P2ry4 Cas9-CKO Strategy

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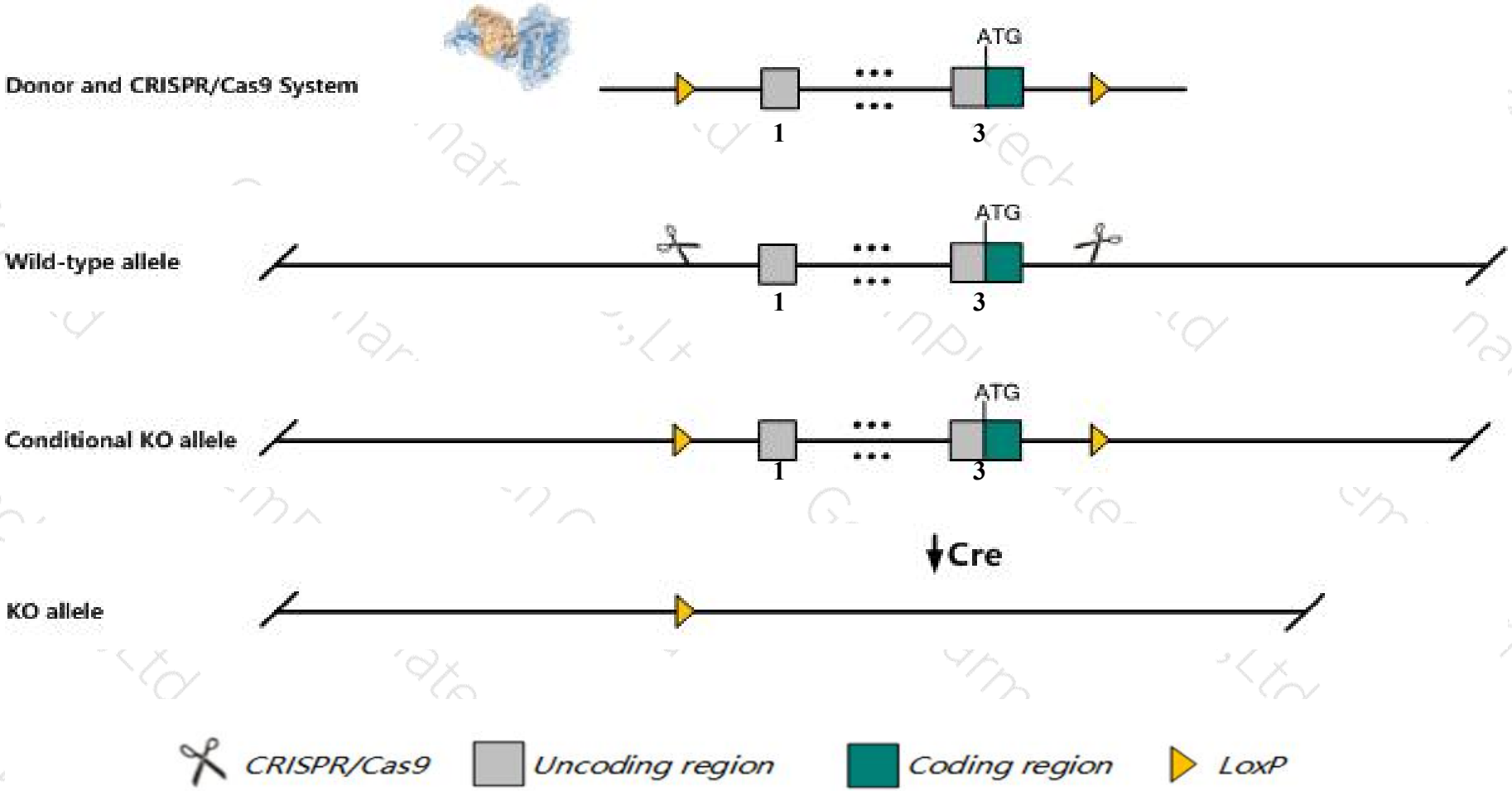
Project Overview

Project Name	***P2ry4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *P2ry4* gene. The schematic diagram is as follows:



Technical routes

- The *P2ry4* gene has 1 transcript. According to the structure of *P2ry4* gene, exon1-exon3 of *P2ry4-201* (ENSMUST00000053373.1) transcript is recommended as the knockout region.The region contains most of the coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *P2ry4* 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,Homozygous and hemizygous null mice display abnormal digestive secretion and homozygous null females display partially penetrant lethality.

➢ The *P2ry4* 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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P2ry4 pyrimidinergic receptor P2Y, G-protein coupled, 4 [Mus musculus (house mouse)]

Gene ID: 57385, updated on 31-Jan-2019

Summary

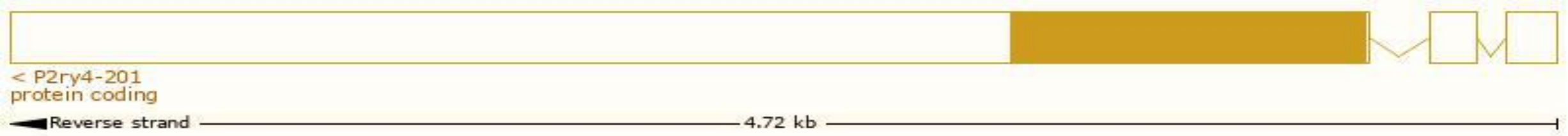
Official Symbol	P2ry4 provided by MGI
Official Full Name	pyrimidinergic receptor P2Y, G-protein coupled, 4 provided by MGI
Primary source	MGI:MGI:1926594
See related	Ensembl:ENSMUSG00000044359
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	P2Y4, P2Y4R
Expression	Biased expression in liver adult (RPKM 7.1), large intestine adult (RPKM 3.5) and 5 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

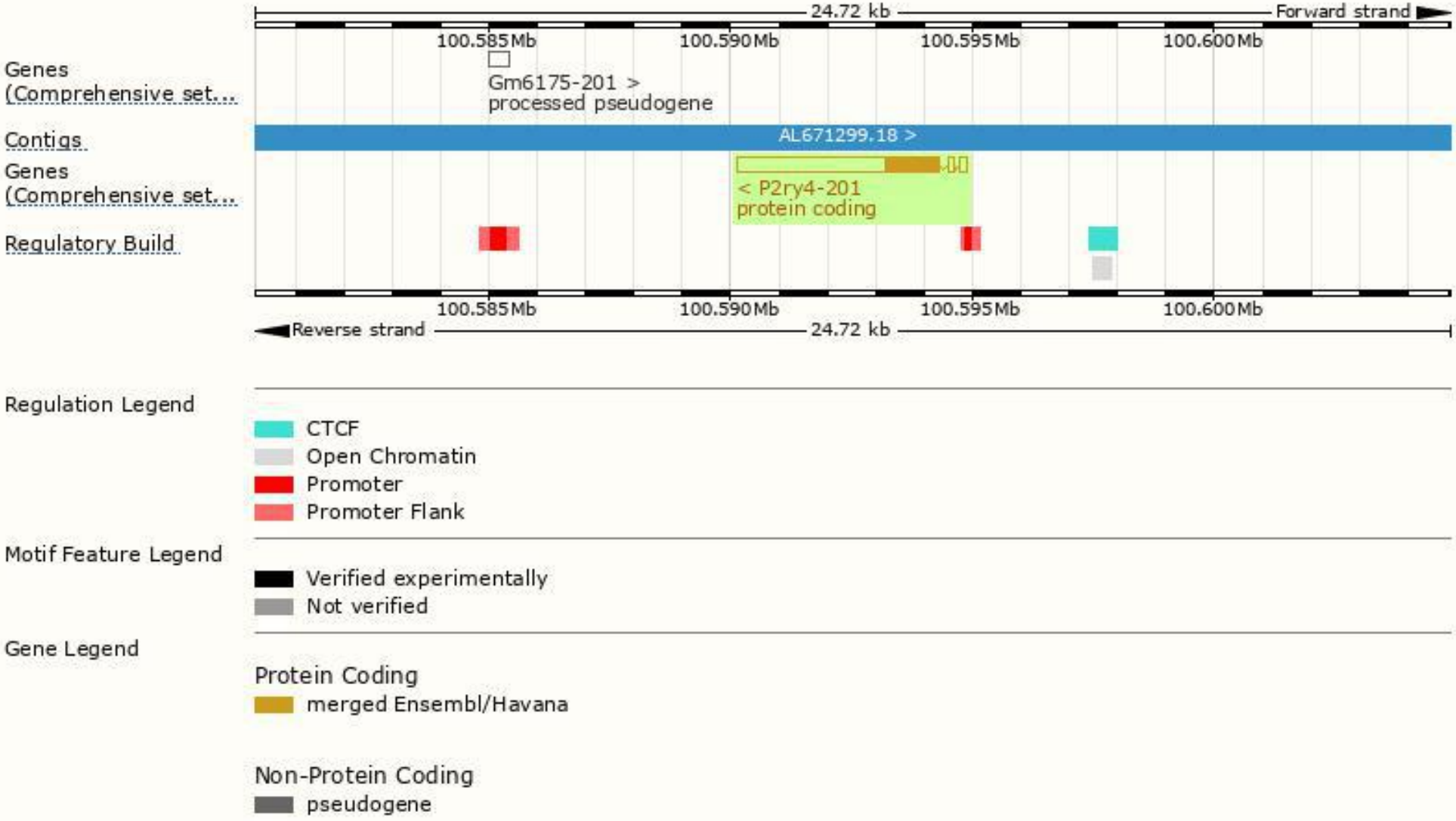
The gene has 1 transcript,and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
P2ry4-201	ENSMUST00000053373.1	4434	361aa	Protein coding	CCDS30303	Q0VBT7 Q9JJS7	TSL:1 GENCODE basic APPRIS P1

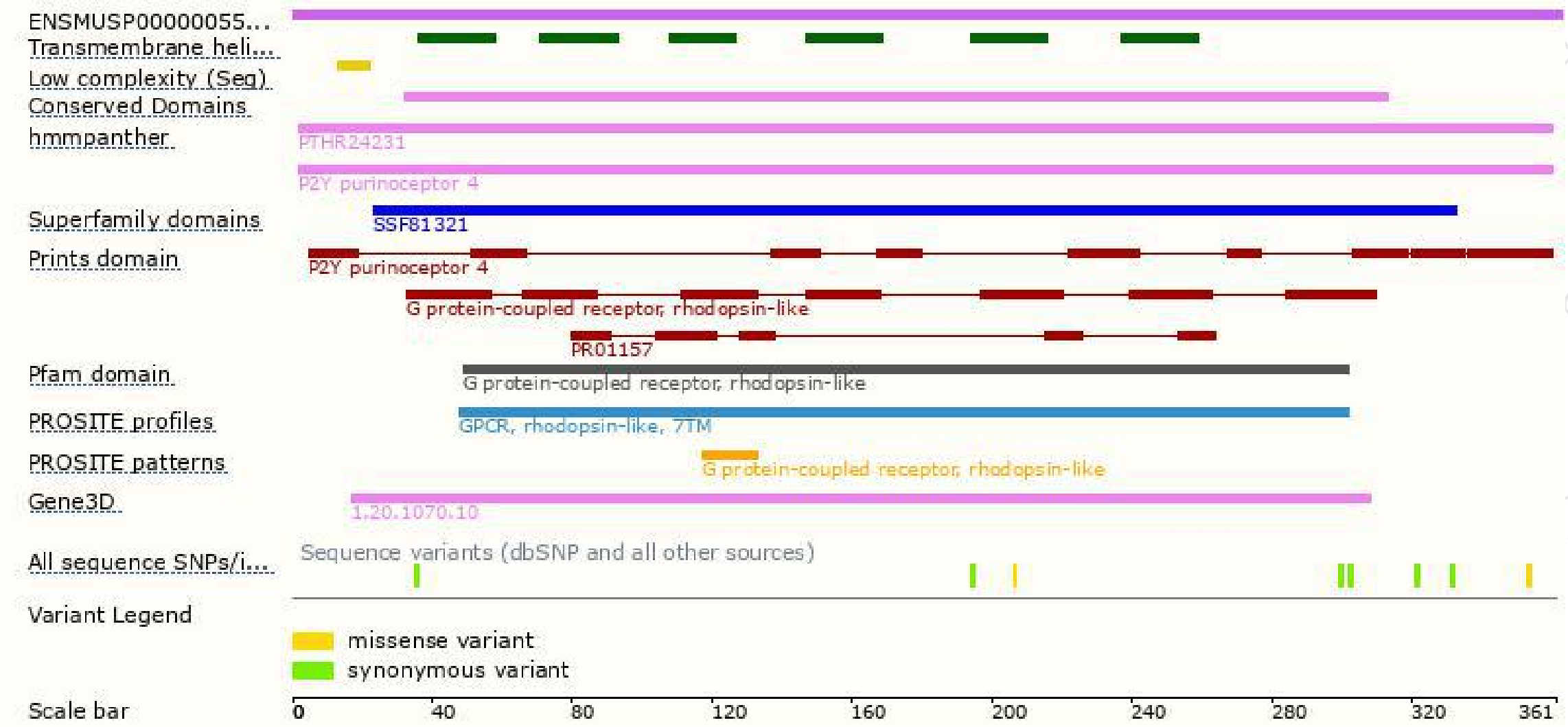
The strategy is based on the design of *P2ry4-201* transcript,The transcription is shown below



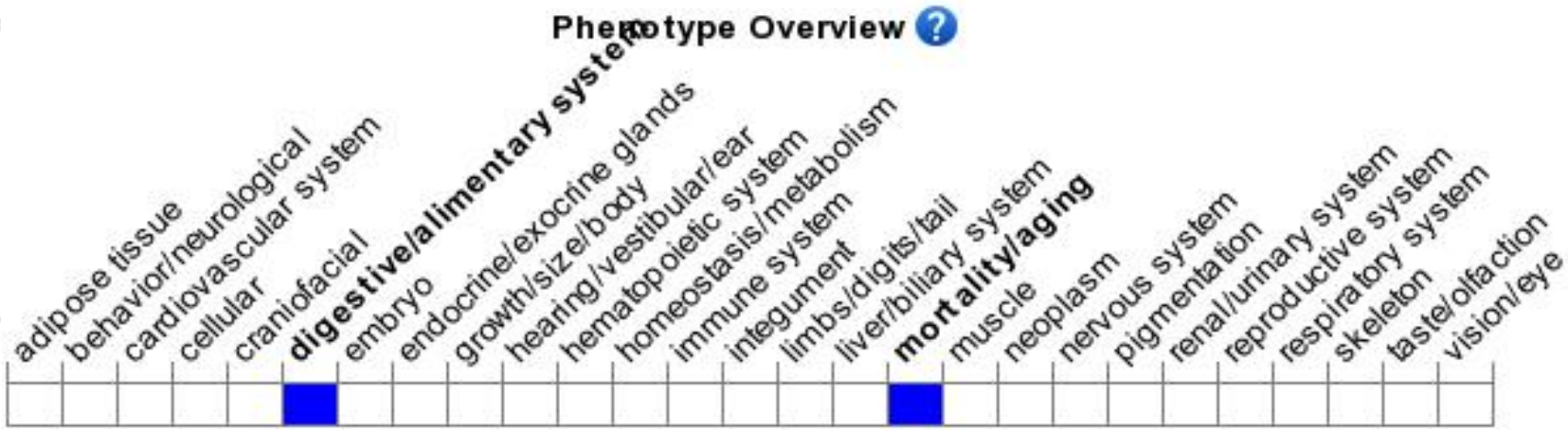
Genomic location distribution



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/).

According to the existing MGI data,Homozygous and hemizygous null mice display abnormal digestive secretion and homozygous null females display partially penetrant lethality.

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

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