Ctla4 Cas9-CKO Strategy

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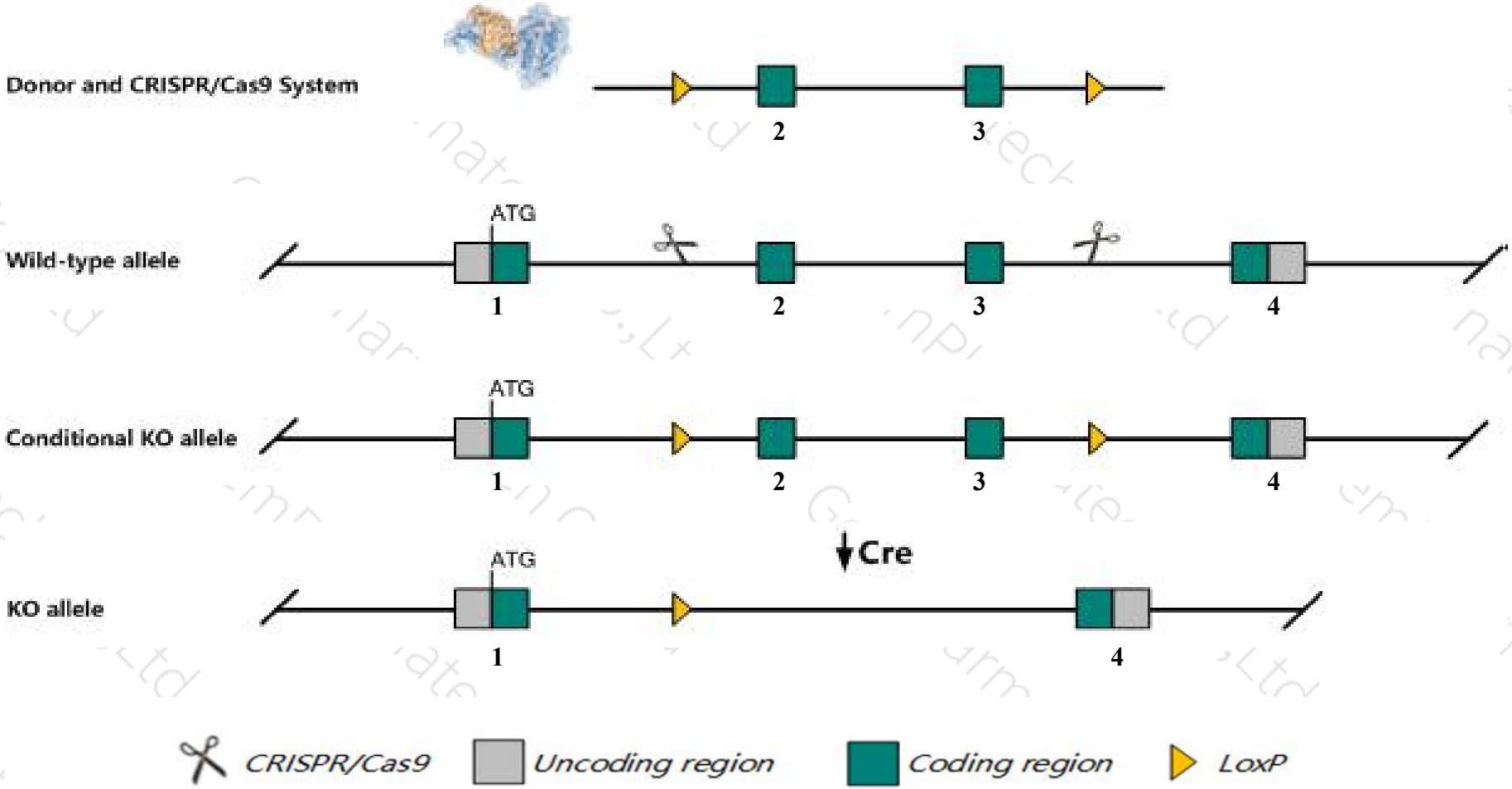
Project Overview

Project Name	***Ctla4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ctla4* gene. The schematic diagram is as follows:



Technical routes

- The *Ctla4* gene has 3 transcripts. According to the structure of *Ctla4* gene, exon2-exon3 of *Ctla4-201* (ENSMUST00000027164.8) transcript is recommended as the knockout region.The region contains 458bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Ctla4* 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.

Notice

➢ According to the existing MGI data,Mice homozygous for a knock-out allele exhibit lethality at 3 to 4 weeks of age, decreased T cell numbers, abnormal T cell physiology, inflammation in mutliple organs, abnormal thymus morphology, and lymph node hypoplasia.

➢ The *Ctla4* gene is located on the Chr1. 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）

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Ctla4 cytotoxic T-lymphocyte-associated protein 4 [Mus musculus (house mouse)]

Gene ID: 12477, updated on 9-Apr-2019

Summary

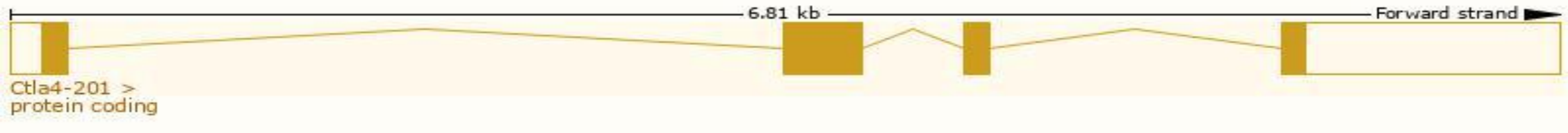
Official Symbol	Ctla4 provided by MGI
Official Full Name	cytotoxic T-lymphocyte-associated protein 4 provided byMGI
Primary source	MGI:MGI:88556
See related	Ensembl:ENSMUSG00000026011
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Cd152, Ctla-4, Ly-56
Summary	This gene is a member of the immunoglobulin superfamily, and encodes a protein that functions as a negative regulator of T-cell responses. Alternatively spliced transcript variants encoding different isoforms have been described for this gene. [provided by RefSeq, Aug 2013]
Expression	Biased expression in thymus adult (RPKM 1.4), spleen adult (RPKM 0.8) and 6 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

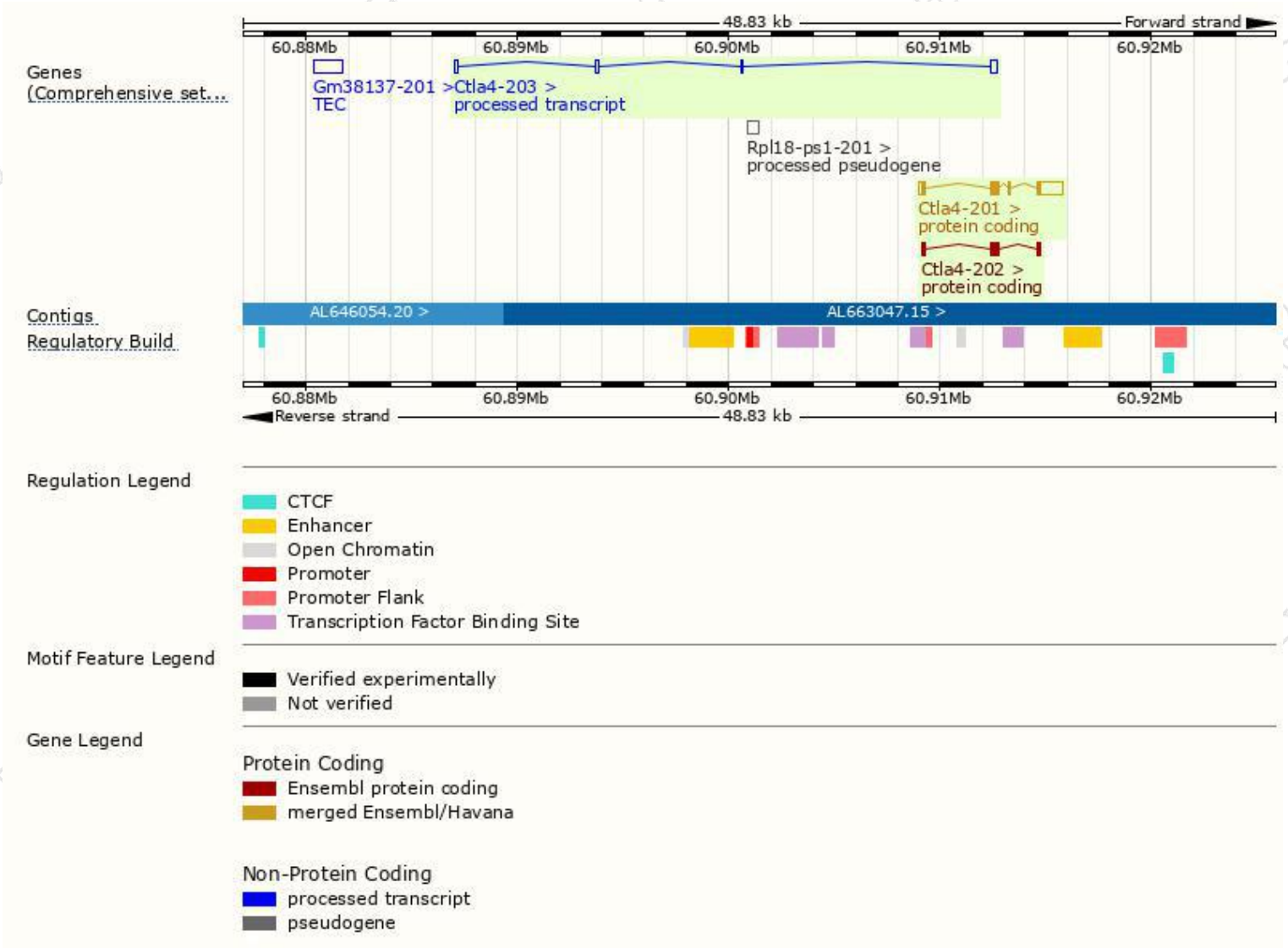
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ctla4-201	ENSMUST00000027164.8	1933	223aa	Protein coding	CCDS14993	Q6GTR6	TSL:1 GENCODE basic APPRIS P1
Ctla4-202	ENSMUST00000097720.3	614	174aa	Protein coding	CCDS69893	Q5SSM0	TSL:5 GENCODE basic
Ctla4-203	ENSMUST00000124816.1	691	No protein	Processed transcript	-	-	TSL:3

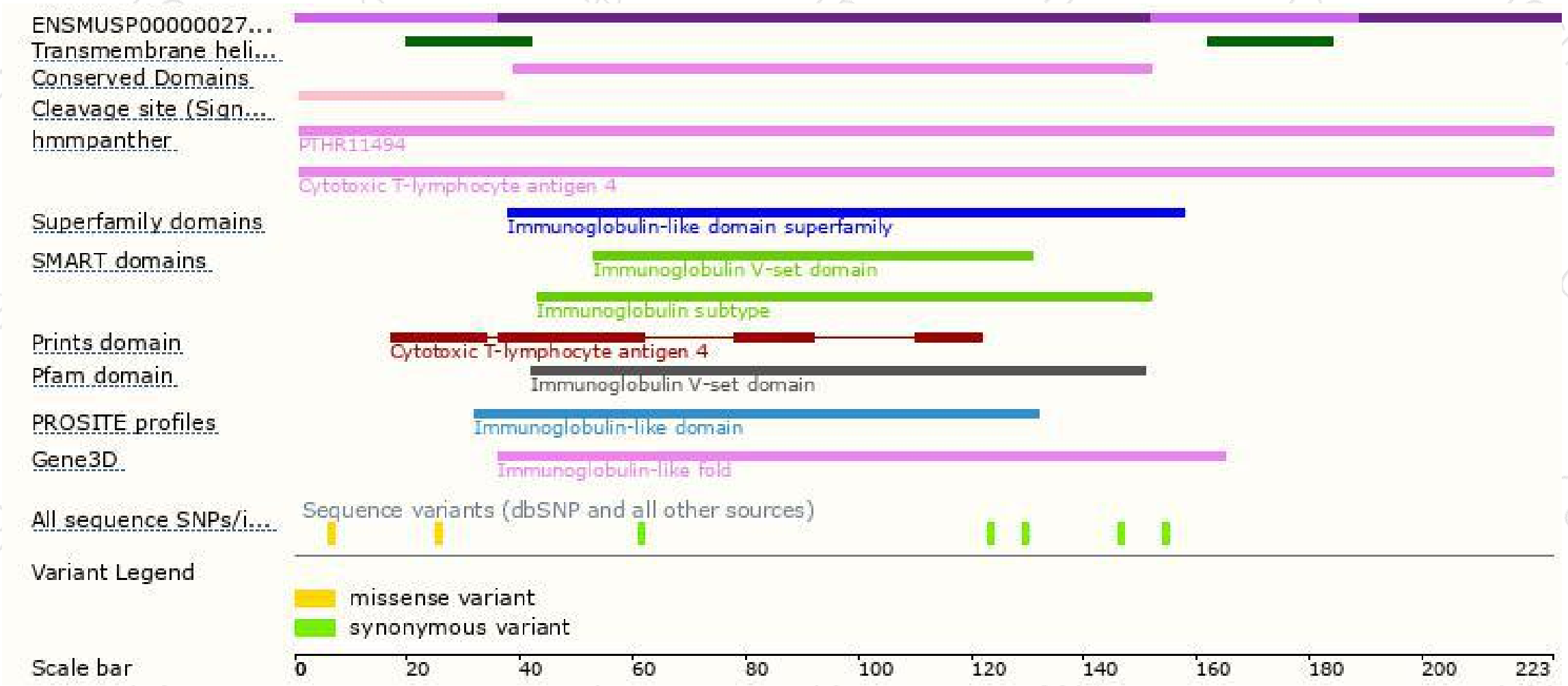
The strategy is based on the design of *Ctla4-201* transcript,The transcription is shown below



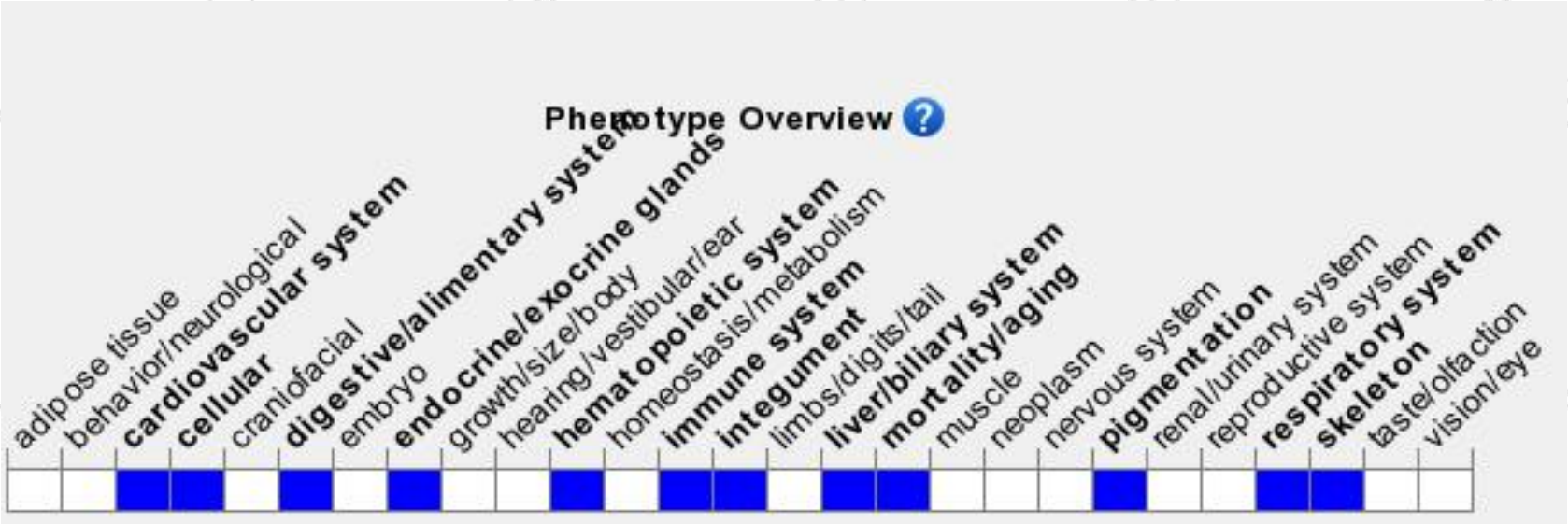
Genomic location distribution



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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