

***Car12* Cas9-CKO Strategy**

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Project Overview

Project Name

Car12

Project type

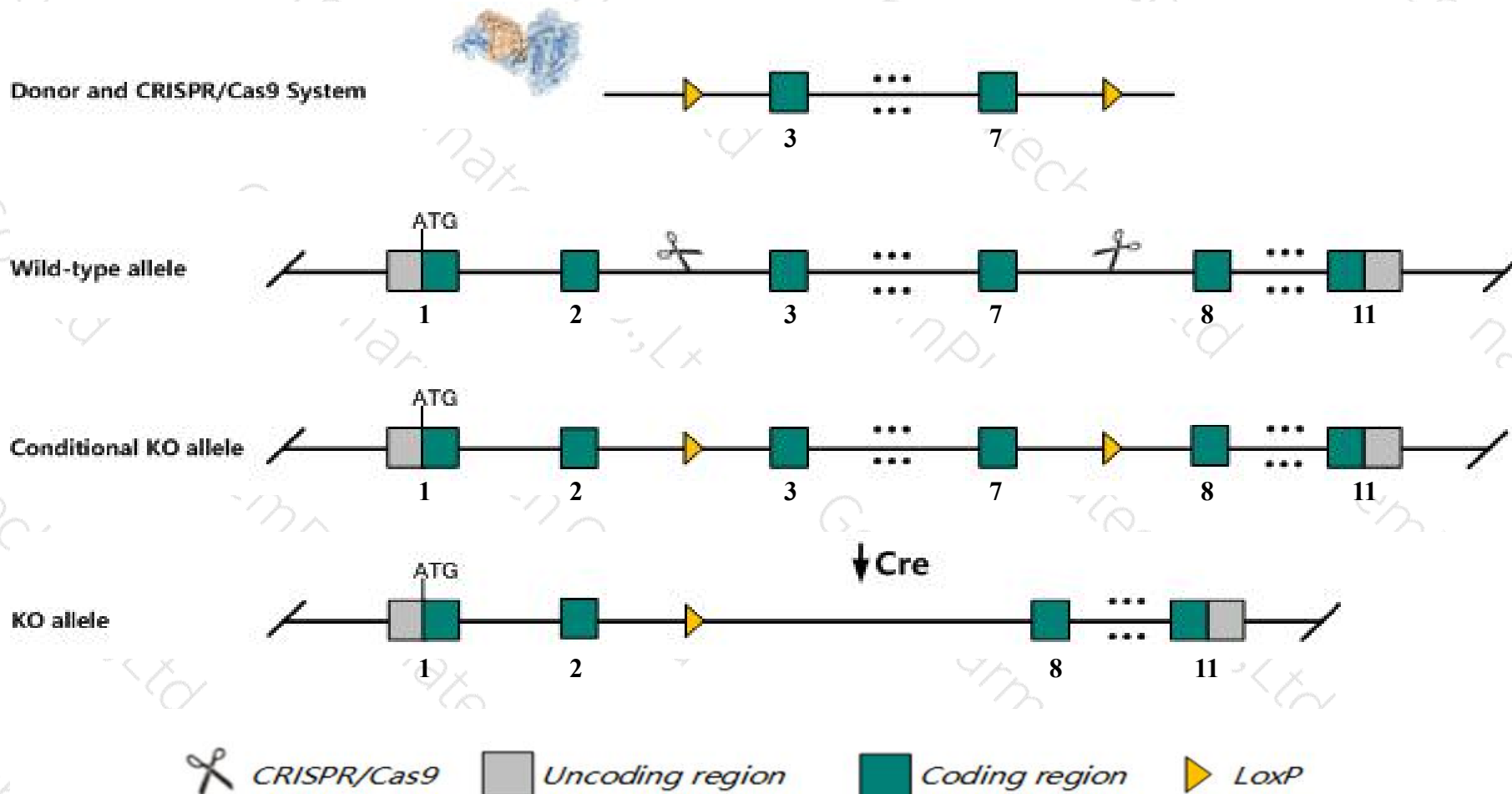
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Car12* gene. The schematic diagram is as follows:



Technical routes

- The *Car12* gene has 6 transcripts. According to the structure of *Car12* gene, exon3-exon7 of *Car12-201* (ENSMUST00000071889.12) transcript is recommended as the knockout region. The region contains 644bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Car12* 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.

- According to the existing MGI data, Mice homozygous for a transposon-induced mutation that inactivates this gene display reduced fitness.
- Transcript *Car12-206* may not be affected.
- The effect on transcript *Car12-204* is unknown.
- The *Car12* gene is located on the Chr9. 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)



Car12 carbonic anhydrase 12 [*Mus musculus* (house mouse)]

Gene ID: 76459, updated on 17-Dec-2019

Summary

- Official Symbol

Car12 provided by MGI
- Official Full Name

carbonic anhydrase 12 provided by MGI
- Primary source

MGI:MGI:1923709
- See related

Ensembl:ENSMUSG00000032373
- Gene type

protein coding
- RefSeq status

REVIEWED
- Organism

Mus musculus
- Lineage

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
- Also known as

Ca12; CA-XII; AI314958; 2310047E01Rik
- Summary

This gene encodes a membrane-bound member of the alpha carbonic anhydrase family of enzymes that catalyze the reversible hydration of carbon dioxide to bicarbonate. These proteins participate in a variety of biological processes, including respiration, calcification, acid-base balance, bone resorption, and the formation of aqueous humor, cerebrospinal fluid, saliva, and gastric acid. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Apr 2015]
- Expression

Biased expression in kidney adult (RPKM 57.2), limb E14.5 (RPKM 7.3) and 5 other tissues [See more](#)
- Orthologs

[human](#) [all](#)

Genomic context

Location: 9; 9 C

Exon count: 11

See Car12 in [Genome Data Viewer](#)

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	9	NC_000075.6 (66712296..66766845)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	9	NC_000075.5 (66561493..66614652)

Transcript information (Ensembl)

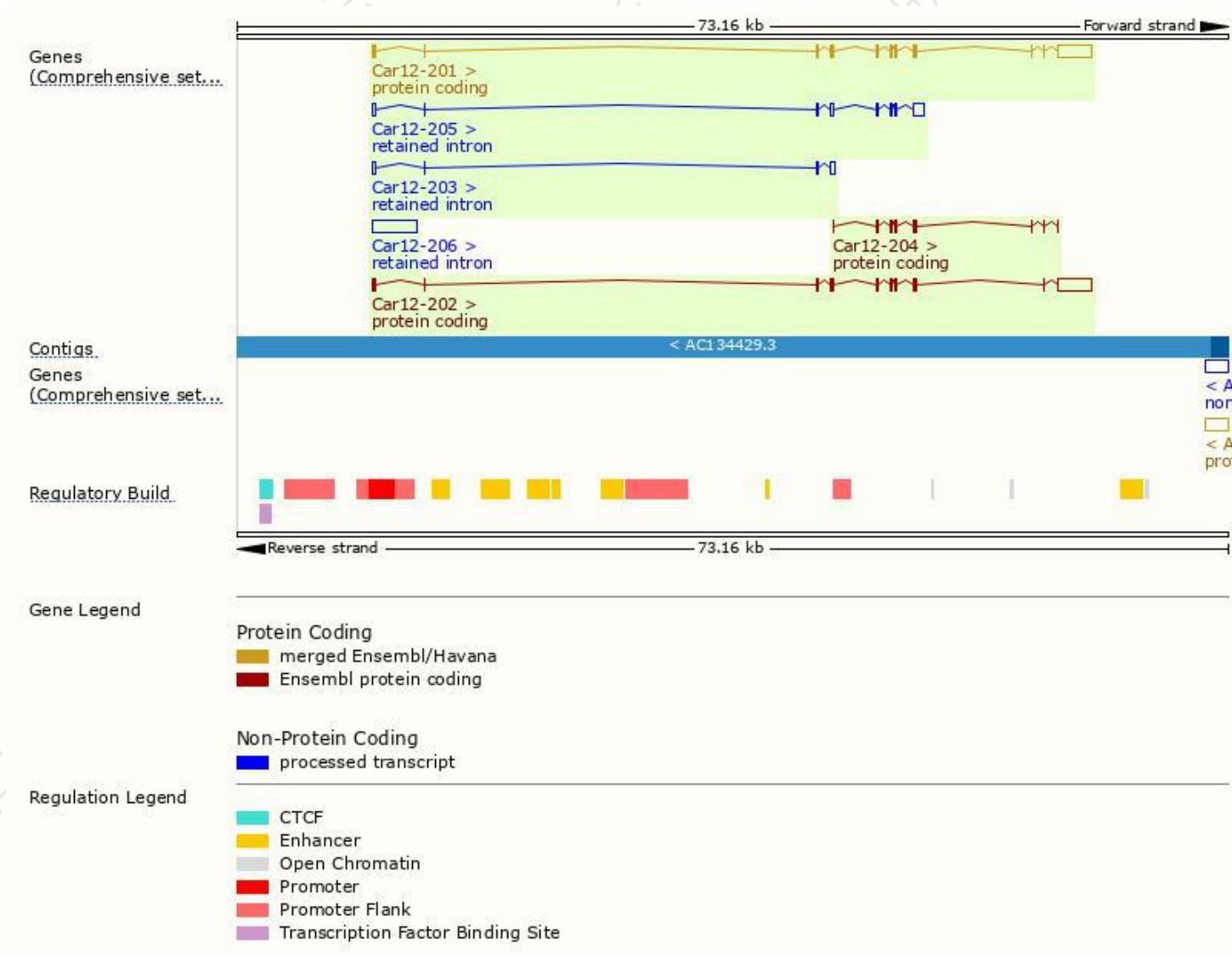
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Car12-201	ENSMUST00000071889.12	3716	354aa	Protein coding	CCDS23306	A0A0R4J0W4	TSL:1 GENCODE basic APPRIS P3
Car12-202	ENSMUST00000085420.11	3639	344aa	Protein coding	CCDS81026	Q8K2J1	TSL:1 GENCODE basic APPRIS ALT2
Car12-204	ENSMUST00000134829.1	614	204aa	Protein coding	-	F6W0I8	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Car12-206	ENSMUST00000217394.1	3263	No protein	Retained intron	-	-	TSL:NA
Car12-205	ENSMUST00000152011.7	1652	No protein	Retained intron	-	-	TSL:2
Car12-203	ENSMUST00000123195.1	710	No protein	Retained intron	-	-	TSL:2

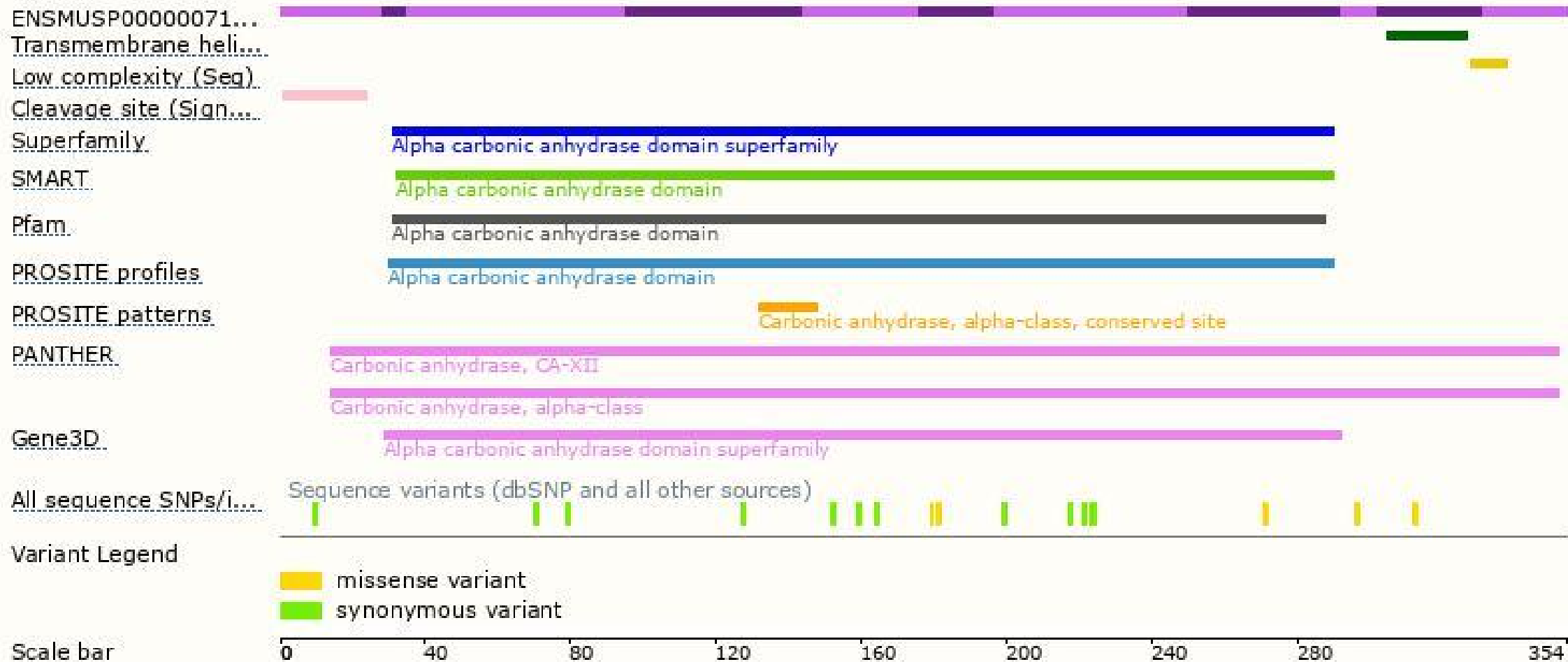
The strategy is based on the design of *Car12-201* transcript,The transcription is shown below



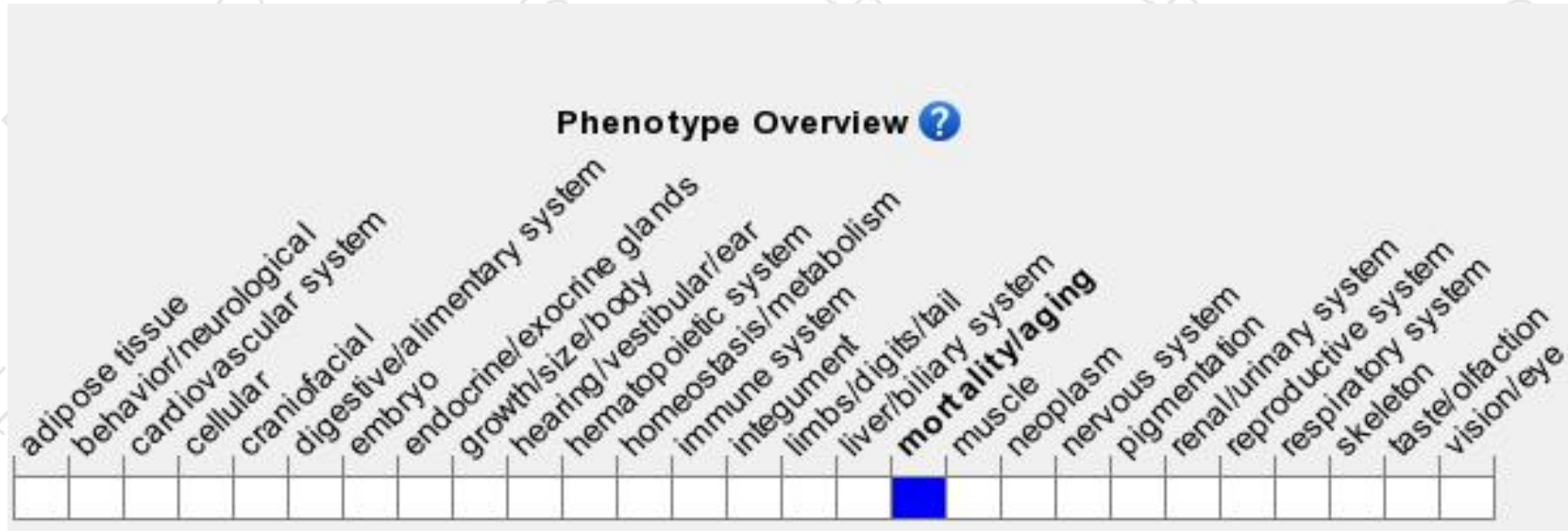
Genomic location distribution



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, Mice homozygous for a transposon-induced mutation that inactivates this gene display reduced fitness.

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

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