

Rab4a Cas9-CKO Strategy

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Reviewer:

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Project Overview

Project Name

Rab4a

Project type

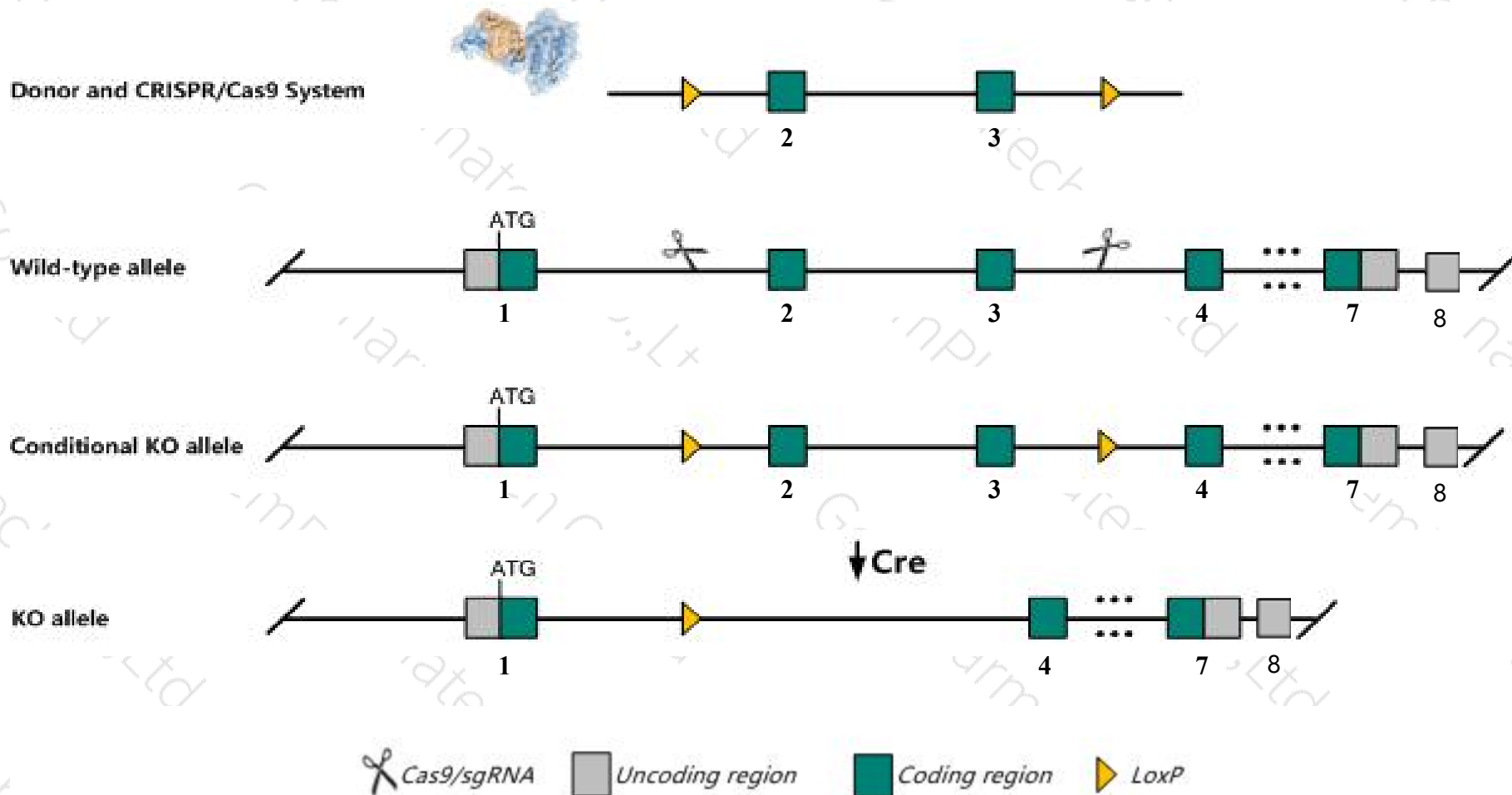
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rab4a* gene. The schematic diagram is as follows:



- The *Rab4a* gene has 3 transcripts. According to the structure of *Rab4a* gene, exon2-exon3 of *Rab4a-202* (ENSMUST00000118535.7) transcript is recommended as the knockout region. The region contains 196bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rab4a* 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

- The *Rab4a* gene is located on the Chr8. 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.
- The KO region contains functional region of the *Gm20388* gene. Knockout the region may affect the function of *Gm20388* gene.
- 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)

Rab4a RAB4A, member RAS oncogene family [*Mus musculus* (house mouse)]

Gene ID: 19341, updated on 12-Aug-2019

Summary

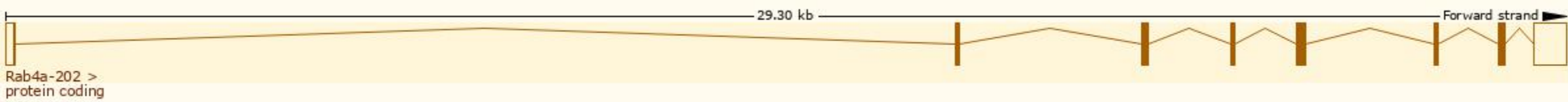
Official Symbol	Rab4a provided by MGI
Official Full Name	RAB4A, member RAS oncogene family provided by MGI
Primary source	MGI:MGI:105069
See related	Ensembl:ENSMUSG00000019478
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Rab4; AI848268
Expression	Ubiquitous expression in large intestine adult (RPKM 26.3), cerebellum adult (RPKM 22.2) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

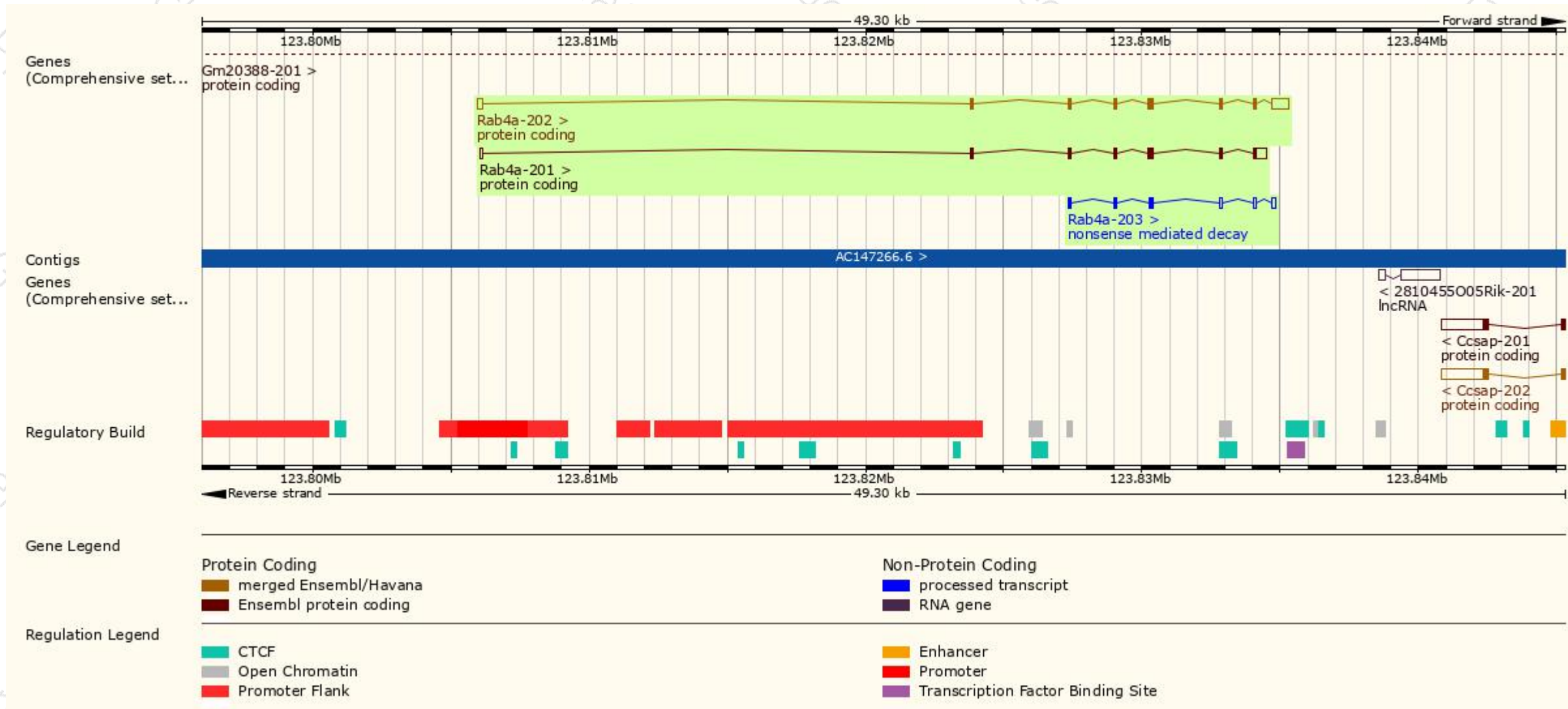
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rab4a-202	ENSMUST00000118535.7	1419	218aa	Protein coding	CCDS52703	P56371	TSL:1 Gencode basic APPRIS P1
Rab4a-201	ENSMUST00000117702.1	1080	218aa	Protein coding	CCDS52703	P56371	TSL:1 Gencode basic APPRIS P1
Rab4a-203	ENSMUST00000212846.1	640	96aa	Nonsense mediated decay	-	A0A1D5RMH1	CDS 5' incomplete TSL:3

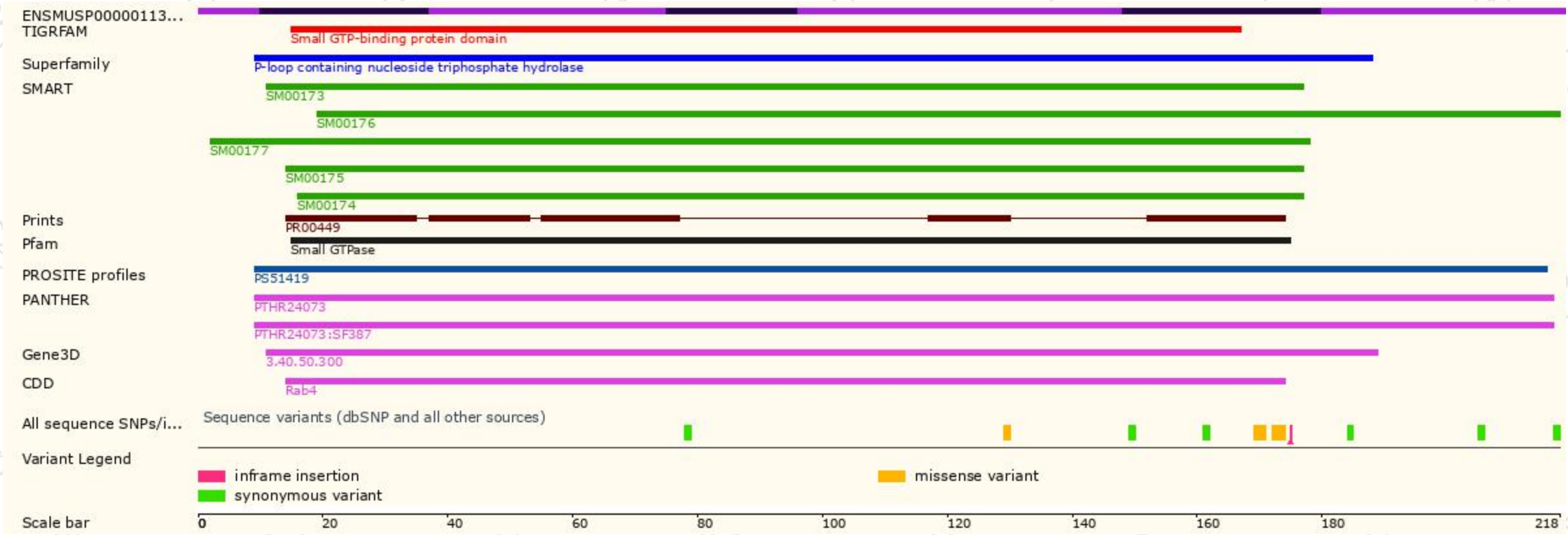
The strategy is based on the design of *Rab4a-202* transcript, the transcription is shown below:



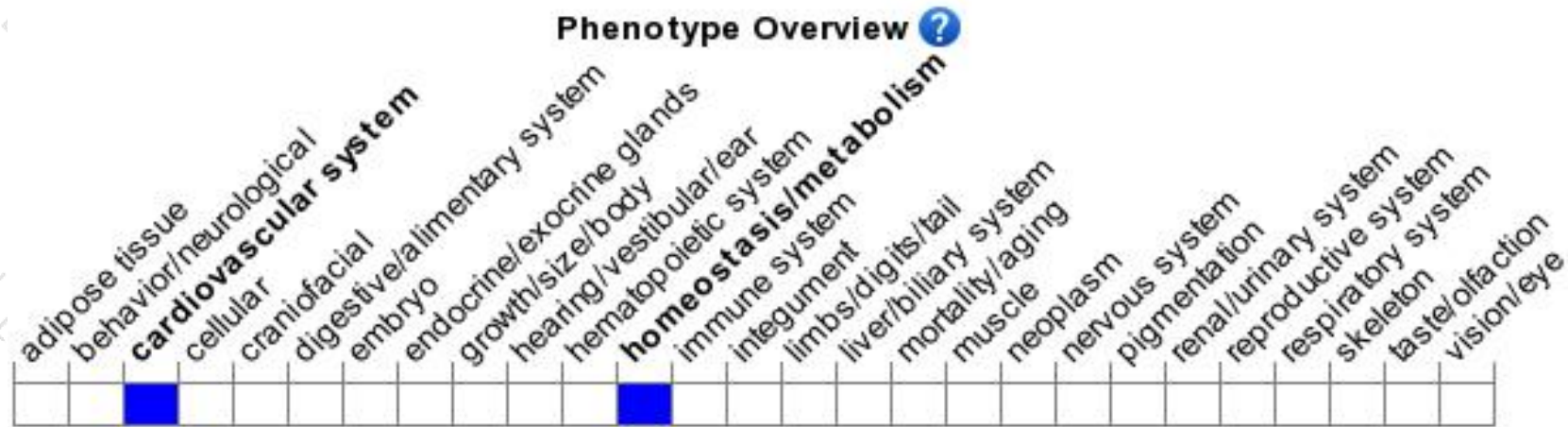
Genomic location distribution



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/>).

If you have any questions, you are welcome to inquire.

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