Traf6 Cas9-CKO Strategy

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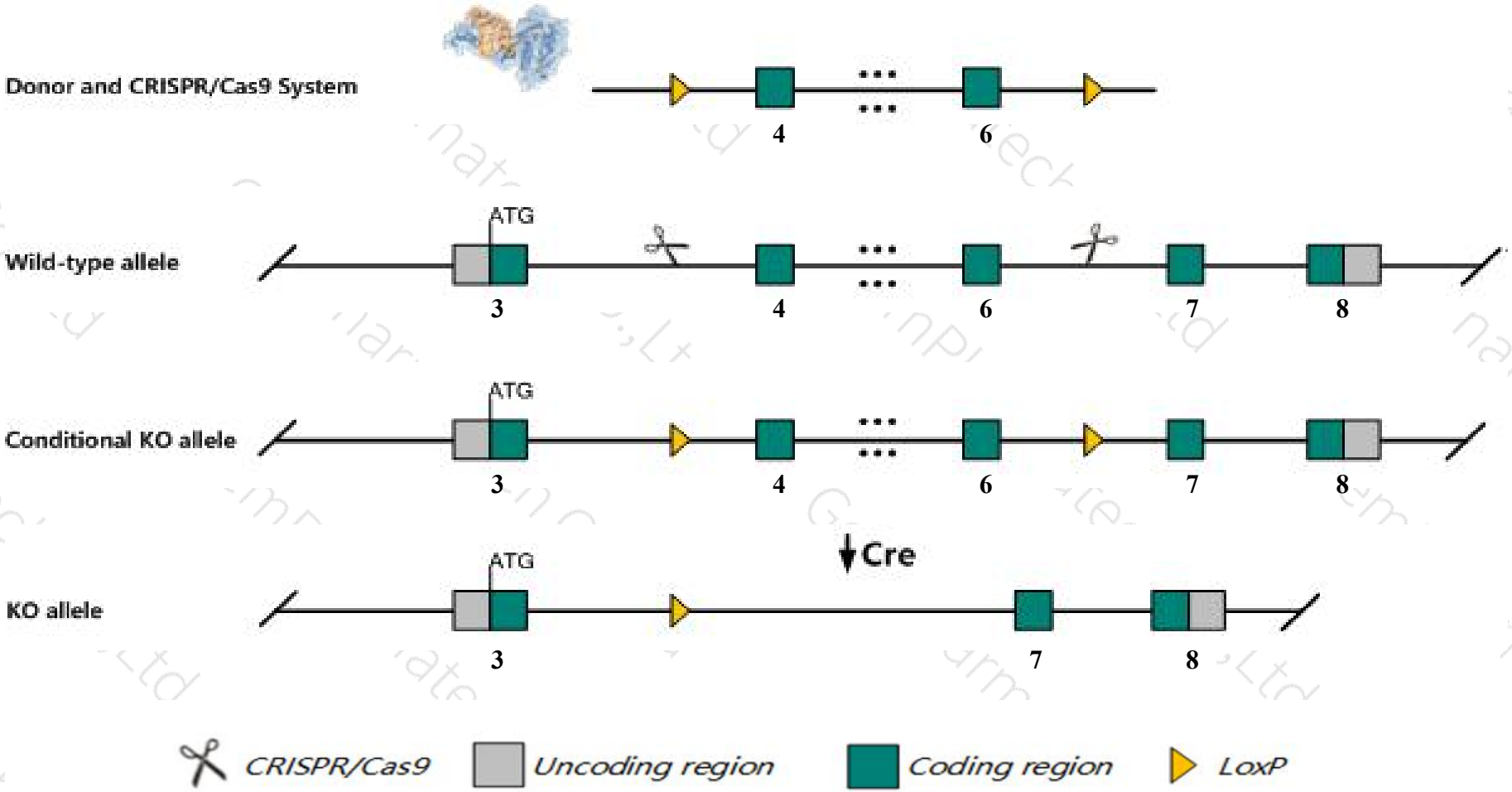
Project Overview

Project Name	***Traf6***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Traf6* gene. The schematic diagram is as follows:



Technical routes

- The *Traf6* gene has 3 transcripts. According to the structure of *Traf6* gene, exon4-exon6 of *Traf6-201* (ENSMUST00000004949.7) transcript is recommended as the knockout region.The region contains 382bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Traf6* 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,Viability is reduced in mice lacking both functional copies of this gene, with death occuring just before birth or around weaning. Mutants exhibit osteopetrosis and immune defects including abnormal immune cell development and function.

➢ The *Traf6* gene is located on the Chr2. 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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Traf6 TNF receptor-associated factor 6 [*Mus musculus* (house mouse)]

Gene ID: 22034, updated on 3-Sep-2019

Summary

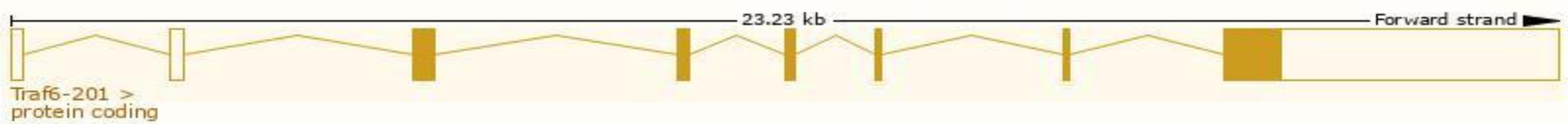
Official Symbol	Traf6 provided by MGI
Official Full Name	TNF receptor-associated factor 6 provided by MGI
Primary source	MGI:MGI:108072
See related	Ensembl:ENSMUSG00000027164
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI851288; 2310003F17Rik; C630032O20Rik
Summary	This gene encodes a member of the TNF receptor associated factor (TRAF) family of adaptor proteins that mediate signaling events from members of the TNF receptor and Toll/IL-1 receptor families to activate transcription factors such as NF-kappa-B and AP-1. The product of this gene is essential for perinatal and postnatal survival. Mice deficient in this protein exhibit osteopetrosis and defective in development of epidermal appendixes, normal B cell differentiation, lymph node organogenesis, interleukin-1 signaling, lipopolysaccharide signaling and neural tube closure. This protein possesses ubiquitin ligase activity. Alternate splicing of this gene results in multiple transcript variants. [provided by RefSeq, Dec 2014]
Expression	Ubiquitous expression in CNS E11.5 (RPKM 2.0), thymus adult (RPKM 2.0) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

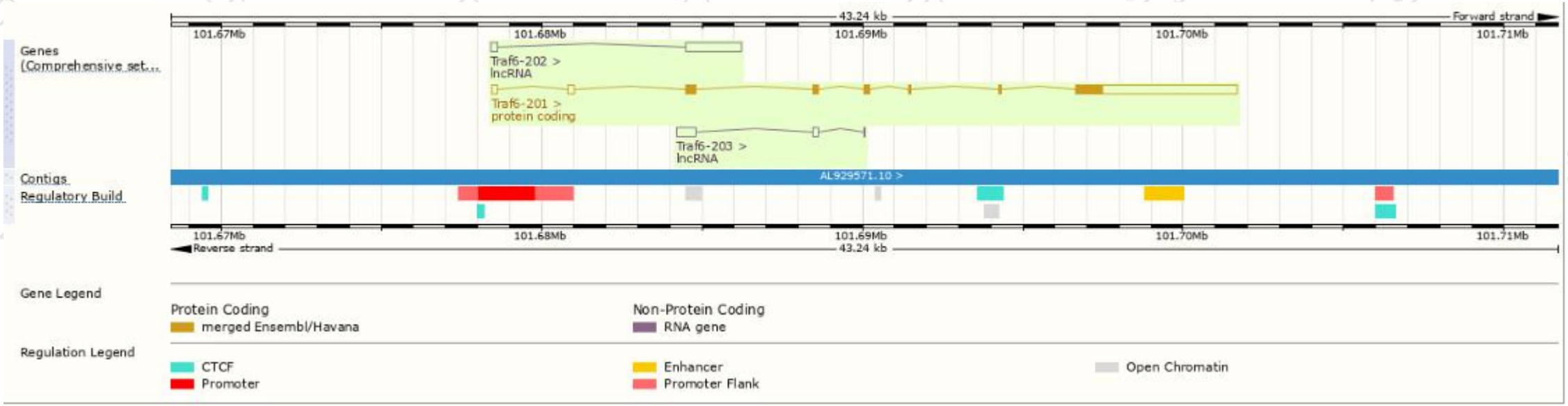
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Traf6-201	ENSMUST00000004949.7	6169	530aa	ENSMUSP00000004949.7	Protein coding	CCDS16464	P70196	TSL:1 GENCODE basic APPRIS P1
Traf6-202	ENSMUST00000143341.1	1949	No protein	-	lncRNA	-	-	TSL:2
Traf6-203	ENSMUST00000144063.1	763	No protein	-	lncRNA	-	-	TSL:3

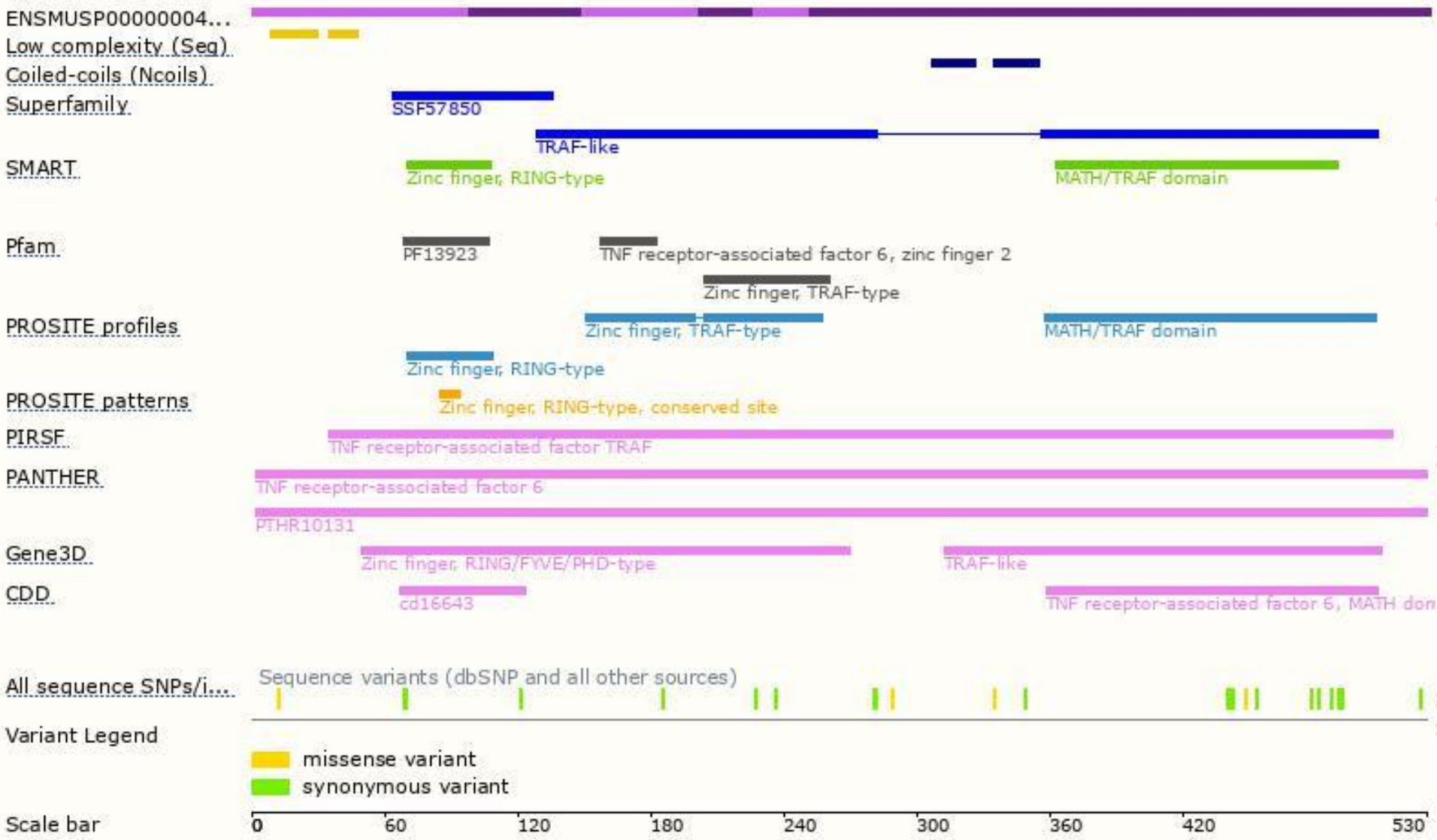
The strategy is based on the design of *Traf6-201* transcript,The transcription is shown below



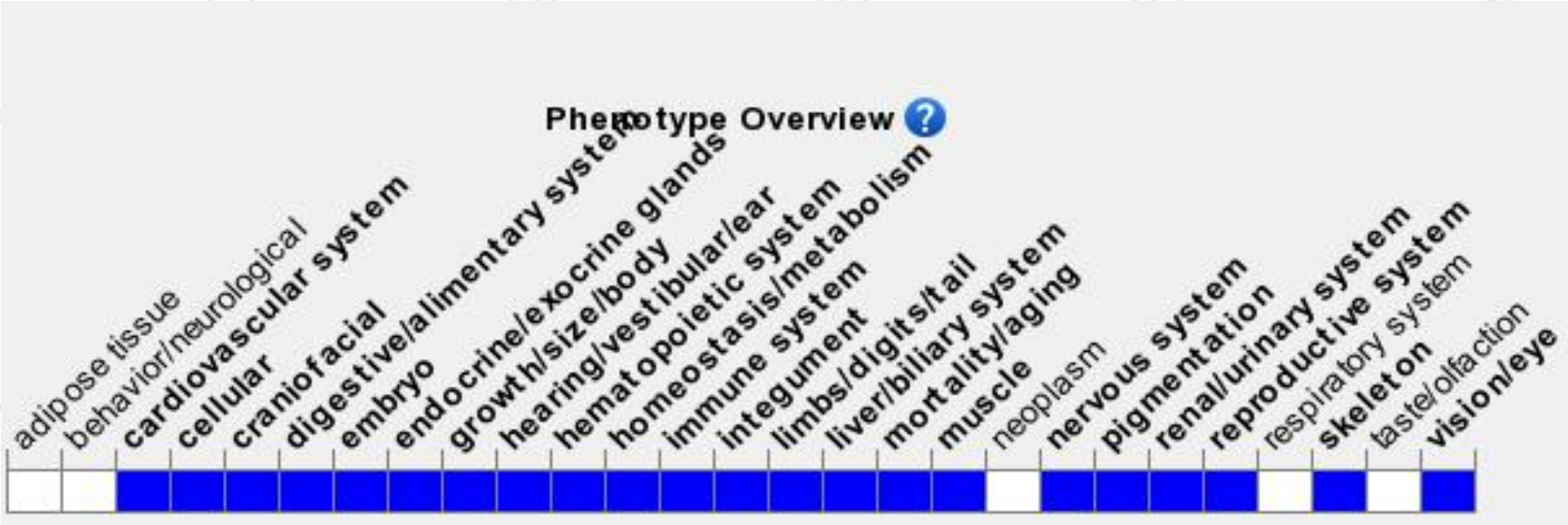
Genomic location distribution



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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