Sema5b Cas9-CKO Strategy

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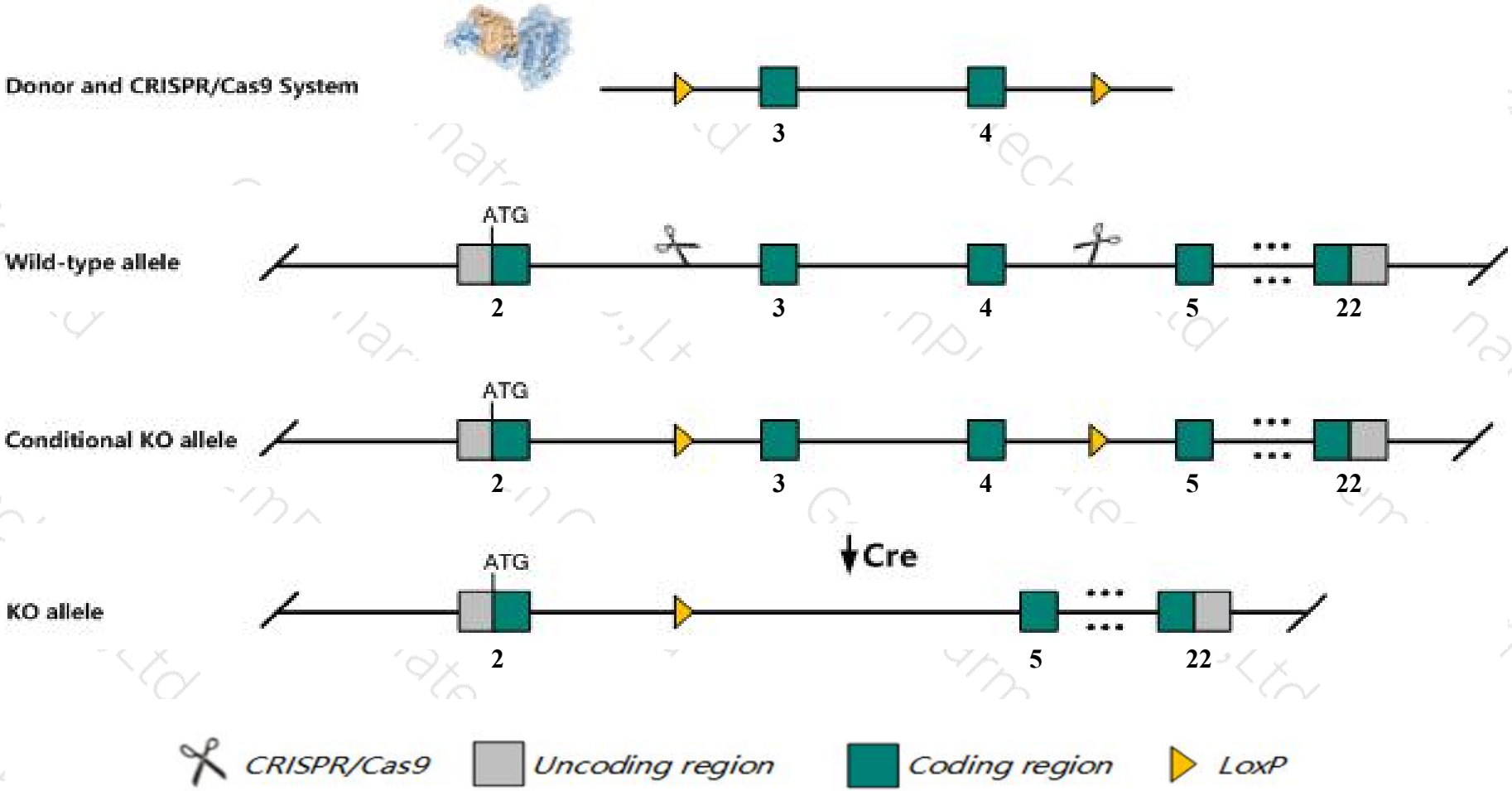
Project Overview

Project Name	***Sema5b***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Sema5b* gene. The schematic diagram is as follows:



Technical routes

- The *Sema5b* gene has 8 transcripts. According to the structure of *Sema5b* gene, exon3-exon4 of *Sema5b-201* (ENSMUST00000050625.14) transcript is recommended as the knockout region.The region contains 146bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Sema5b* 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 null mutation display defects in neurite arborization of multiple retinal cell types.

- The *Sema5b* 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）

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Sema5b sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B [Mus musculus (house mouse)]

Gene ID: 20357, updated on 31-Jan-2019

Summary

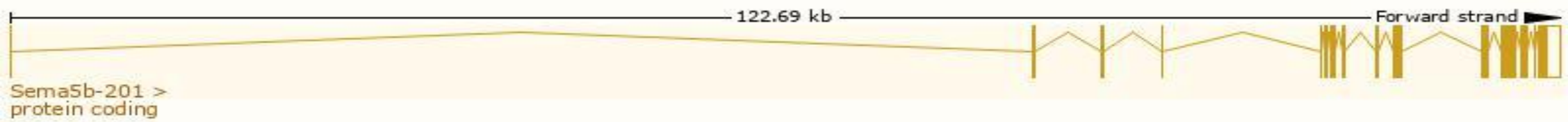
Official Symbol	Sema5b provided by MGI
Official Full Name	sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B provided by MGI
Primary source	MGI:MGI:107555
See related	Ensembl:ENSMUSG00000052133
Gene type	protein 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	AI893641, SemG, Semag, mKIAA1445
Expression	Biased expression in adrenal adult (RPKM 16.2), CNS E11.5 (RPKM 13.7) and 11 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

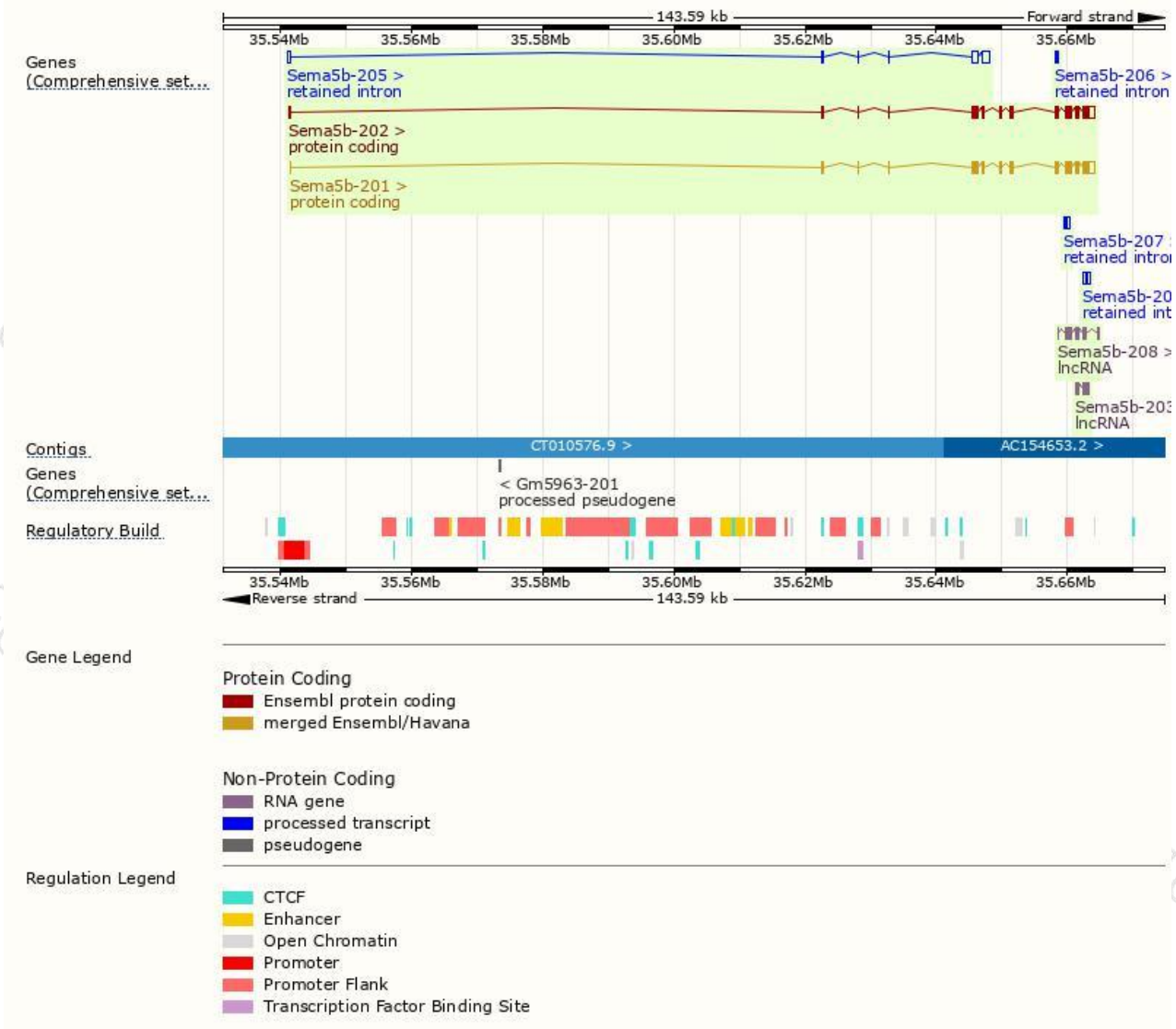
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sema5b-201	ENSMUST00000050625.14	4350	1093aa	Protein coding	CCDS28140	Q60519	TSL:1 GENCODE basic APPRIS P2
Sema5b-202	ENSMUST00000120756.7	4646	1122aa	Protein coding	-	Q60519	TSL:1 GENCODE basic APPRIS ALT2
Sema5b-205	ENSMUST00000133554.1	2928	No protein	Retained intron	-	-	TSL:1
Sema5b-204	ENSMUST00000128966.1	766	No protein	Retained intron	-	-	TSL:2
Sema5b-207	ENSMUST00000149097.1	717	No protein	Retained intron	-	-	TSL:3
Sema5b-206	ENSMUST00000139805.1	268	No protein	Retained intron	-	-	TSL:3
Sema5b-208	ENSMUST00000149855.7	1563	No protein	lncRNA	-	-	TSL:5
Sema5b-203	ENSMUST00000128347.1	694	No protein	lncRNA	-	-	TSL:2

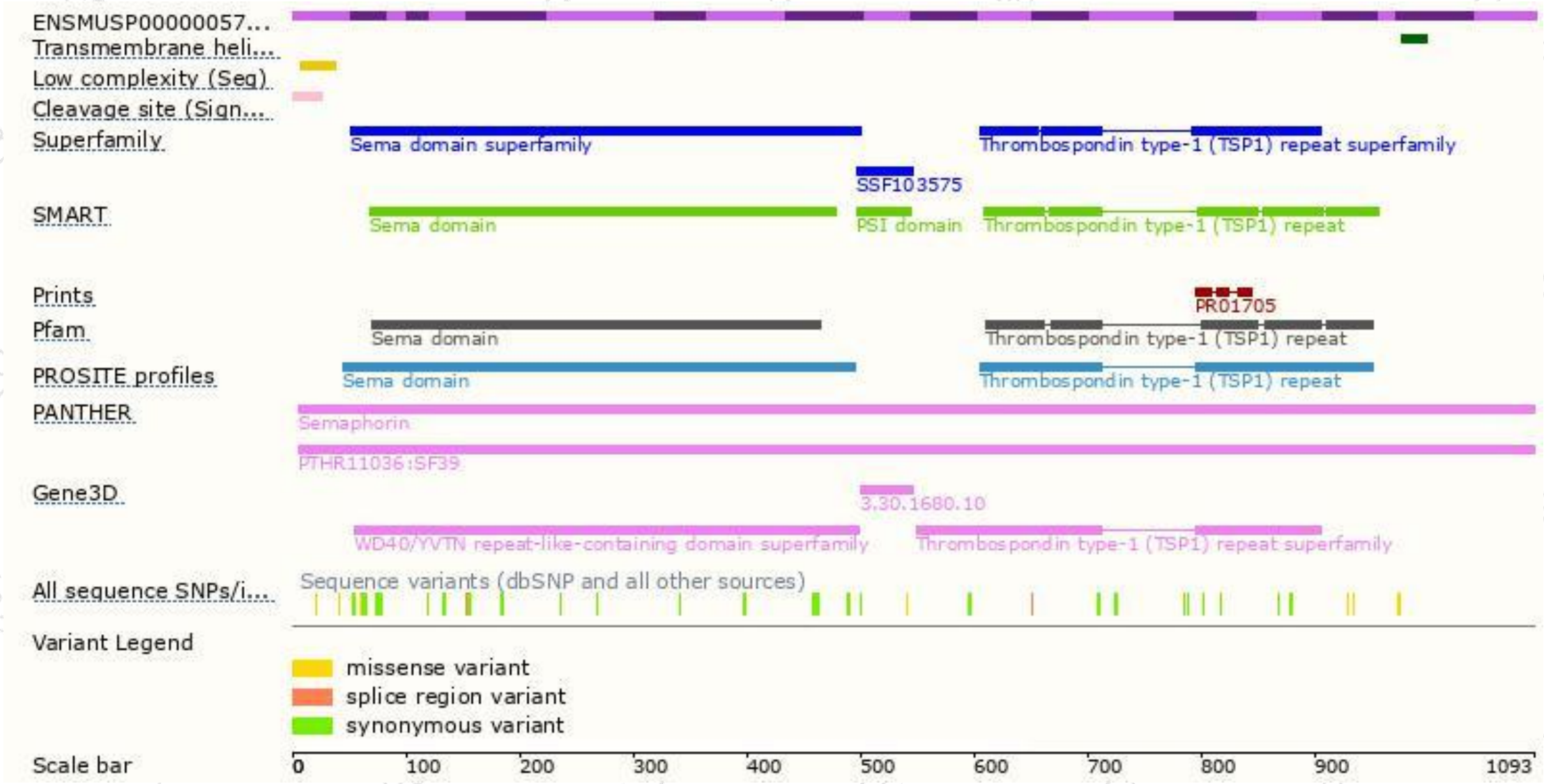
The strategy is based on the design of *Sema5b-201* transcript,The transcription is shown below



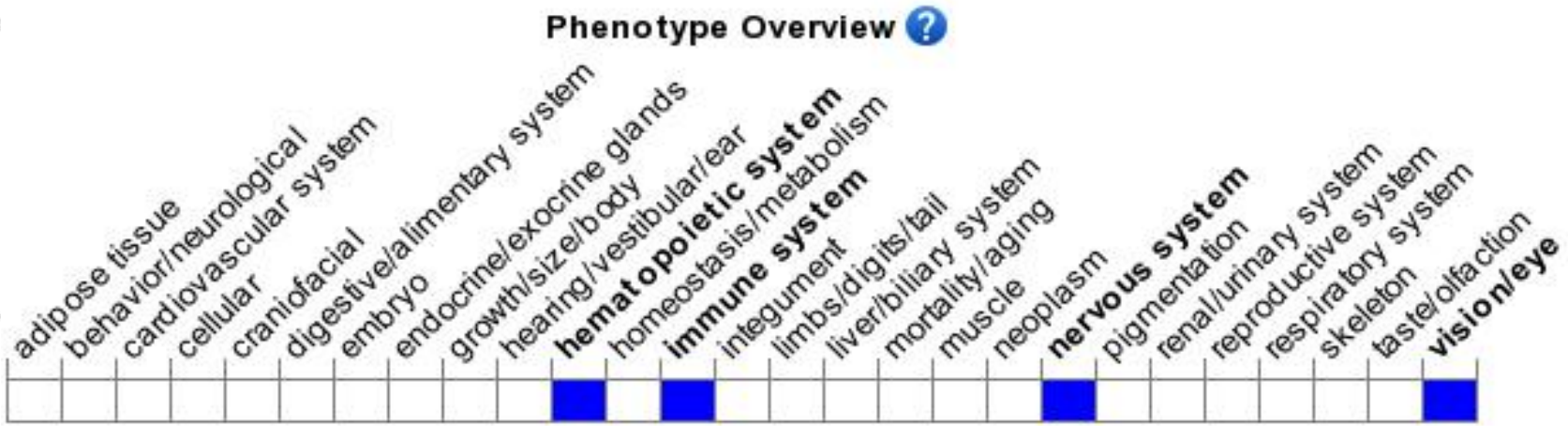
Genomic location distribution



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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