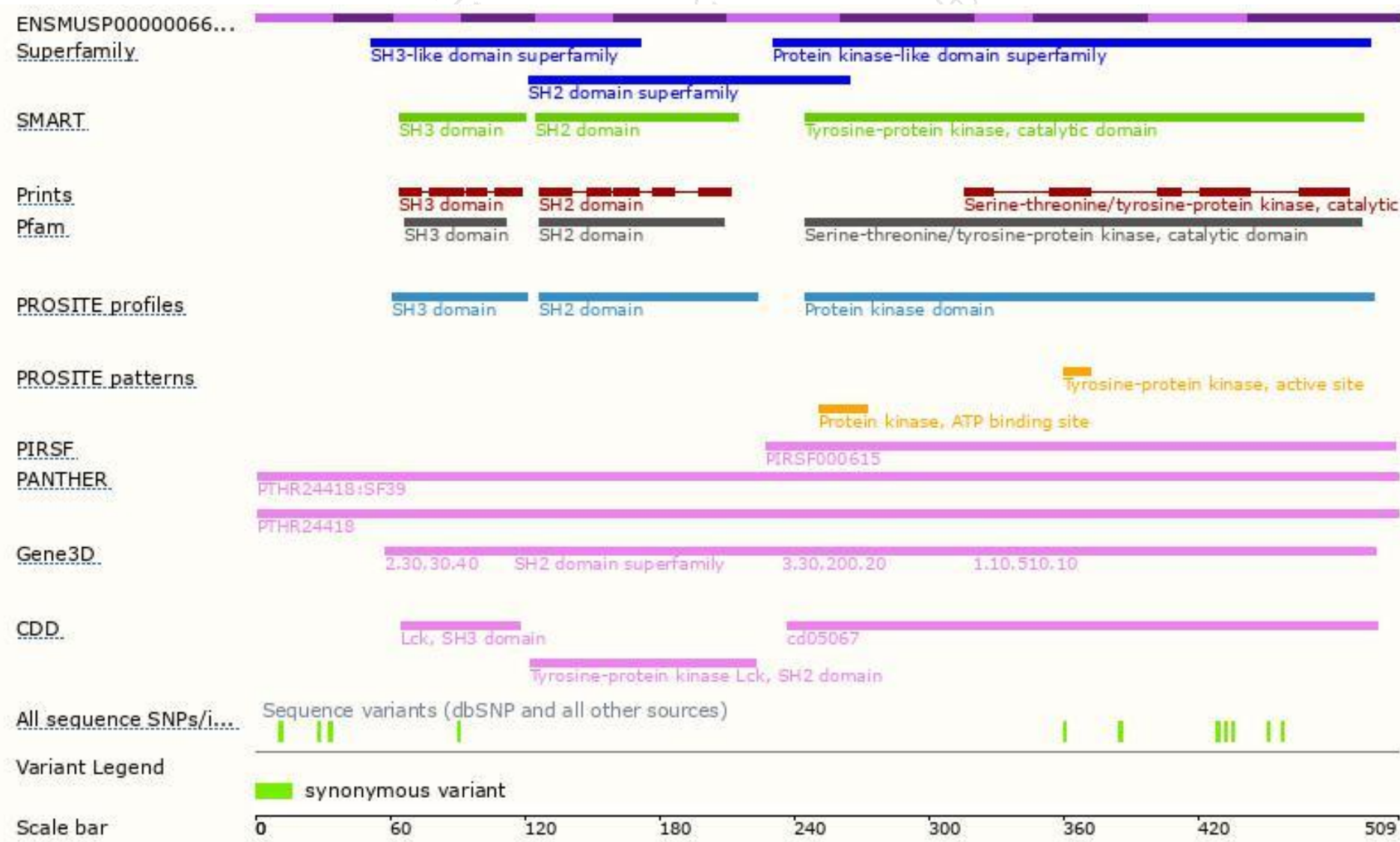
The strategy is based on the design of *Lck-201* transcript,The transcription is shown below



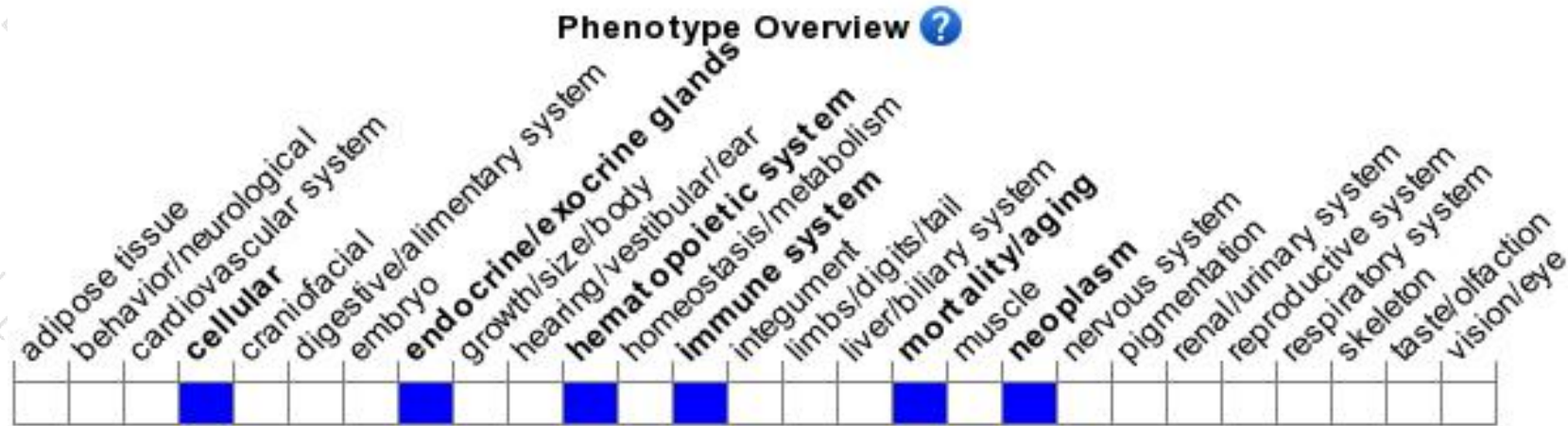
Genomic location distribution



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/>).

According to the existing MGI data, Mice homozygous for mutations of this gene exhibit thymic atrophy with reduced numbers of peripheral T cells. Null mutants have few double positive and no mature single positive (SP) thymocytes. A hypomorph has decreased expression of CD3epsilon chain on SP thymocytes, whose numbers are reduced.

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

Tel: 400-9660890

