

Car3 Cas9-CKO Strategy

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Project Overview

Project Name

Car3

Project type

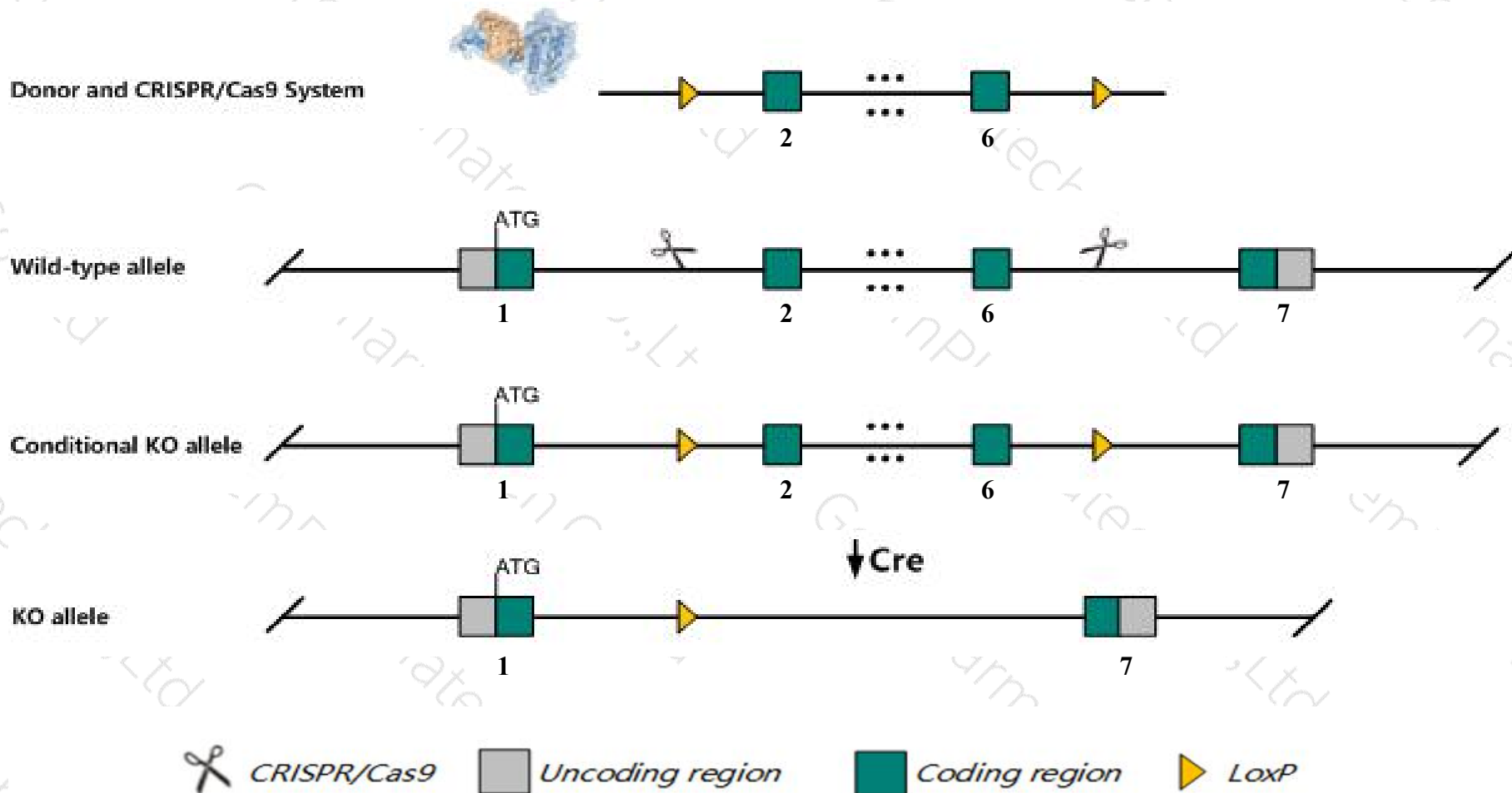
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Car3* gene. The schematic diagram is as follows:



- The *Car3* gene has 3 transcripts. According to the structure of *Car3* gene, exon2-exon6 of *Car3-201* (ENSMUST00000029076.5) transcript is recommended as the knockout region. The region contains 629bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Car3* 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 knock-out allele are viable and fertile and exhibit a normal life span and a normal response to hyperoxic challenge. Mutant muscles display shorter half-relaxation times for both single and tetanic twitches but show normal fatigability.
- The *Car3* gene is located on the Chr3. 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)

Car3 carbonic anhydrase 3 [*Mus musculus* (house mouse)]

Gene ID: 12350, updated on 17-Dec-2019

Summary

- Official Symbol** Car3 provided by [MGI](#)
- Official Full Name** carbonic anhydrase 3 provided by [MGI](#)
- Primary source** [MGI:MGI:88270](#)
- See related** [Ensembl:ENSMUSG00000027559](#)
- Gene type** protein coding
- RefSeq status** PROVISIONAL
- Organism** [Mus musculus](#)
- Lineage** Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
- Also known as** Ca3; Car-3; BB219044
- Expression** Biased expression in subcutaneous fat pad adult (RPKM 4525.3), mammary gland adult (RPKM 3129.6) and 3 other tissues
[See more](#)
- Orthologs** [human](#) [all](#)

Genomic context

Location: 3 A1; 3 3.22 cM

See Car3 in [Genome Data Viewer](#)

Exon count: 7

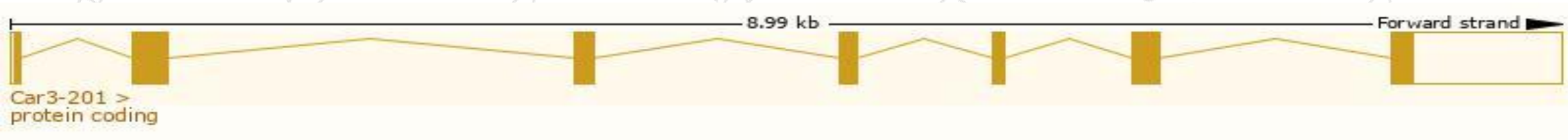
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	3	NC_000069.6 (14863538..14872381)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	3	NC_000069.5 (14863538..14872381)

Transcript information (Ensembl)

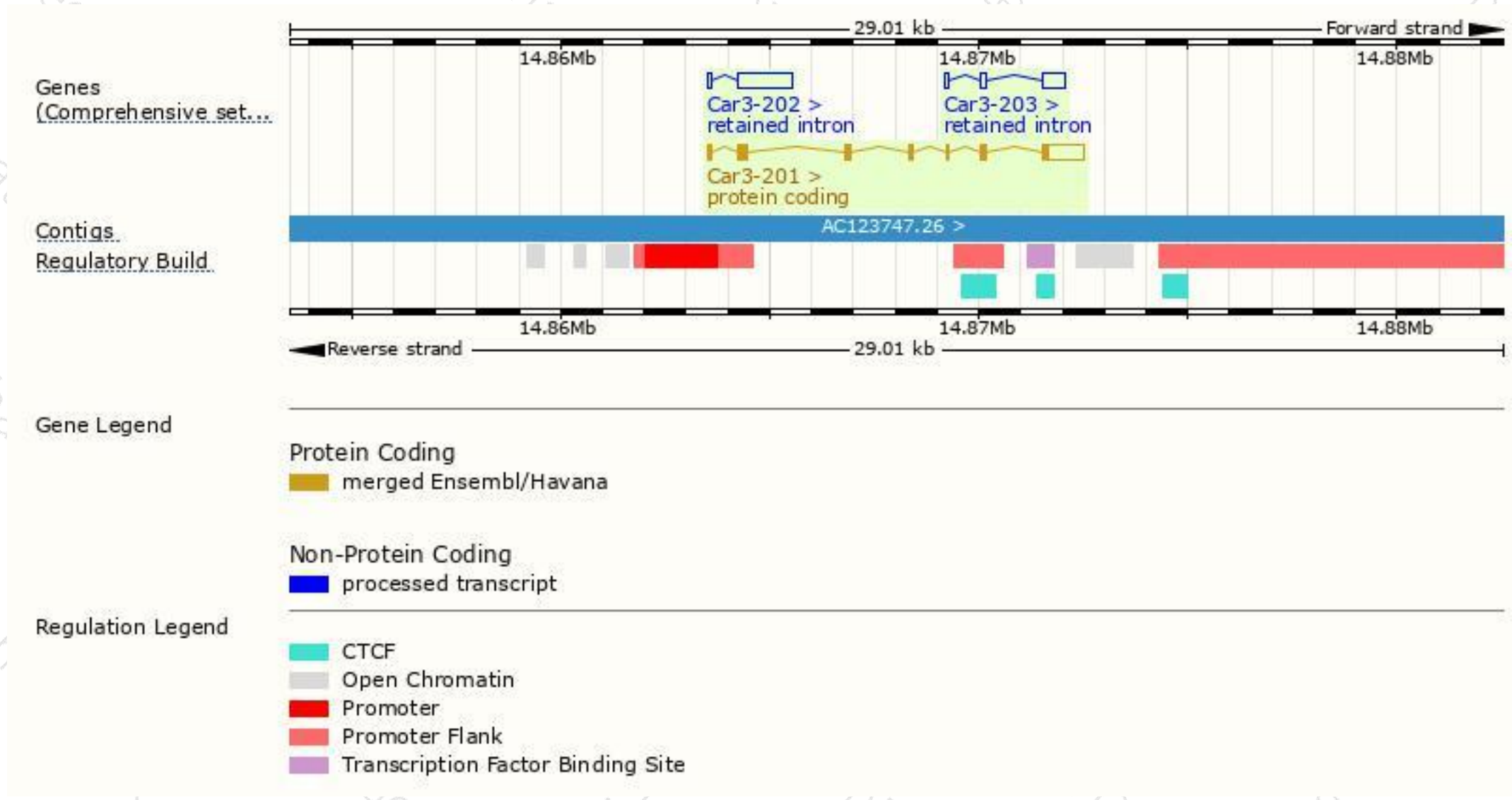
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Car3-201	ENSMUST00000029076.5	1679	260aa	Protein coding	CCDS17250	P16015	TSL:1 GENCODE basic APPRIS P1
Car3-202	ENSMUST00000195575.1	1395	No protein	Retained intron	-	-	TSL:1
Car3-203	ENSMUST00000195834.1	804	No protein	Retained intron	-	-	TSL:2

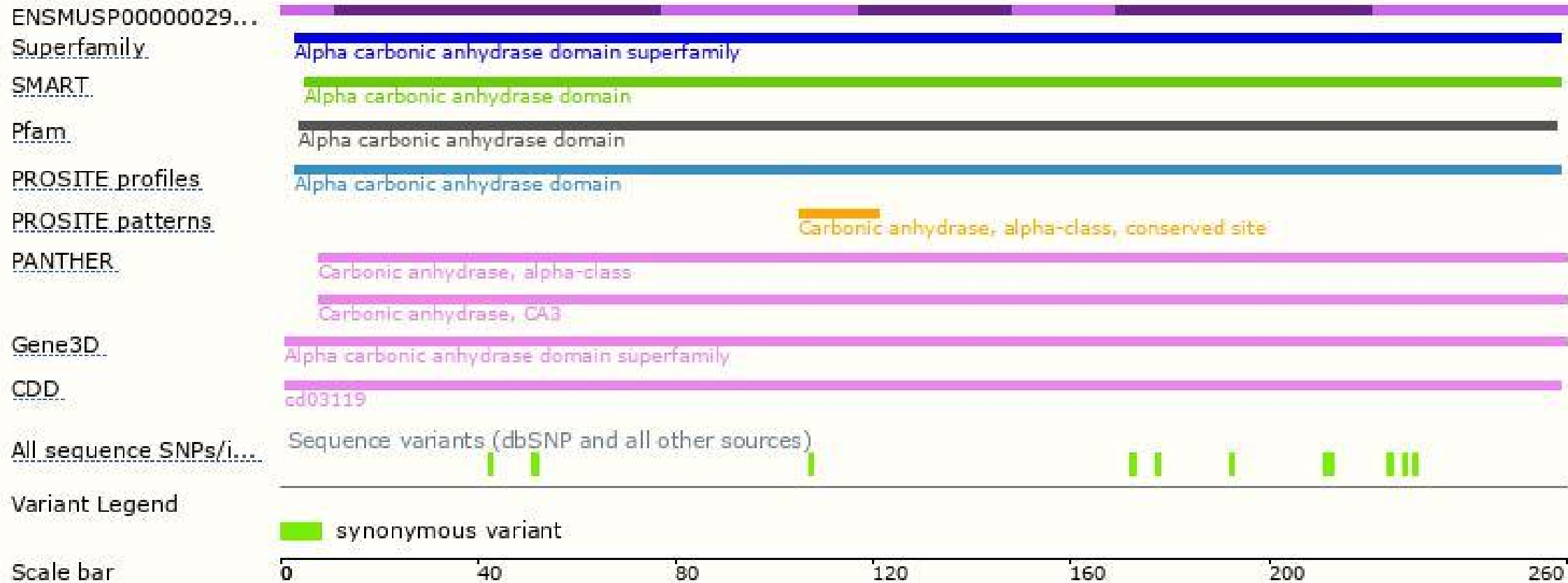
The strategy is based on the design of *Car3-201* transcript,The transcription is shown below



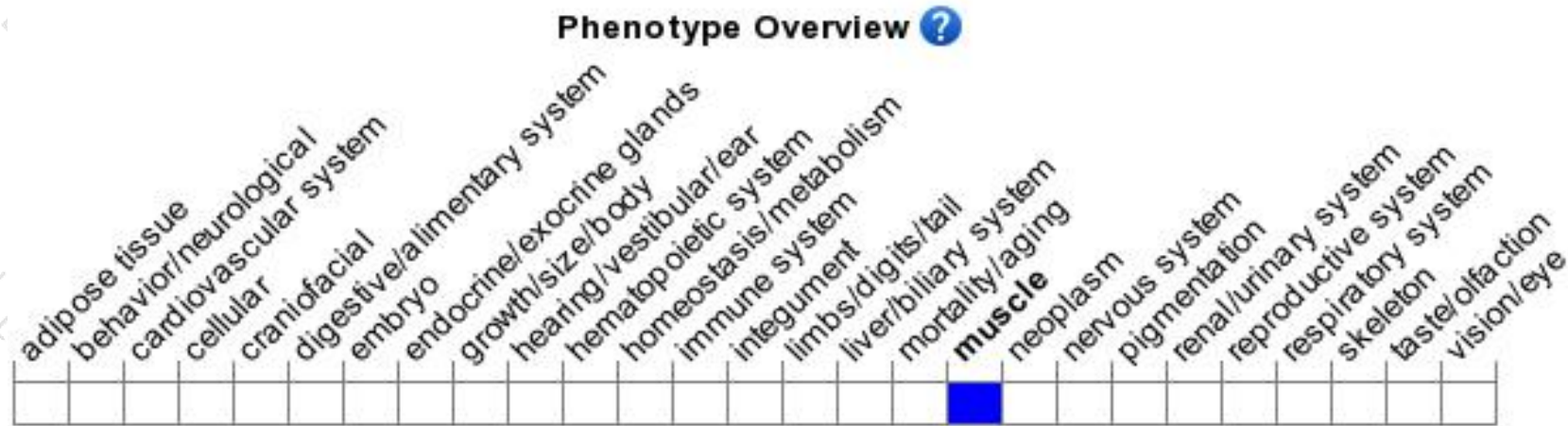
Genomic location distribution



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 knock-out allele are viable and fertile and exhibit a normal life span and a normal response to hyperoxic challenge. Mutant muscles display shorter half-relaxation times for both single and tetanic twitches but show normal fatigability.

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

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