Pax4 Cas9-CKO Strategy

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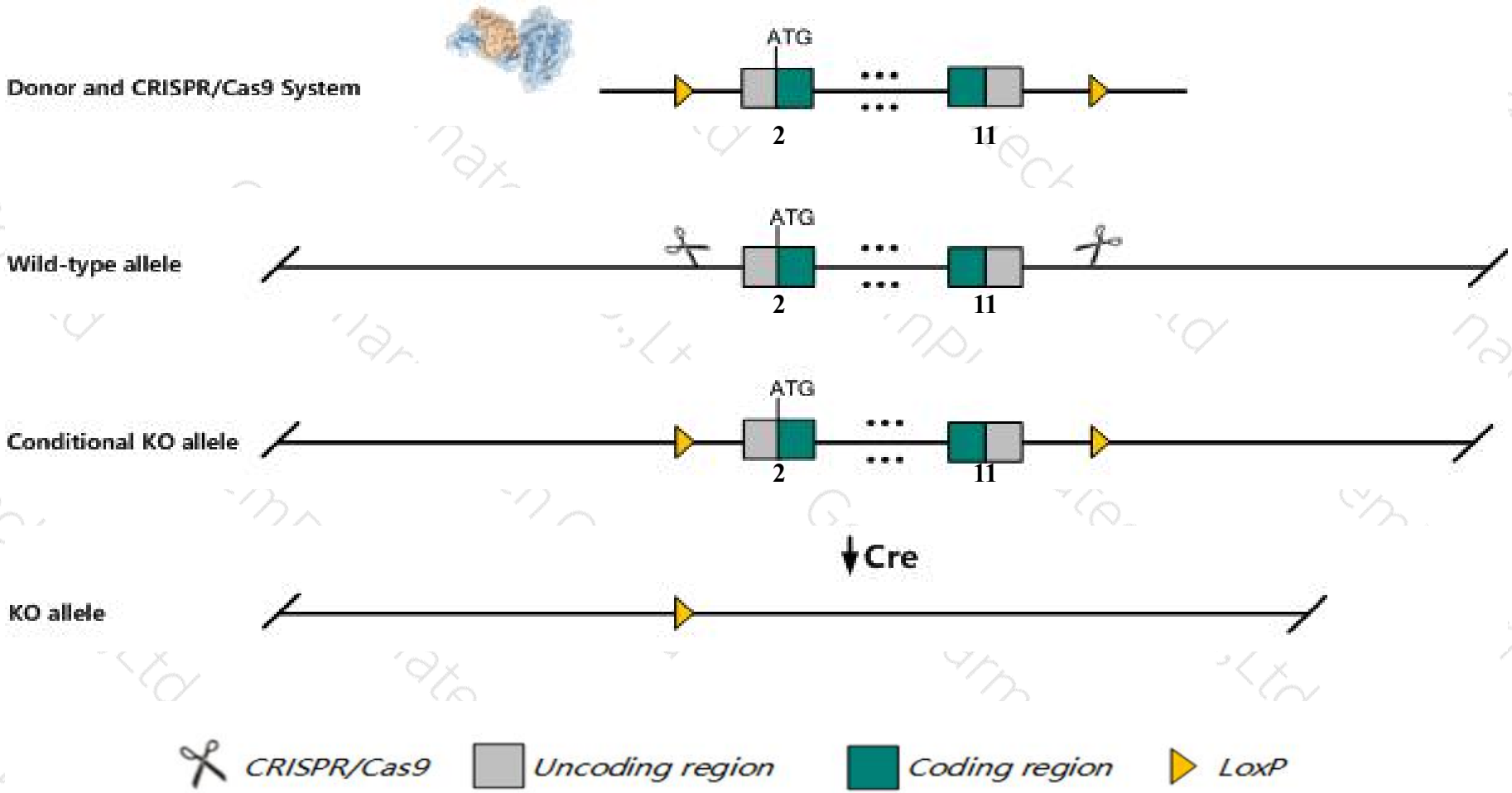
Design Date:2019-8-9

Project Overview

Project Name	***Pax4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pax4* gene. The schematic diagram is as follows:



Technical routes

- The *Pax4* gene has 6 transcripts. According to the structure of *Pax4* gene, exon2-exon11 of *Pax4-201* (ENSMUST00000031718.13) transcript is recommended as the knockout region.The region contains all 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 *Pax4* 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 this targeted mutation lack mature insulin- and somatostatin-producing cells (beta and delta, respectively) in the pancreas, but contain glucagon-producing alpha cells in considerably higher numbers relative to wild-type mice.

➢ The *Pax4* gene is located on the Chr6. 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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Pax4 paired box 4 [Mus musculus (house mouse)]

Gene ID: 18506, updated on 31-Jan-2019

Summary

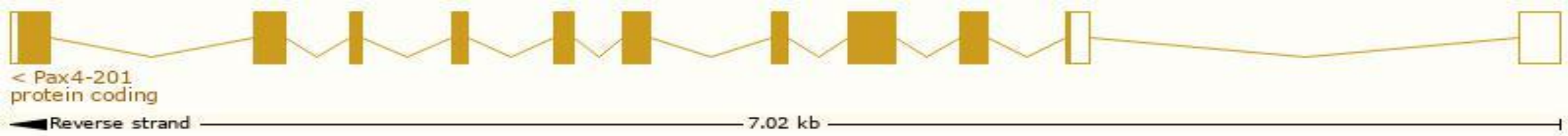
Official Symbol	Pax4 provided by MGI
Official Full Name	paired box 4 provided byMGI
Primary source	MGI:MGI:97488
See related	Ensembl:ENSMUSG00000029706
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Pax-4
Expression	Low expression observed in reference datasetSee more
Orthologs	human all

Transcript information（Ensembl）

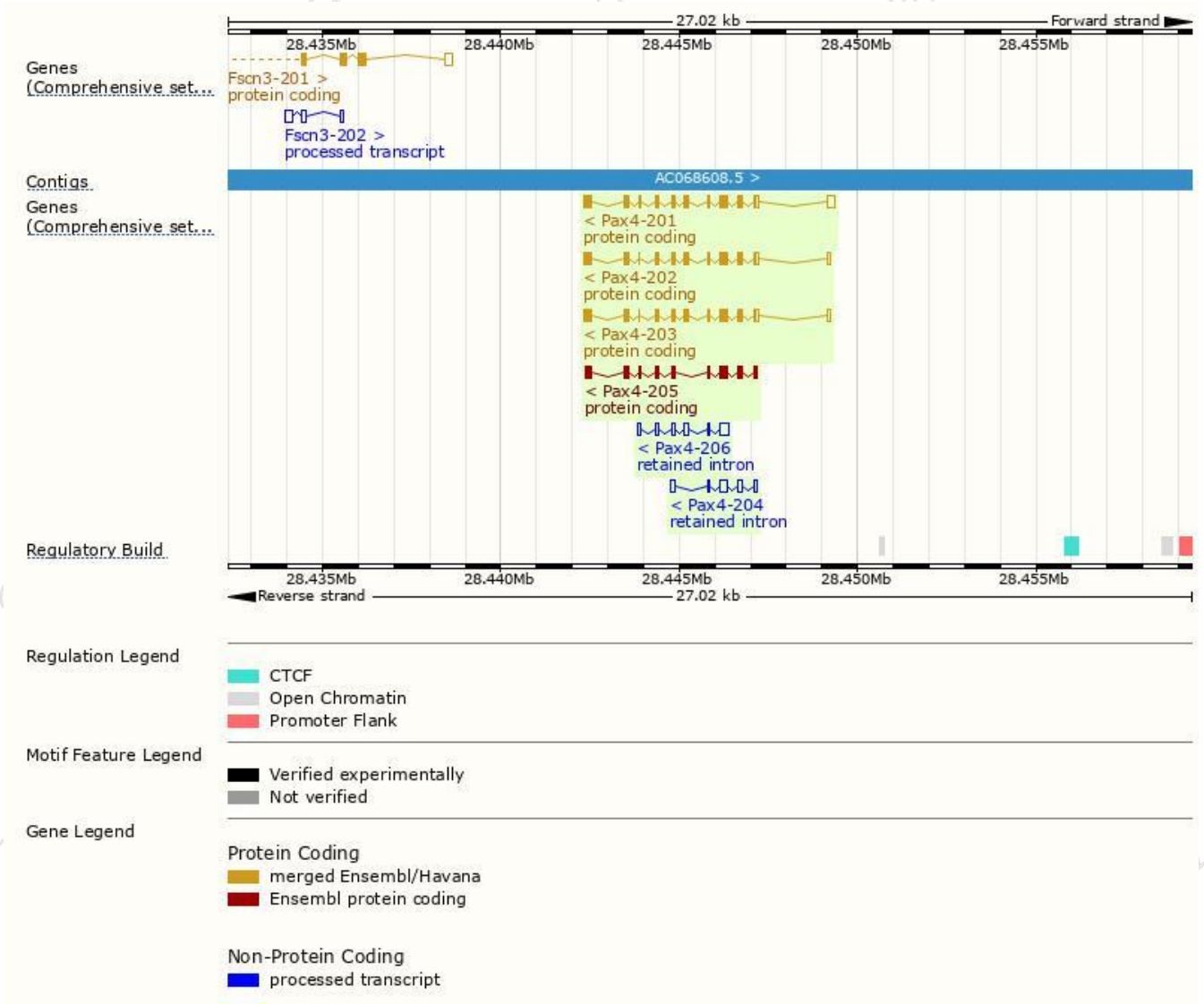
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pax4-201	ENSMUST00000031718.13	1370	349aa	Protein coding	CCDS39447	A2RRY5 P32115	TSL:1 GENCODE basic APPRIS P3
Pax4-202	ENSMUST00000164519.8	1229	336aa	Protein coding	CCDS51732	P32115	TSL:1 GENCODE basic APPRIS ALT2
Pax4-203	ENSMUST00000171089.8	1221	335aa	Protein coding	CCDS51731	B7ZNC8	TSL:1 GENCODE basic APPRIS ALT2
Pax4-205	ENSMUST00000174194.1	974	307aa	Protein coding	-	G3UZE8	TSL:5 GENCODE basic APPRIS ALT2
Pax4-206	ENSMUST00000174423.7	673	No protein	Retained intron	-	-	TSL:5
Pax4-204	ENSMUST00000174036.2	595	No protein	Retained intron	-	-	TSL:5

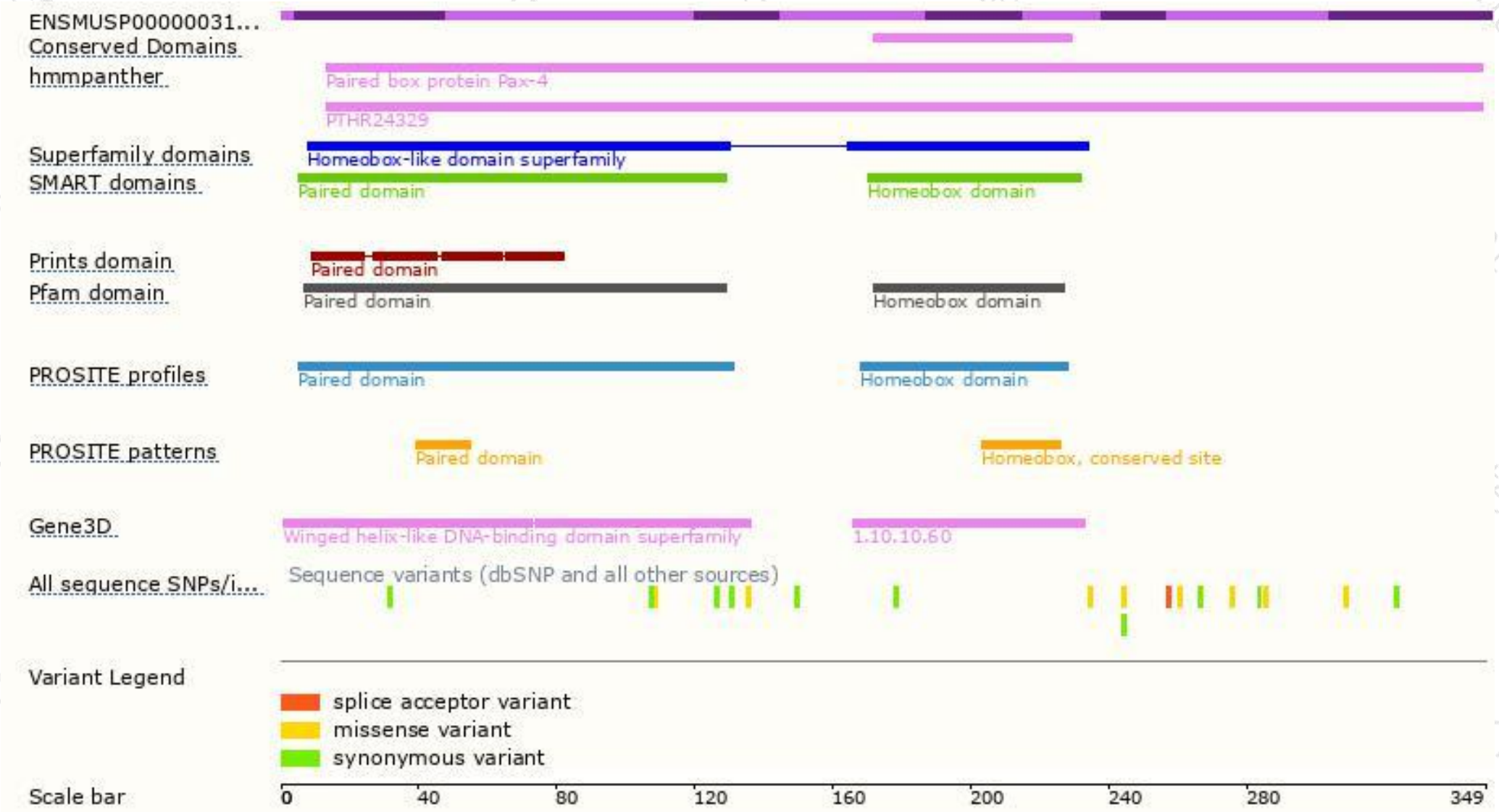
The strategy is based on the design of *Pax4-201* transcript,The transcription is shown below



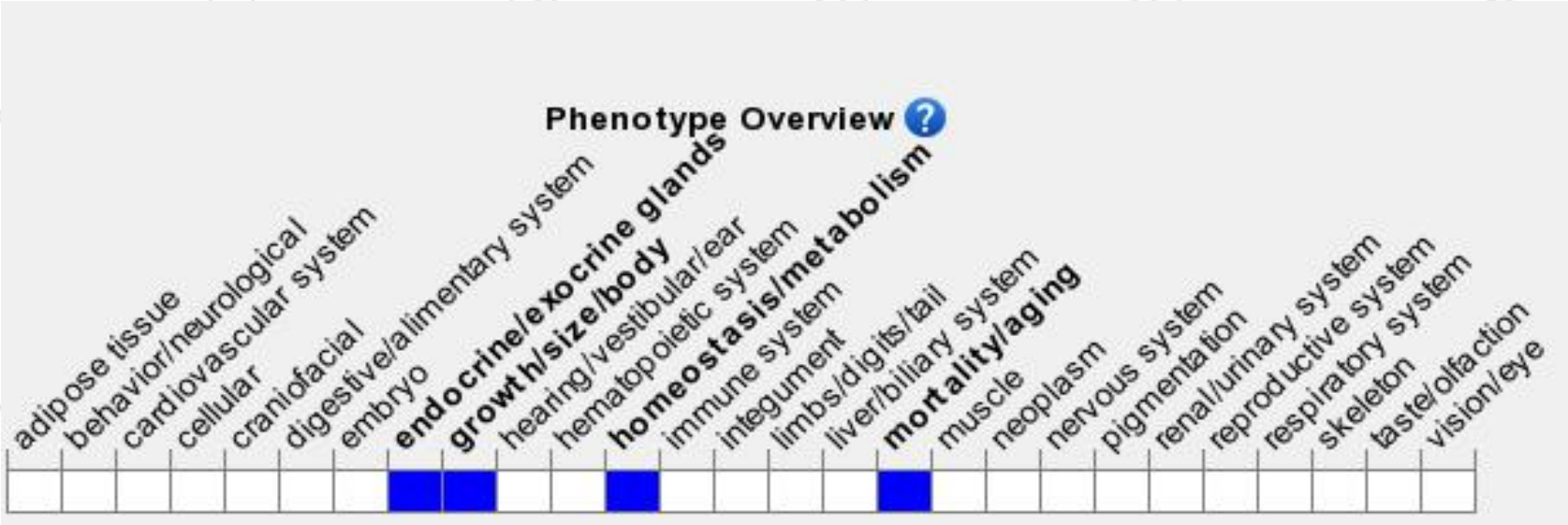
Genomic location distribution



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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