

***Cblb* Cas9-CKO Strategy**

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Project Overview

Project Name

Cblb

Project type

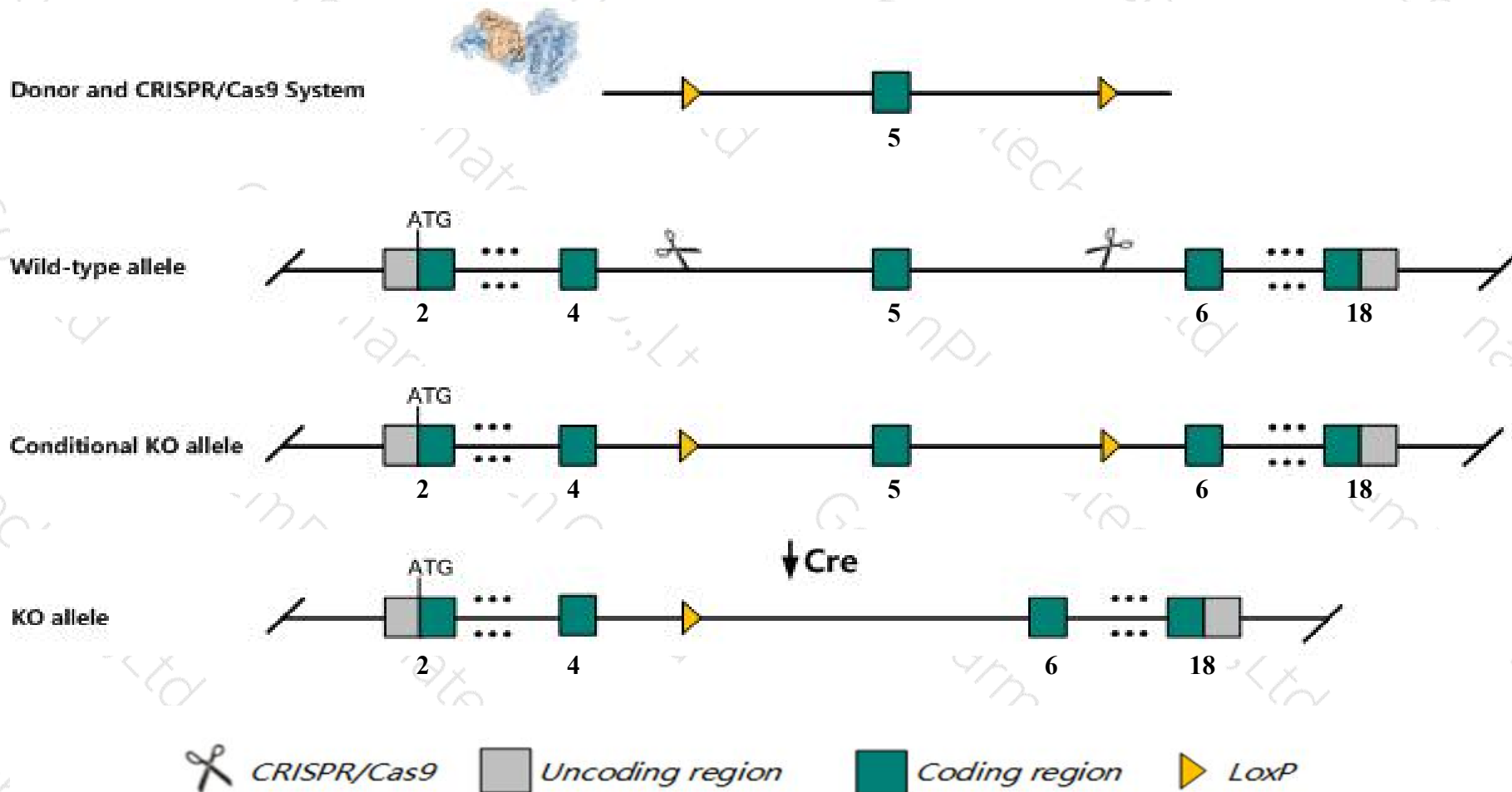
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Cblb* gene has 7 transcripts. According to the structure of *Cblb* gene, exon5 of *Cblb-201* (ENSMUST00000114471.2) transcript is recommended as the knockout region. The region contains 157bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cblb* 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, Homozygotes for targeted null mutations exhibit elevated IL2 production by T cells, develop spontaneous autoimmunity, and are highly susceptible to experimental autoimmune encephalomyelitis.
- The *Cblb* gene is located on the Chr16. 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)

Cblb Casitas B-lineage lymphoma b [Mus musculus (house mouse)]

Gene ID: 208650, updated on 31-Jan-2019

Summary



Official Symbol	Cblb provided by MGI
Official Full Name	Casitas B-lineage lymphoma b provided by MGI
Primary source	MGI:MGI:2146430
See related	Ensembl:ENSMUSG00000022637
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	AI429560, AI851073, Cbl-b
Expression	Ubiquitous expression in CNS E11.5 (RPKM 7.7), CNS E14 (RPKM 6.1) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

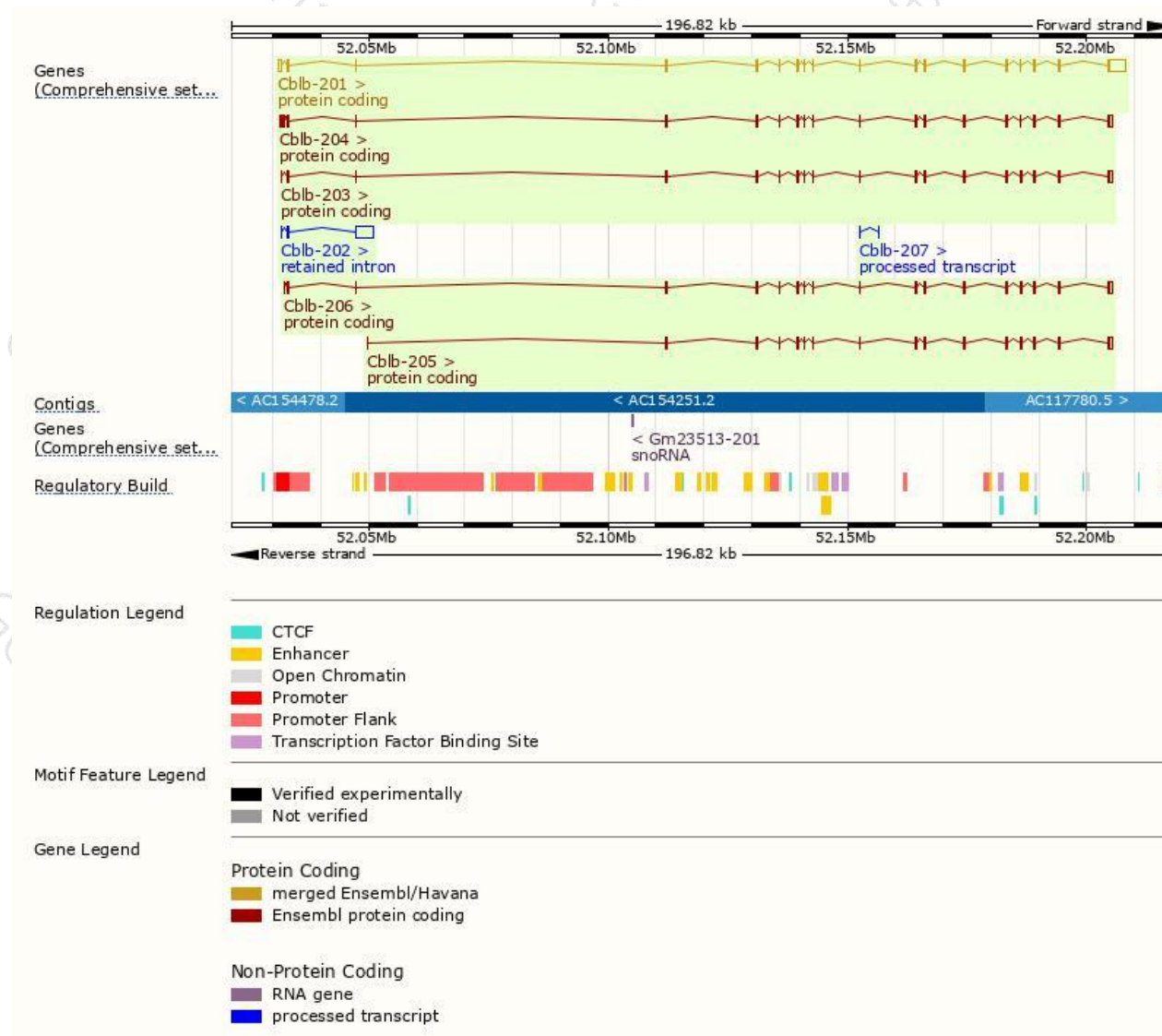
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cblb-201	ENSMUST00000114471.2	6648	938aa	Protein coding	CCDS37355	B9EKI5	TSL:1 GENCODE basic APPRIS P2
Cblb-204	ENSMUST00000227062.1	3728	938aa	Protein coding	CCDS37355	B9EKI5	GENCODE basic APPRIS P2
Cblb-206	ENSMUST00000227879.1	3866	982aa	Protein coding	-	Q3TTA7	GENCODE basic APPRIS ALT2
Cblb-203	ENSMUST00000226593.1	3864	982aa	Protein coding	-	Q3TTA7	GENCODE basic APPRIS ALT2
Cblb-205	ENSMUST00000227756.1	3378	830aa	Protein coding	-	Q3TTA7	GENCODE basic
Cblb-207	ENSMUST00000228247.1	307	No protein	Processed transcript	-	-	
Cblb-202	ENSMUST00000226253.1	3930	No protein	Retained intron	-	-	

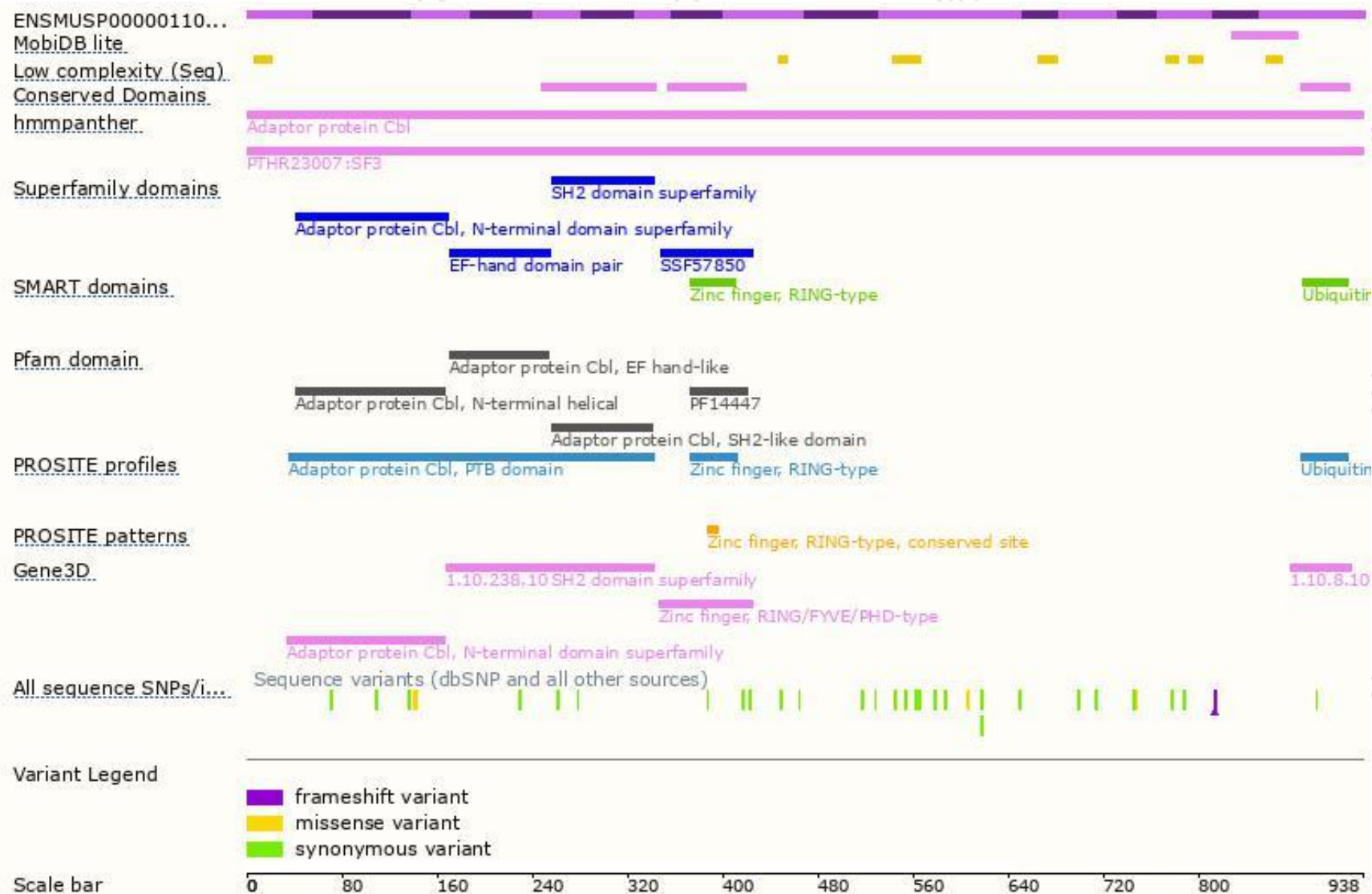
The strategy is based on the design of *Cblb-201* transcript,The transcription is shown below



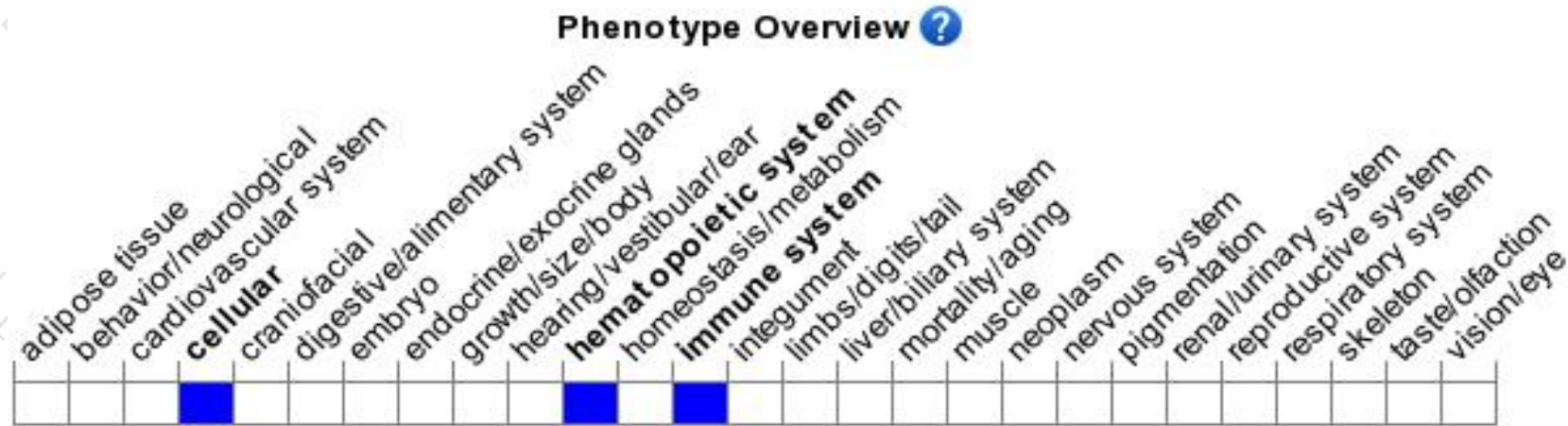
Genomic location distribution



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, Homozygotes for targeted null mutations exhibit elevated IL2 production by T cells, develop spontaneous autoimmunity, and are highly susceptible to experimental autoimmune encephalomyelitis.

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

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