Casp8 Cas9-CKO Strategy

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Design Date:2018-06-27

Project Overview

Project Name	***Casp8***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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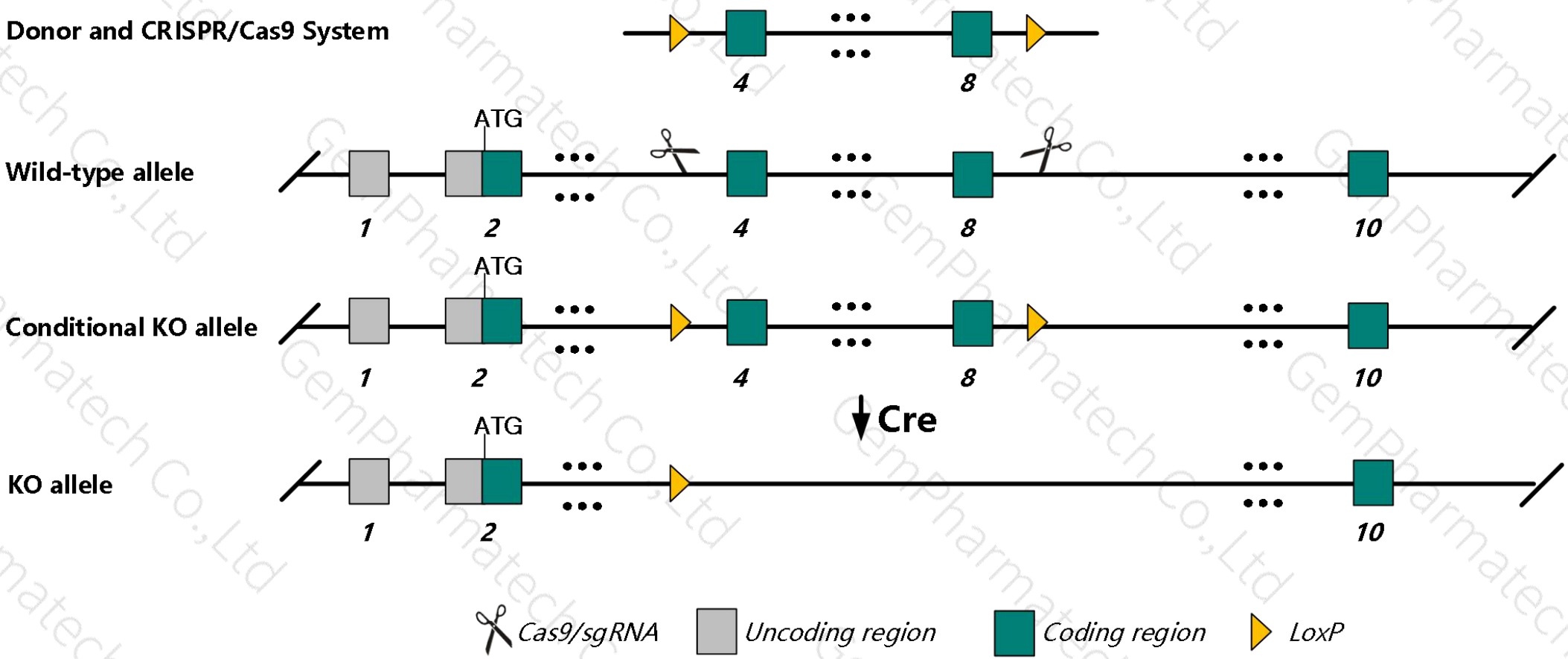
This model will use CRISPR/Cas9 technology to edit the *Casp8* gene. The schematic diagram is as follows:



Technical routes

- The *Casp8* gene has 4 transcripts. According to the structure of *Casp8* gene, exon4-exon8 of *Casp8-203* (ENSMUST00000190213.1) transcript is recommended as the knockout region.The region contains 503bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Casp8* 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,Homozygotes for a targeted null mutation exhibit impaired cardiac muscle development, cardiac erythrocyte congestion, low numbers of colony-forming cells, and prenatal lethality. T-cell restricted knockout mice are viable, but immunodeficient.

➢ The *Casp8* 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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Casp8 caspase 8 [Mus musculus (house mouse)]

Gene ID: 12370, updated on 9-Apr-2019

Summary

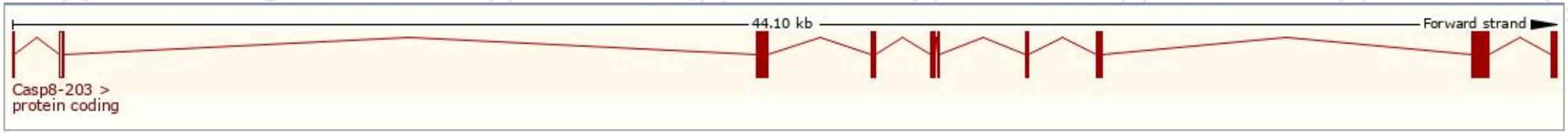
Official Symbol	Casp8 provided by MGI
Official Full Name	caspase 8 provided by MGI
Primary source	MGI:MGI:1261423
See related	Ensembl:ENSMUSG00000026029
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CASP-8, FLICE, MACH, Mch5
Summary	This gene is part of a family of caspases, aspartate-specific cysteine proteases well studied for their involvement in immune and apoptosis signaling. This protein, an initiator of apoptotic cell death, is activated by death-inducing tumor necrosis family receptors and targets downstream effectors. In mouse deficiency of this gene can cause embryonic lethality. This protein may have a role in embryogenesis. Alternative splicing results in multiple transcript variants that encode different protein isoforms. [provided by RefSeq, Apr 2013]
Expression	Ubiquitous expression in large intestine adult (RPKM 8.9), bladder adult (RPKM 4.8) and 24 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

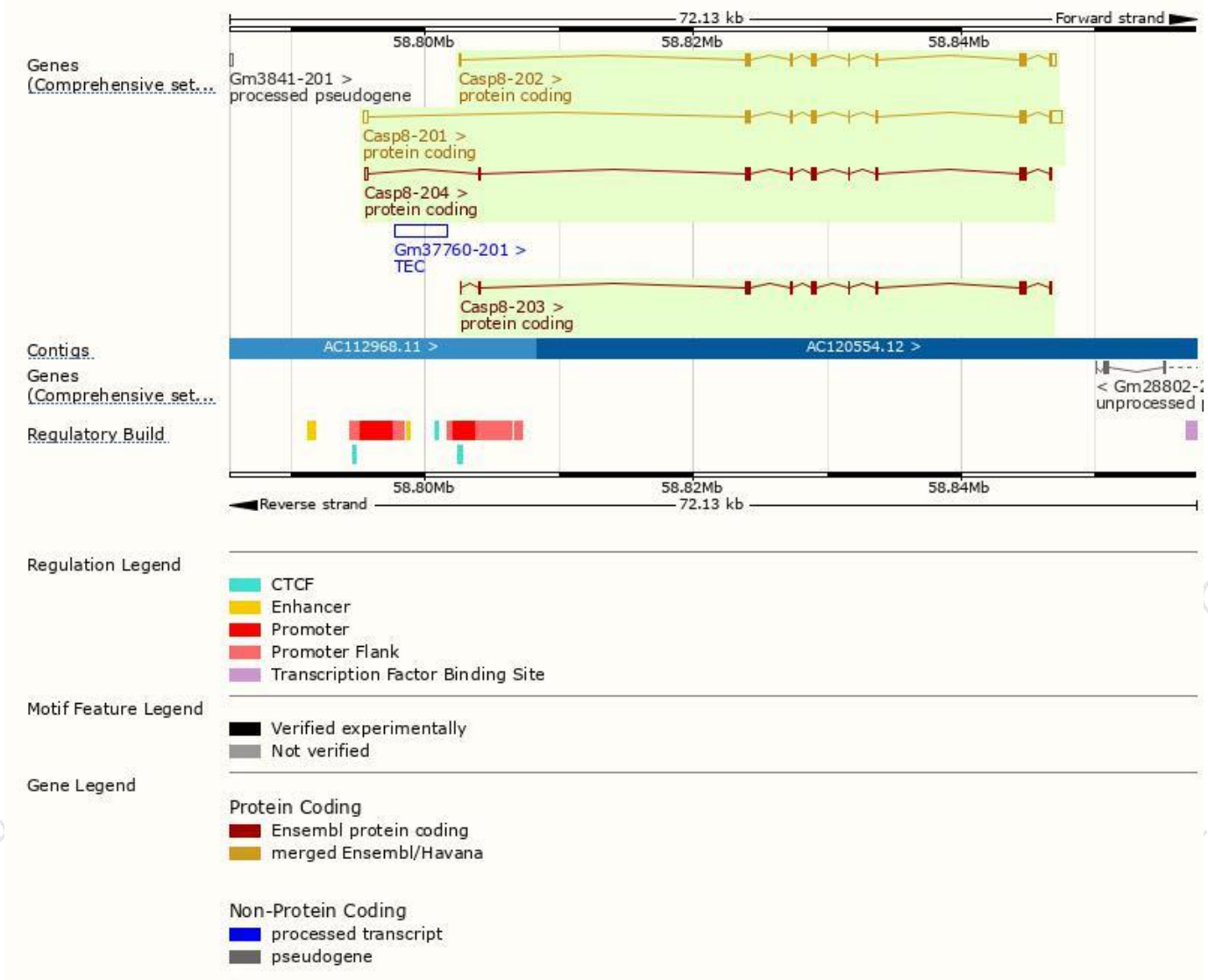
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Casp8-201	ENSMUST00000027189.14	2585	480aa	Protein coding	CCDS14979	O89110 Q3U607	TSL:1 GENCODE basic APPRIS P3
Casp8-202	ENSMUST00000165549.7	1917	480aa	Protein coding	CCDS14979	O89110 Q3U607	TSL:1 GENCODE basic APPRIS P3
Casp8-204	ENSMUST00000191201.6	1849	500aa	Protein coding	CCDS78590	A0A087WQT6	TSL:5 GENCODE basic APPRIS ALT2
Casp8-203	ENSMUST00000190213.1	1628	500aa	Protein coding	CCDS78590	A0A087WQT6	TSL:5 GENCODE basic APPRIS ALT2

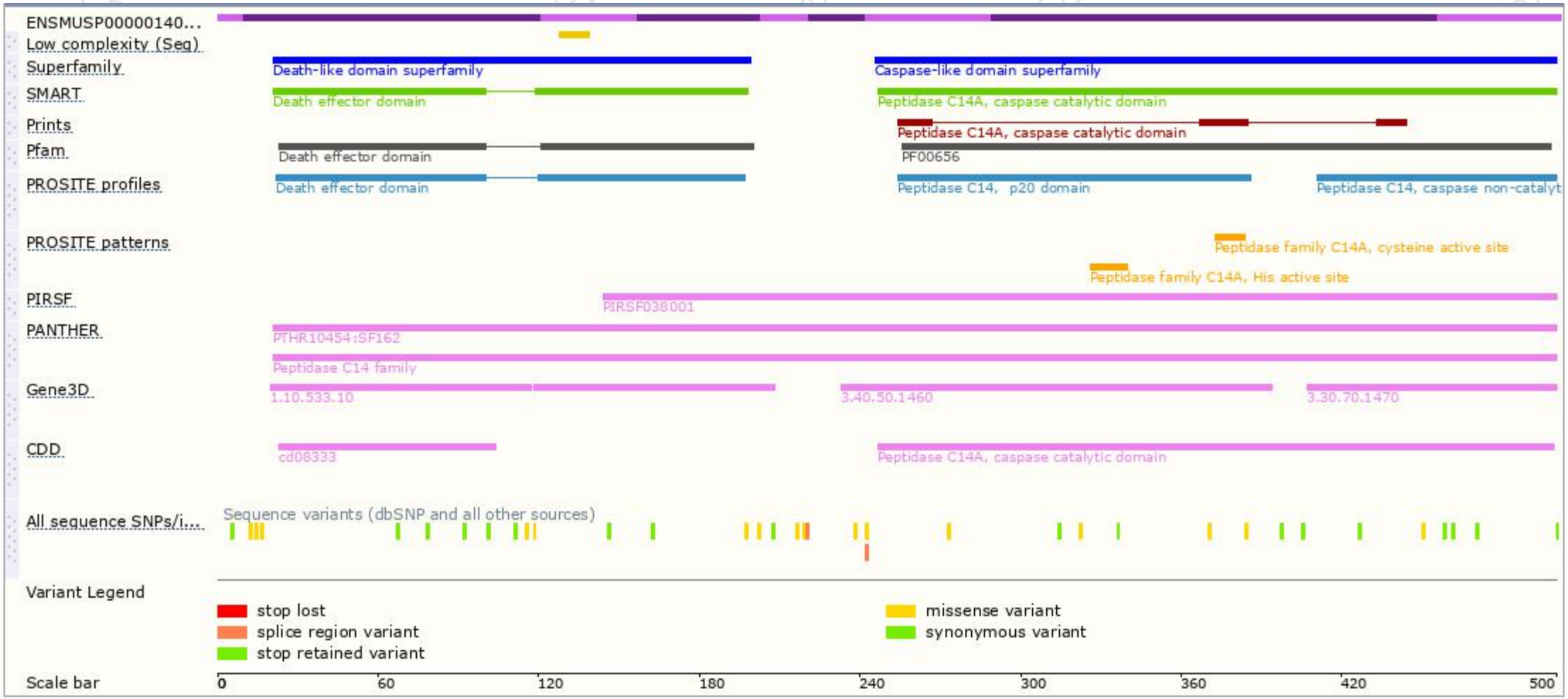
The strategy is based on the design of *Casp8-203* transcript,The transcription is shown below



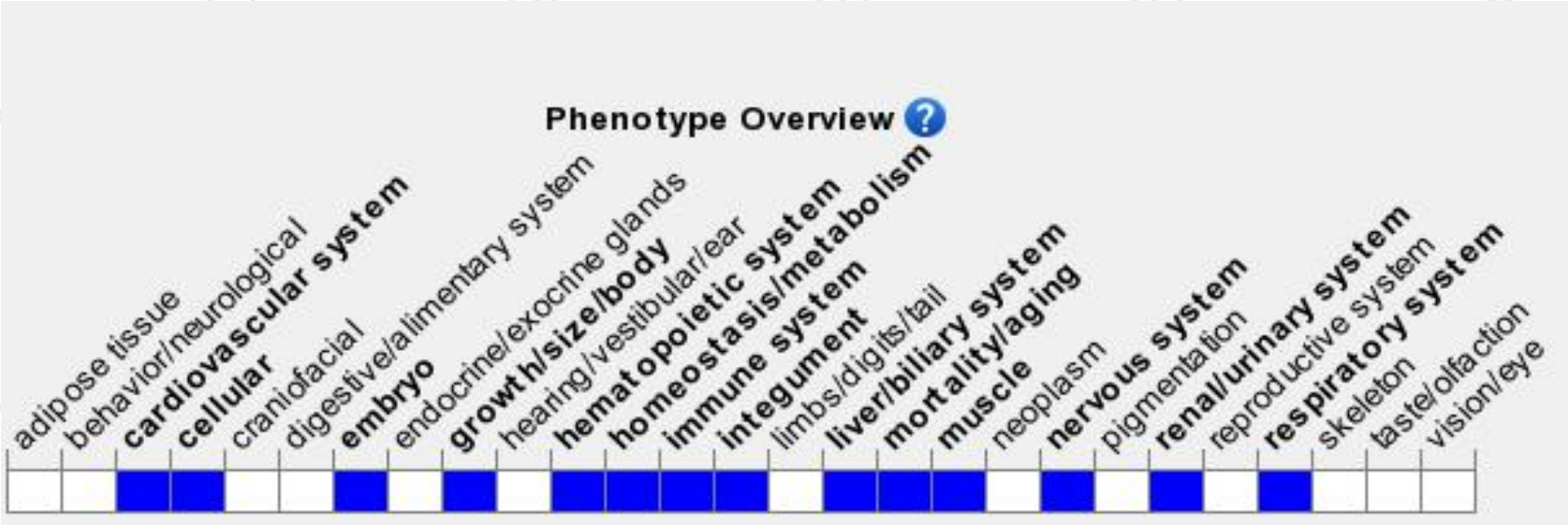
Genomic location distribution



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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