F9 Cas9-CKO Strategy

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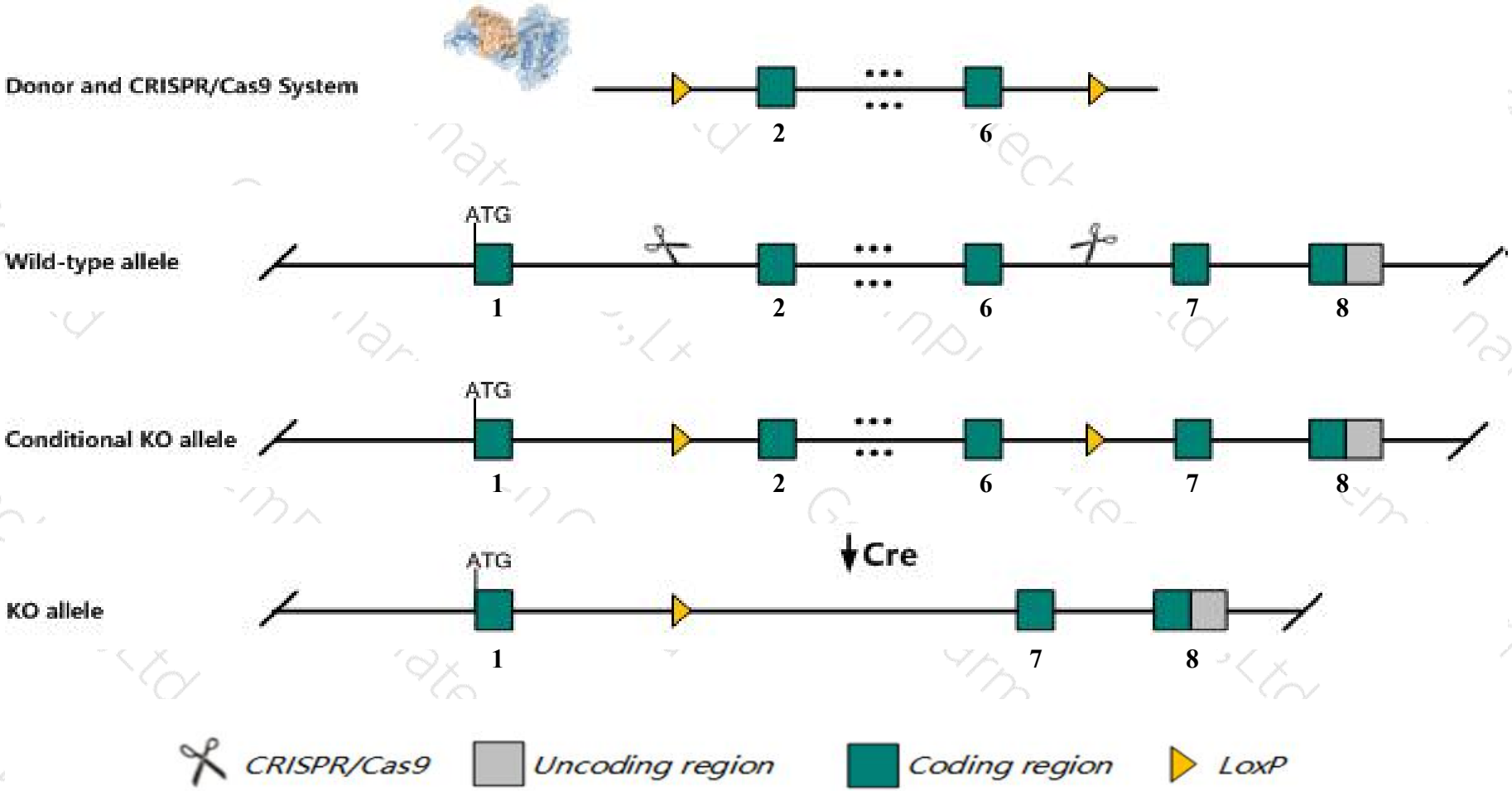
Project Overview

Project Name	***F9***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *F9* gene. The schematic diagram is as follows:



Technical routes

- The *F9* gene has 1 transcript. According to the structure of *F9* gene, exon2-exon6 of *F9-201* (ENSMUST00000033477.4) transcript is recommended as the knockout region.The region contains 665bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *F9* 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,Male hemizygotes for targeted null mutations are subject to fatal blood loss after tail snipping, and some affected males spontaneously die from umbilical cord bleeding. Carrier females show reduced levels of factor IX.

➢ The *F9* gene is located on the ChrX. 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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F9 coagulation factor IX [Mus musculus (house mouse)]

Gene ID: 14071, updated on 5-Mar-2019

Summary

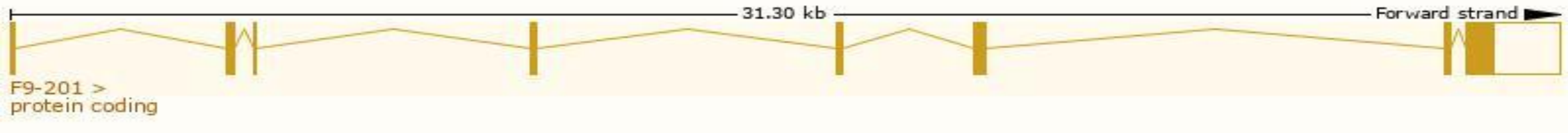
Official Symbol	F9 provided by MGI
Official Full Name	coagulation factor IX provided by MGI
Primary source	MGI:MGI:88384
See related	Ensembl:ENSMUSG00000031138
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW111646, Cf-9, Cf9
Summary	This gene encodes a vitamin K-dependent serine protease that plays a critical role in the intrinsic pathway of blood coagulation. The encoded protein is an inactive zymogen that is activated by coagulation factor XIa to generate factor IXa, a heterodimer containing heavy and light chains. In association with factor VIII, membrane phospholipids and calcium ions, factor IXa cleaves the inactive zymogen factor X to generate active factor Xa. Genetic deletion of this gene in mice results in a severe bleeding phenotype. Alternative splicing of this gene results in multiple transcript variants. [provided by RefSeq, Apr 2015]
Expression	Biased expression in liver E18 (RPKM 11.7), liver adult (RPKM 11.4) and 2 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

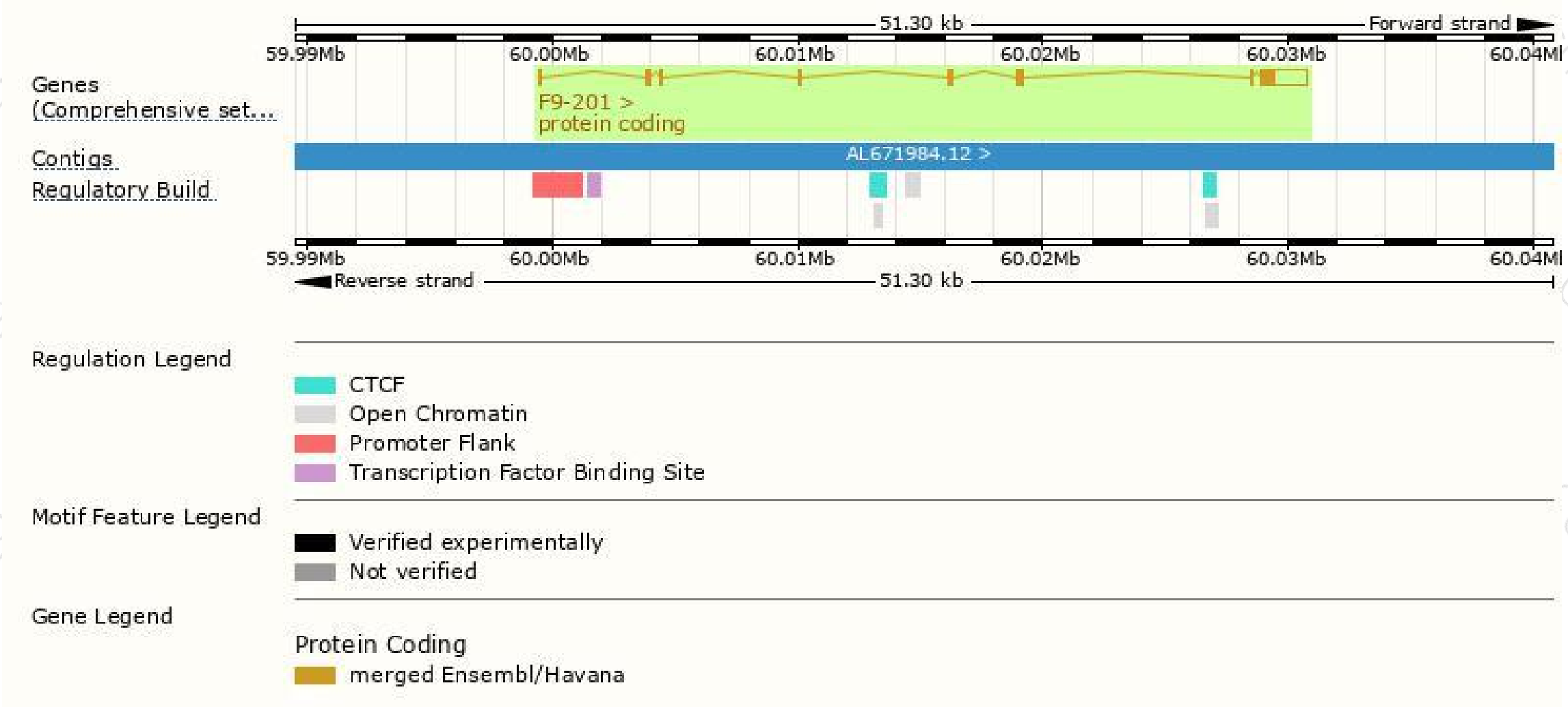
The gene has 1 transcript,and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
F9-201	ENSMUST00000033477.4	2734	471aa	Protein coding	CCDS30158	P16294	TSL:1 GENCODE basic APPRIS P1

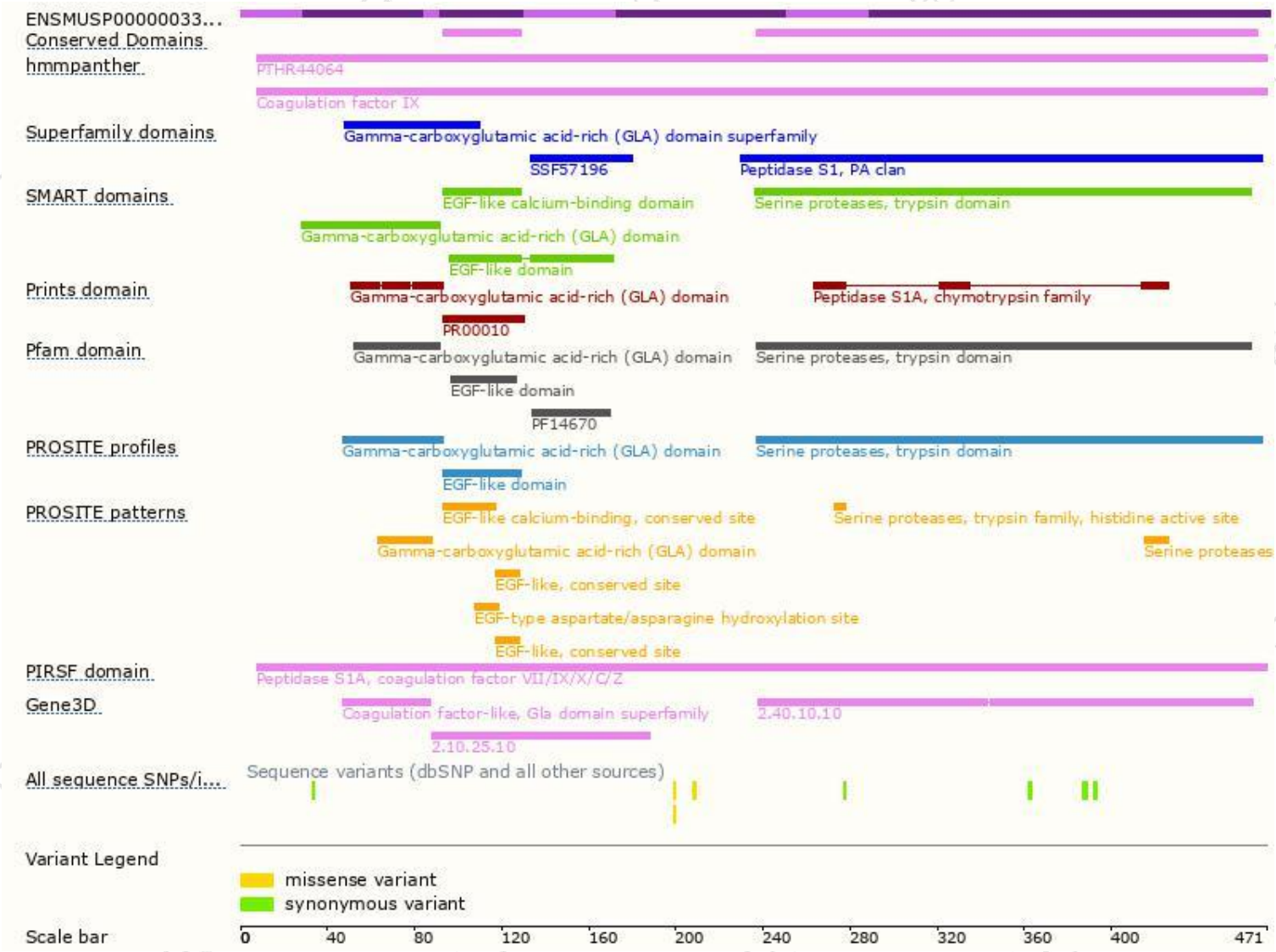
The strategy is based on the design of *F9-201* transcript,The transcription is shown below



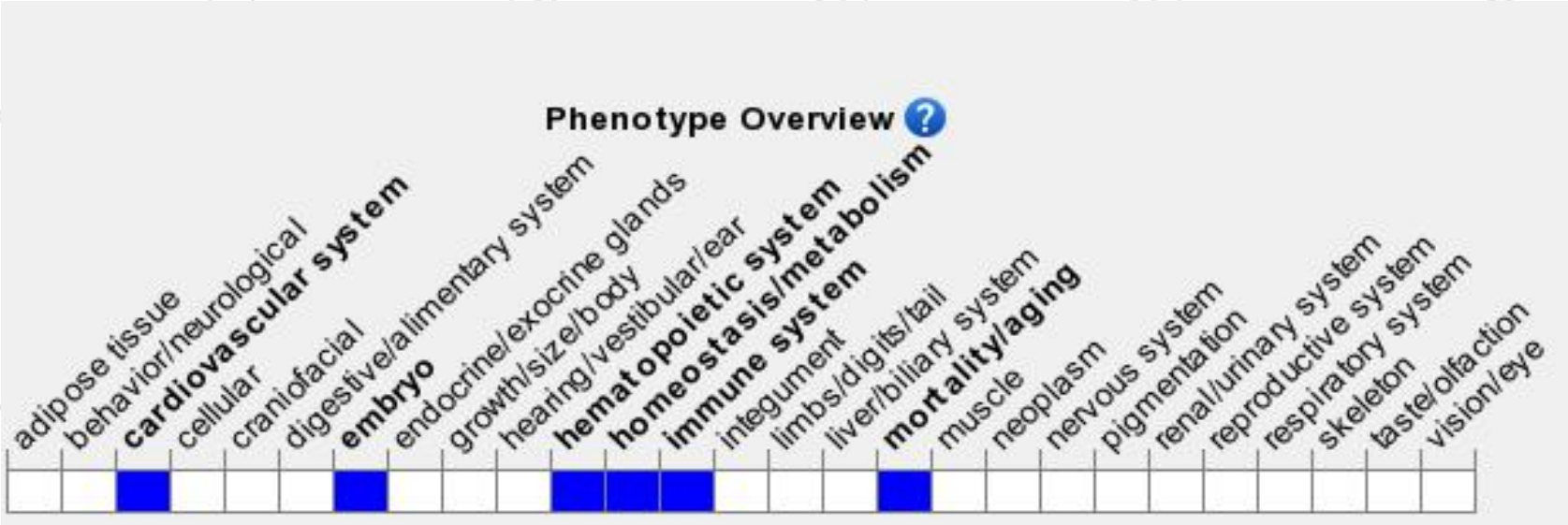
Genomic location distribution



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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