Car4 Cas9-KO Strategy

Designer: Yang Zeng
Reviewer: Ruirui Zhang

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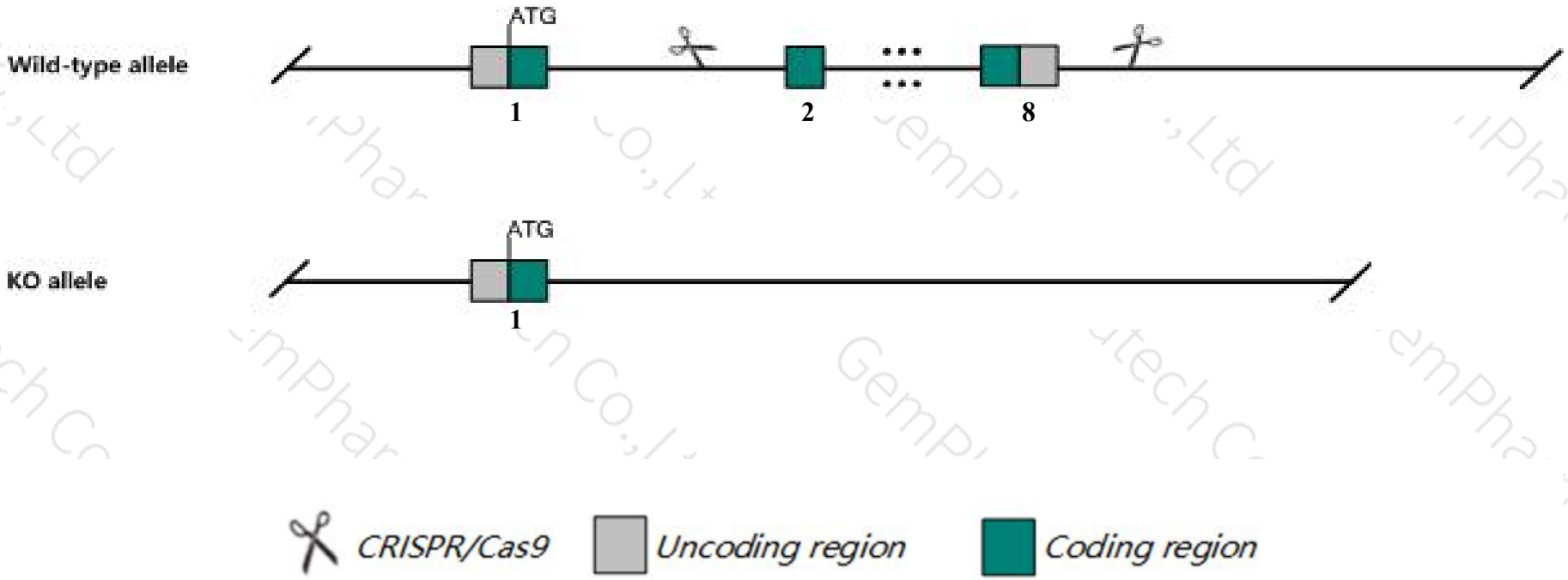
Project Overview

Project Name	***Car4***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Car4* gene. The schematic diagram is as follows:



Technical routes

➢ The *Car4* gene has 6 transcripts. According to the structure of *Car4* gene, exon2-exon8 of *Car4-201* (ENSMUST00000103194.9) transcript is recommended as the knockout region.The region contains most of coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Car4* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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.

Notice

- According to the existing MGI data,Homozygous null mice are produced in lower than expected numbers, with females preferentially lost in the fetal or early postnatal period. Surviving homozygotes are healthy and fertile when crossed with wild-type partners; however, homozygous intercrosses yield small litters and pups do not survive.

- The *Car4* gene is located on the Chr11. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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Car4 carbonic anhydrase 4 [*Mus musculus* (house mouse)]

Gene ID: 12351, updated on 12-Aug-2019

Summary

Official Symbol	Car4 provided by MGI
Official Full Name	carbonic anhydrase 4 provided by MGI
Primary source	MGI:MGI:1096574
See related	Ensembl:ENSMUSG00000000805
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	Ca4; AW456718
Expression	Biased expression in colon adult (RPKM 148.4), placenta adult (RPKM 86.4) and 10 other tissues See more
Orthologs	human all

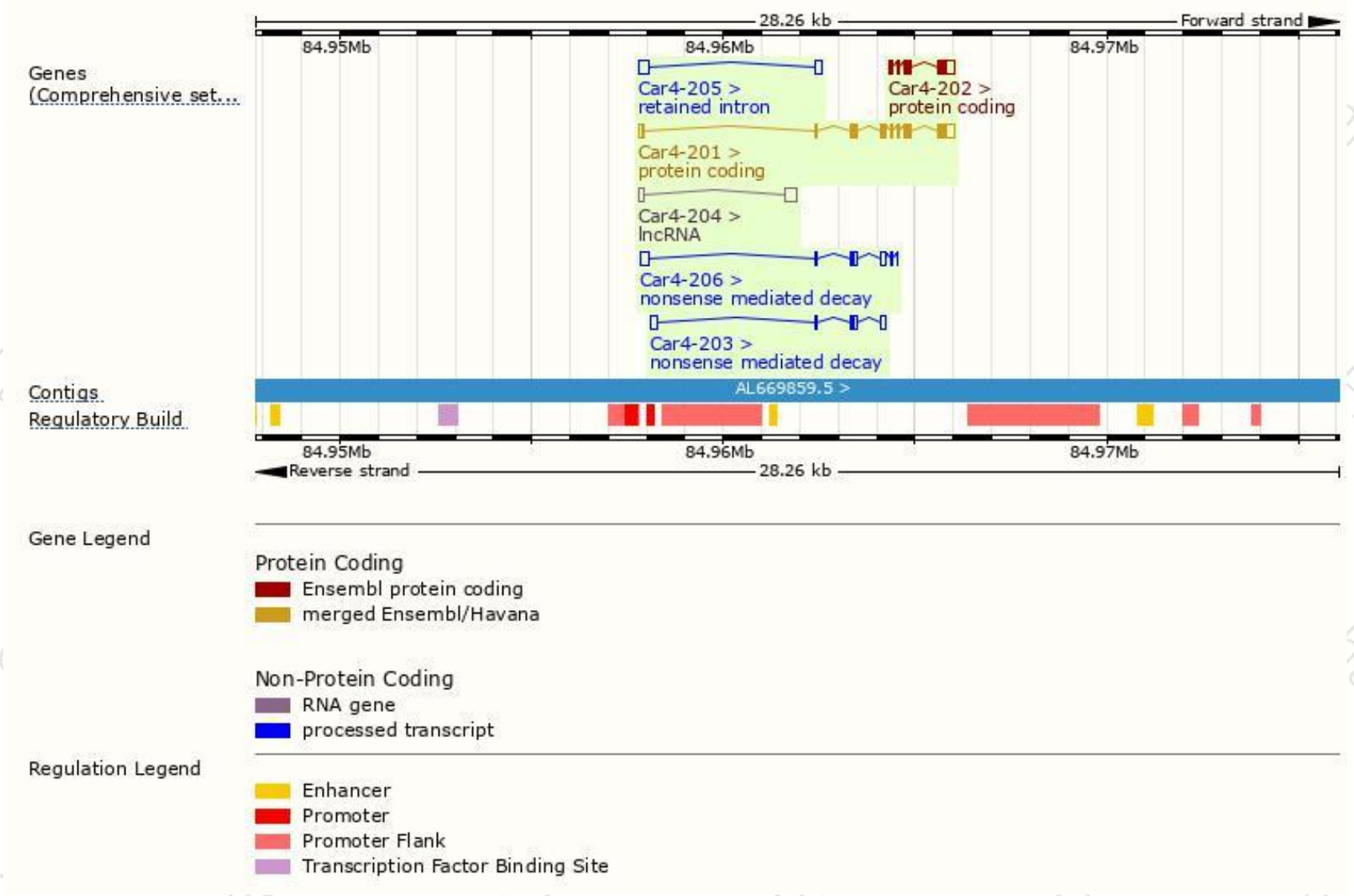
Transcript information（Ensembl）

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

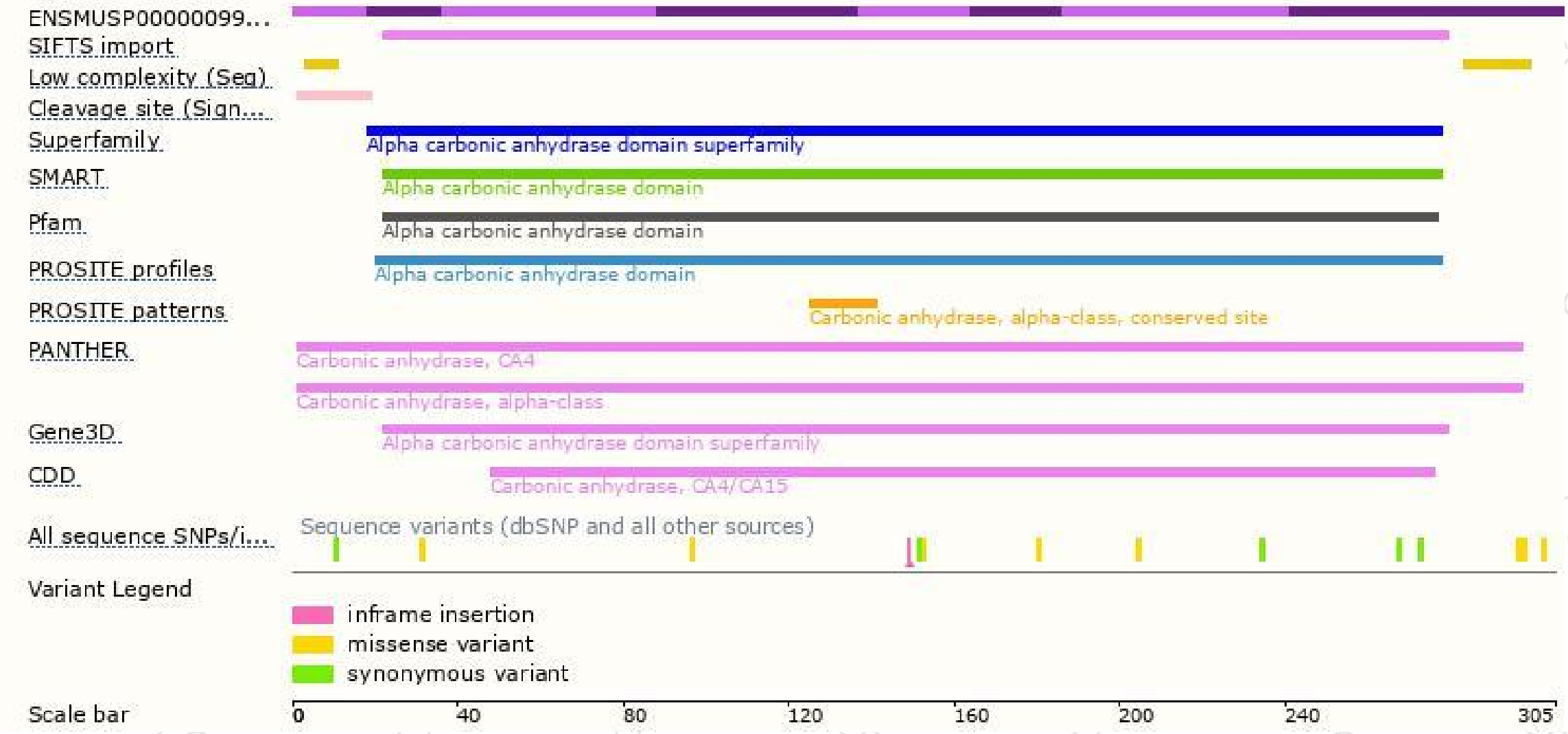
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Car4-201	ENSMUST00000103194.9	1256	305aa	Protein coding	CCDS25190	Q64444	TSL:1 GENCODE basic APPRIS P1
Car4-202	ENSMUST00000108076.2	732	164aa	Protein coding	-	F6ST32	CDS 5' incomplete TSL:3
Car4-206	ENSMUST00000150596.7	692	38aa	Nonsense mediated decay	-	D6RCZ3	TSL:5
Car4-203	ENSMUST00000127827.1	516	38aa	Nonsense mediated decay	-	D6RCZ3	TSL:2
Car4-204	ENSMUST00000138331.1	443	No protein	Processed transcript	-	-	TSL:3
Car4-205	ENSMUST00000139416.1	435	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Car4-201* transcript,The transcription is shown below

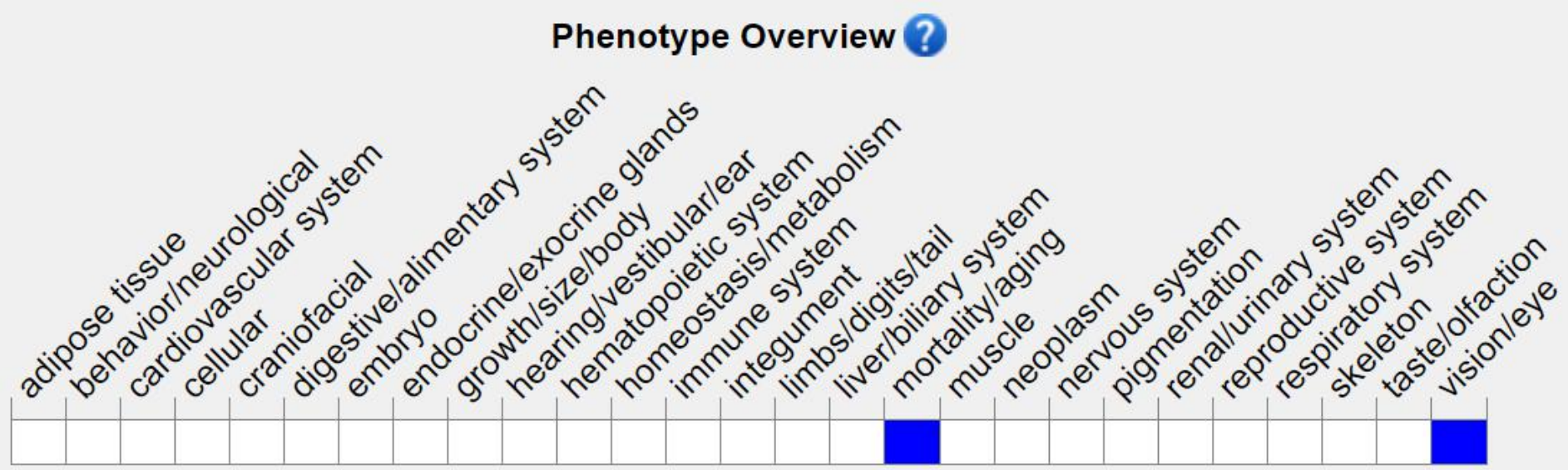
Genomic location distribution



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.

Tel: 400-9660890

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