

Cbl Cas9-CKO Strategy

Designer

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Reviewer

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Design Date

2018-6-8

Project Overview

Project Name

Cbl

Project type

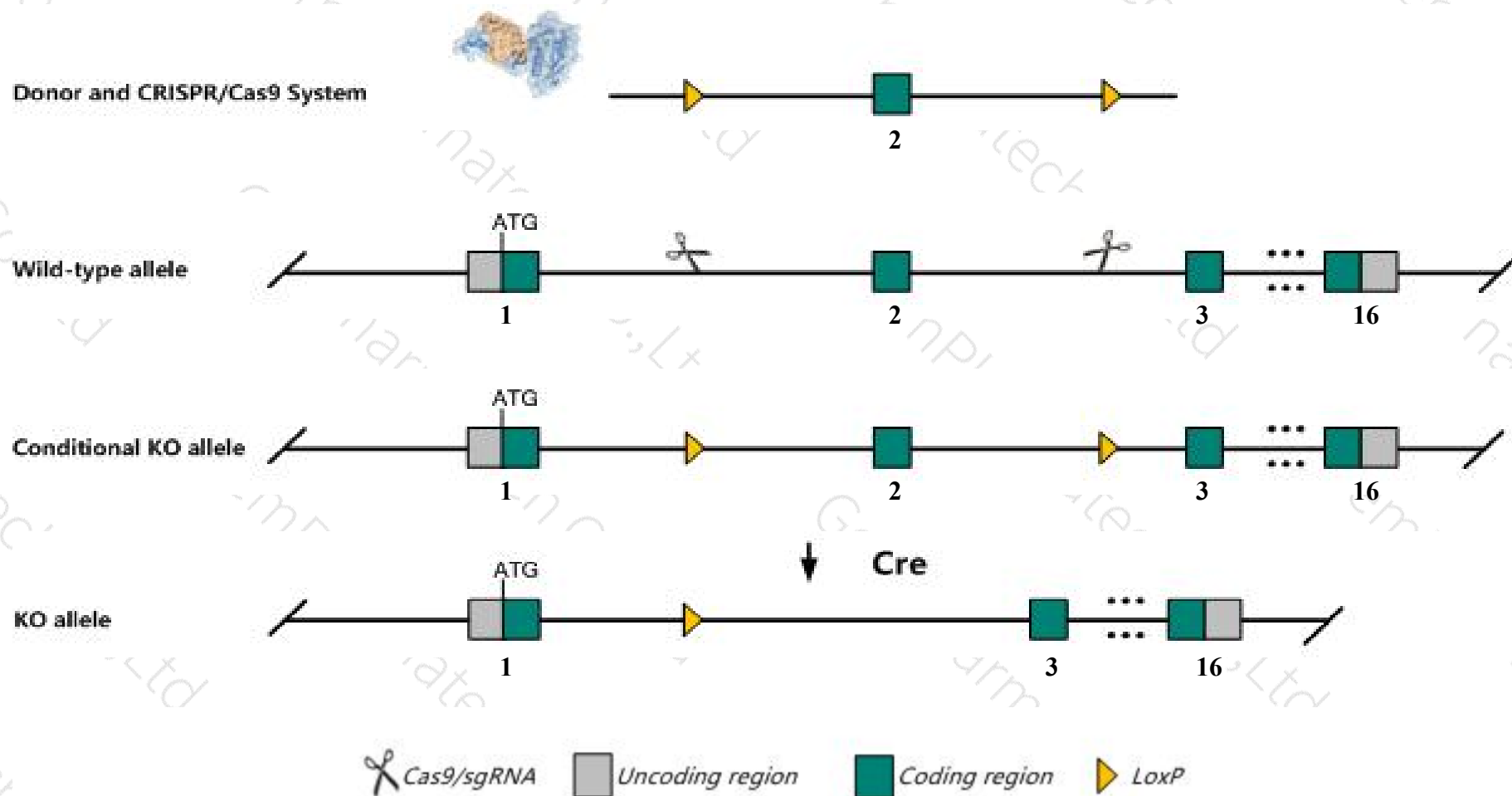
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Cbl* gene has 11 transcripts. According to the structure of *Cbl* gene, exon2 of *Cbl-211* (ENSMUST00000206720.1) transcript is recommended as the knockout region. The region contains 248bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cbl* 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 and cell types.

- According to the existing MGI data, Homozygotes for targeted null mutations exhibit increased thymic CD3 and CD4 expression and tyrosine-phosphorylation, lymphoid hyperplasia, and altered splenic hemopoiesis. Females show increased ductal density and branching in mammary fat pads.
- Transcript *Cbl-202/205/207/208/209/210* may not be affected.
- The *Cbl* gene is located on the Chr9. 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)

Cbl Casitas B-lineage lymphoma [*Mus musculus* (house mouse)]

Gene ID: 12402, updated on 19-Feb-2019

Summary



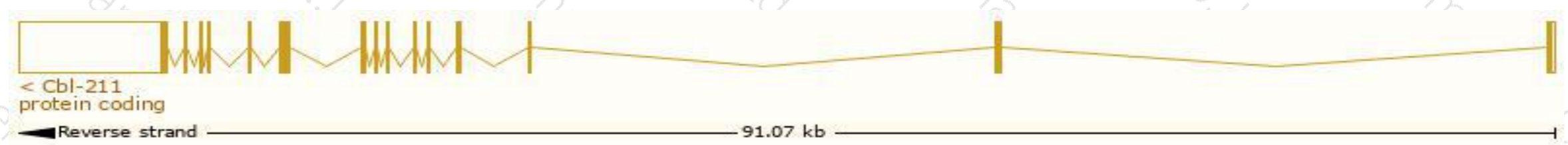
Official Symbol	Cbl provided by MGI
Official Full Name	Casitas B-lineage lymphoma provided by MGI
Primary source	MGI:MGI:88279
See related	Ensembl:ENSMUSG00000034342
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	cbl; Cbl-2; c-Cbl; 4732447J05Rik
Expression	Broad expression in thymus adult (RPKM 39.9), testis adult (RPKM 11.0) and 23 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

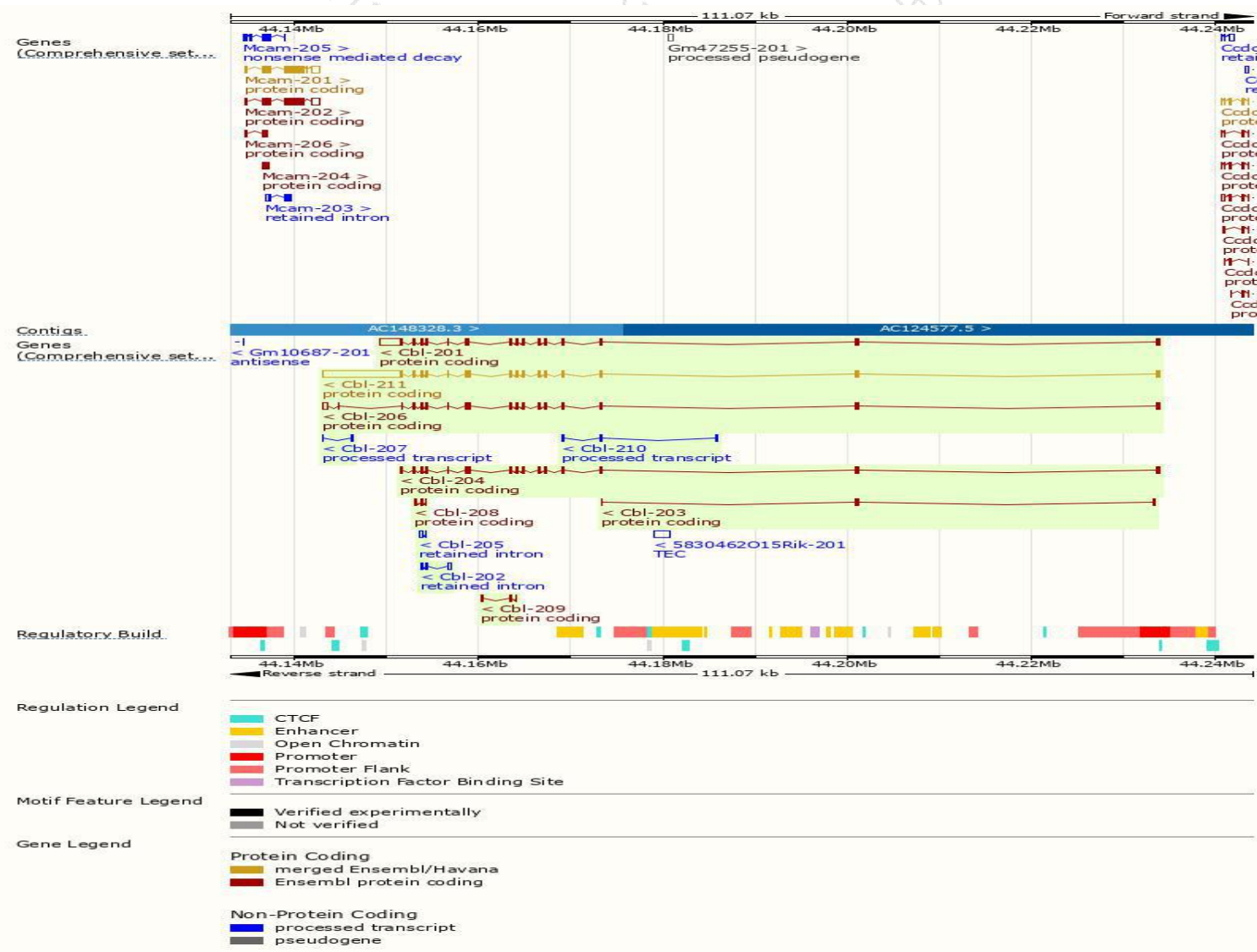
The gene has 11 transcripts,all transcripts are shown below:

Show/hide columns									Filter		
Name ▲	Transcript ID ▲	bp ▲	Protein ▲	Translation ID ▲	Biotype ▲	CCDS ▲	UniProt ▲	Flags ▲			
Cbl-201	ENSMUST00000037644.7	4901	869aa	ENSMUSP00000041902.7	Protein coding	-	A0A0X1KG61	TSL:1 GENCODE basic APPRIS ALT2			
Cbl-202	ENSMUST00000205313.1	664	No protein	-	Retained intron	-	-	TSL:2			
Cbl-203	ENSMUST00000205755.1	457	51aa	ENSMUSP00000145791.1	Protein coding	-	A0A0U1RP17	CDS 3' incomplete TSL:5			
Cbl-204	ENSMUST00000205968.1	2809	896aa	ENSMUSP00000146287.1	Protein coding	-	A0A0U1RQ85	TSL:1 GENCODE basic			
Cbl-205	ENSMUST00000206125.1	283	No protein	-	Retained intron	-	-	TSL:3			
Cbl-206	ENSMUST00000206147.1	3452	895aa	ENSMUSP00000145827.1	Protein coding	-	A0A0U1RP47	TSL:5 GENCODE basic APPRIS ALT2			
Cbl-207	ENSMUST00000206163.1	238	No protein	-	lncRNA	-	-	TSL:3			
Cbl-208	ENSMUST00000206258.1	237	69aa	ENSMUSP00000145538.1	Protein coding	-	A0A0U1RNF1	CDS 5' incomplete TSL:3			
Cbl-209	ENSMUST00000206540.1	298	79aa	ENSMUSP00000145889.1	Protein coding	-	A0A0U1RP95	CDS 5' incomplete TSL:5			
Cbl-210	ENSMUST00000206629.1	443	No protein	-	lncRNA	-	-	TSL:3			
Cbl-211	ENSMUST00000206720.1	11372	913aa	ENSMUSP00000146244.1	Protein coding	CCDS40598	P22682	TSL:1 GENCODE basic APPRIS P2			

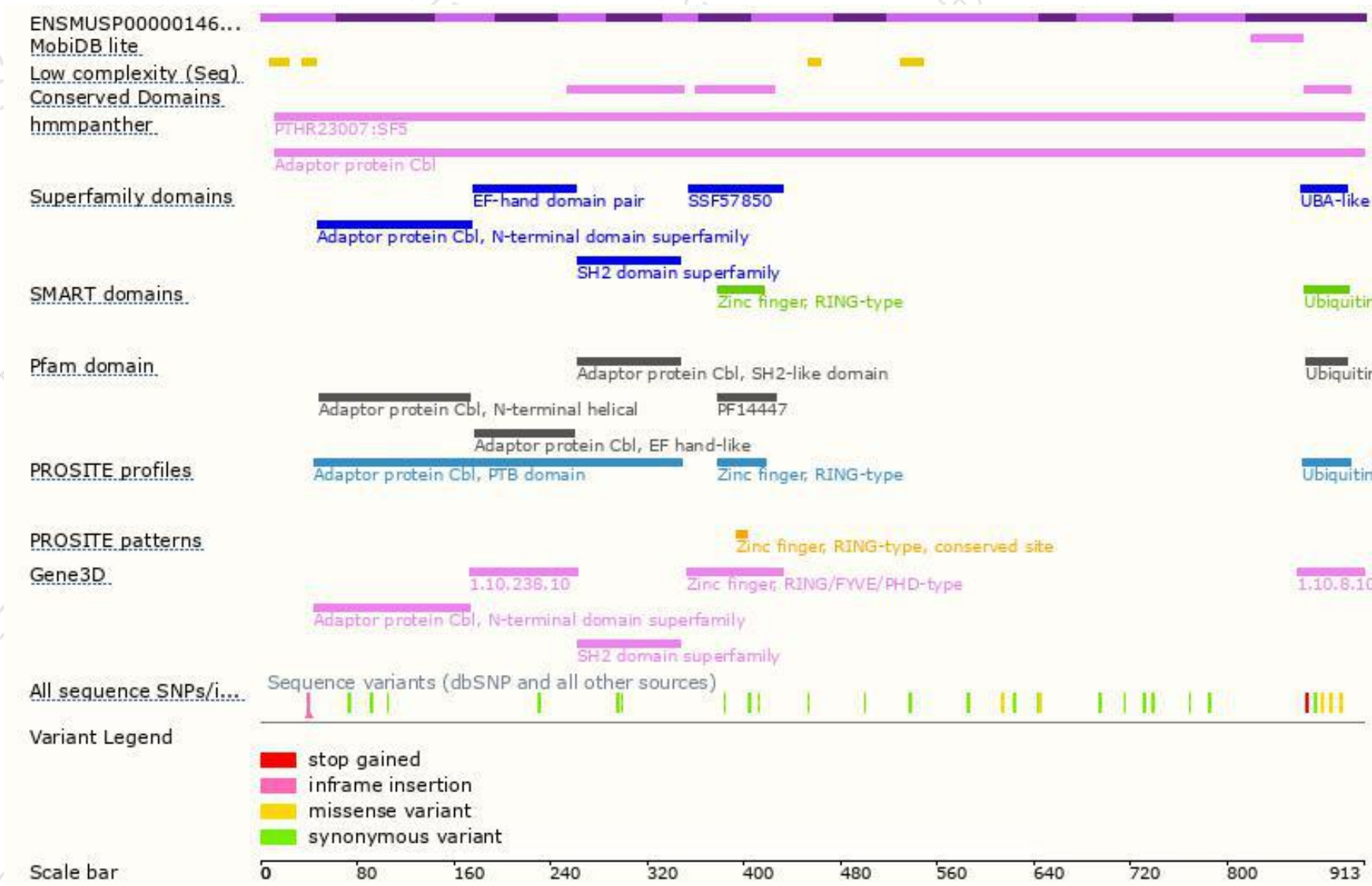
The strategy is based on the design of *Cbl-211* transcript,The transcription is shown below



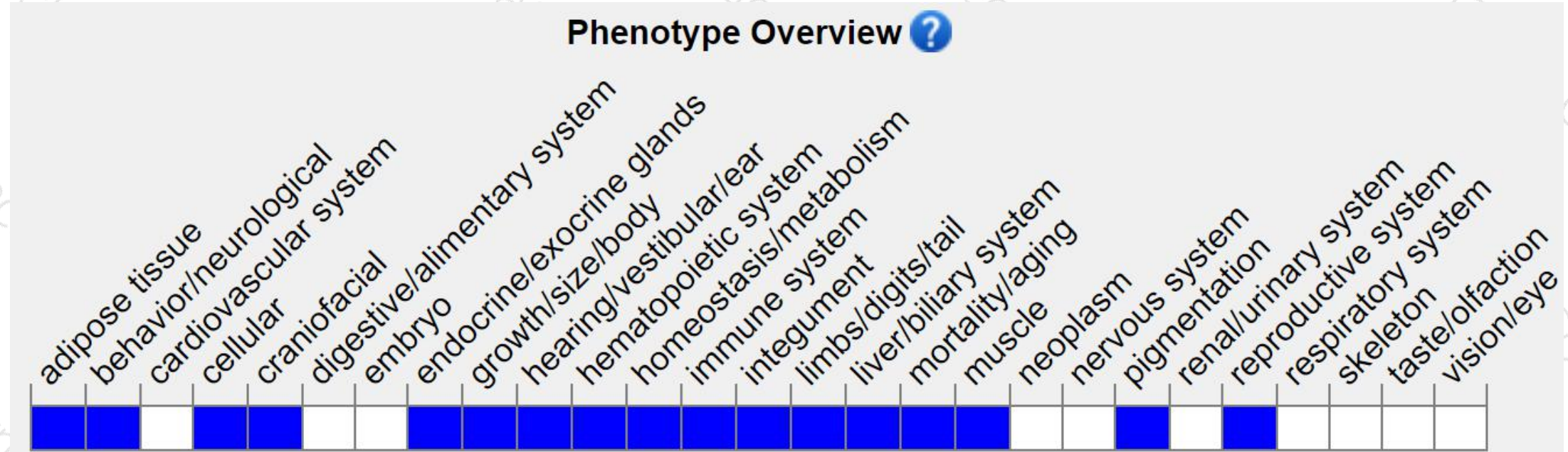
Genomic location distribution



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, Homozygotes for targeted null mutations exhibit increased thymic CD3 and CD4 expression and tyrosine-phosphorylation, lymphoid hyperplasia, and altered splenic hemopoiesis. Females show increased ductal density and branching in mammary fat pads.

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

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