

Mylk3 Cas9-CKO Strategy

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Reviewer:

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Project Overview

Project Name

Mylk3

Project type

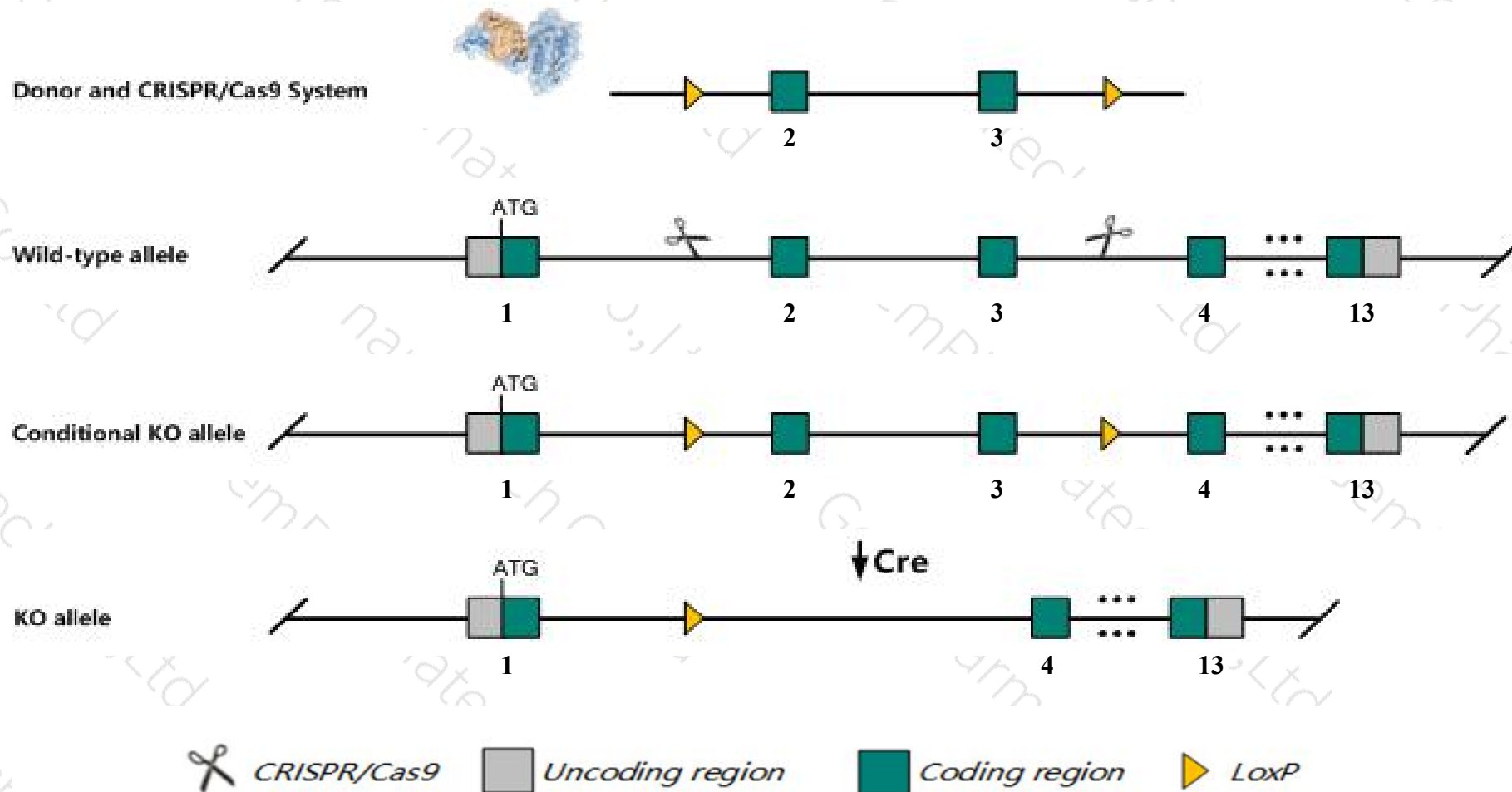
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mylk3* gene. The schematic diagram is as follows:



Technical routes

- The *Mylk3* gene has 5 transcripts. According to the structure of *Mylk3* gene, exon2-exon3 of *Mylk3*-201 (ENSMUST00000034133.13) transcript is recommended as the knockout region. The region contains 503bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Mylk3* 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 exhibit cardiac hypertrophy, reduced cardiac function and increased response of heart to induced stress.
- The *Mylk3* gene is located on the Chr8. 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)

Mylk3 myosin light chain kinase 3 [Mus musculus (house mouse)]

Gene ID: 213435, updated on 13-Mar-2020

Summary



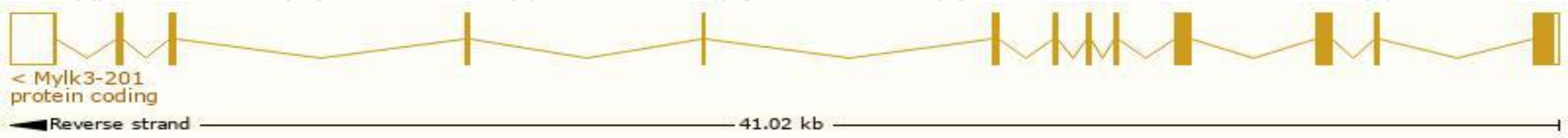
Official Symbol	Mylk3 provided by MGI
Official Full Name	myosin light chain kinase 3 provided by MGI
Primary source	MGI:MGI:2443063
See related	Ensembl:ENSMUSG000000031698
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	D830007F02Rik, MLCK
Expression	Biased expression in heart adult (RPKM 54.9), liver E14 (RPKM 12.4) and 2 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

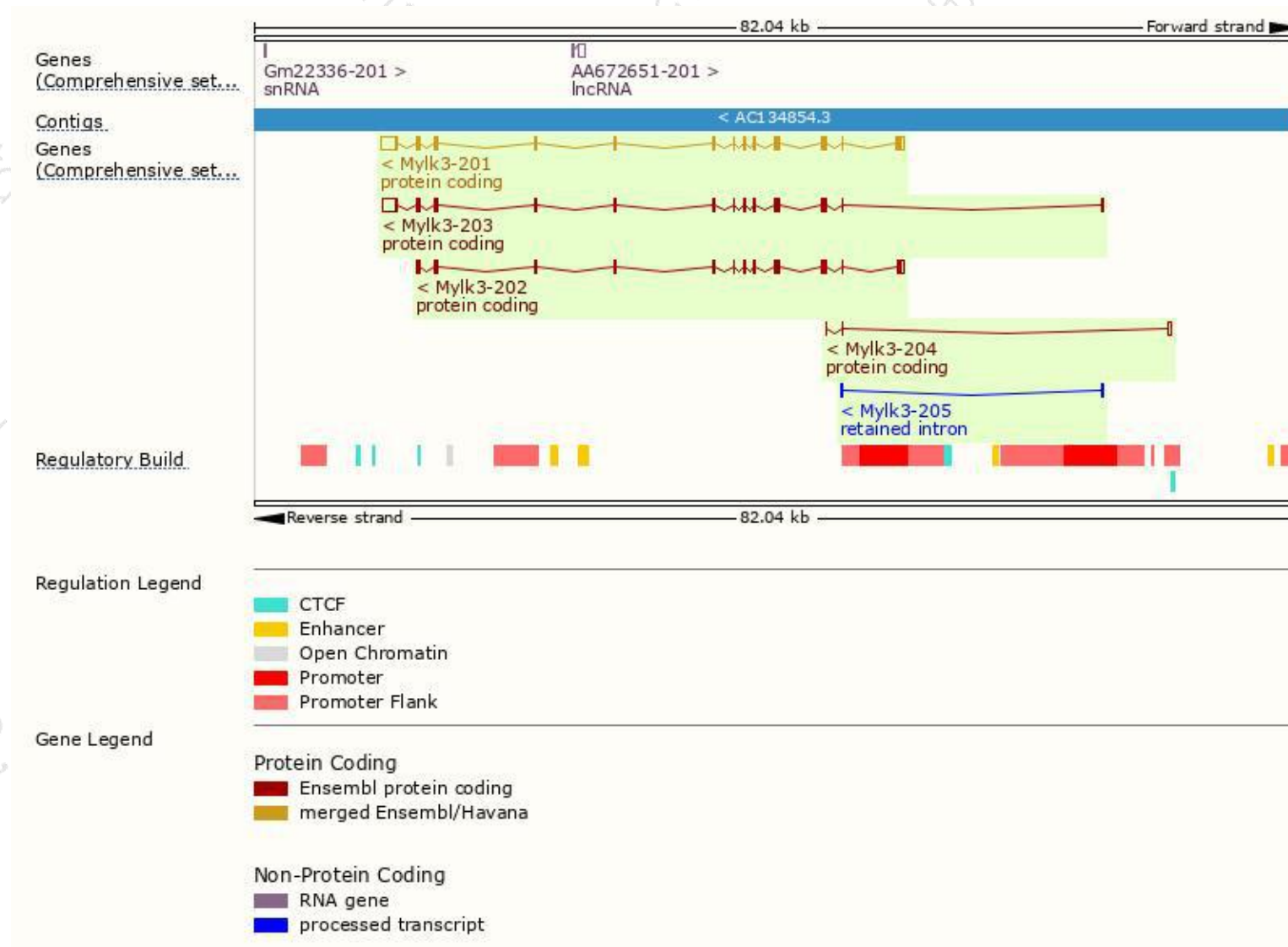
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mylk3-201	ENSMUST00000034133.13	3696	795aa	Protein coding	CCDS40422	Q3UIZ8	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Mylk3-203	ENSMUST00000122452.7	3036	662aa	Protein coding	CCDS80910	D3Z630	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Mylk3-202	ENSMUST00000121972.7	2407	724aa	Protein coding	-	Q3UIZ8	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Mylk3-204	ENSMUST00000137290.1	408	58aa	Protein coding	-	D3YZQ0	CDS 3' incomplete TSL:3
Mylk3-205	ENSMUST00000138825.1	325	No protein	Retained intron	-	-	TSL:2

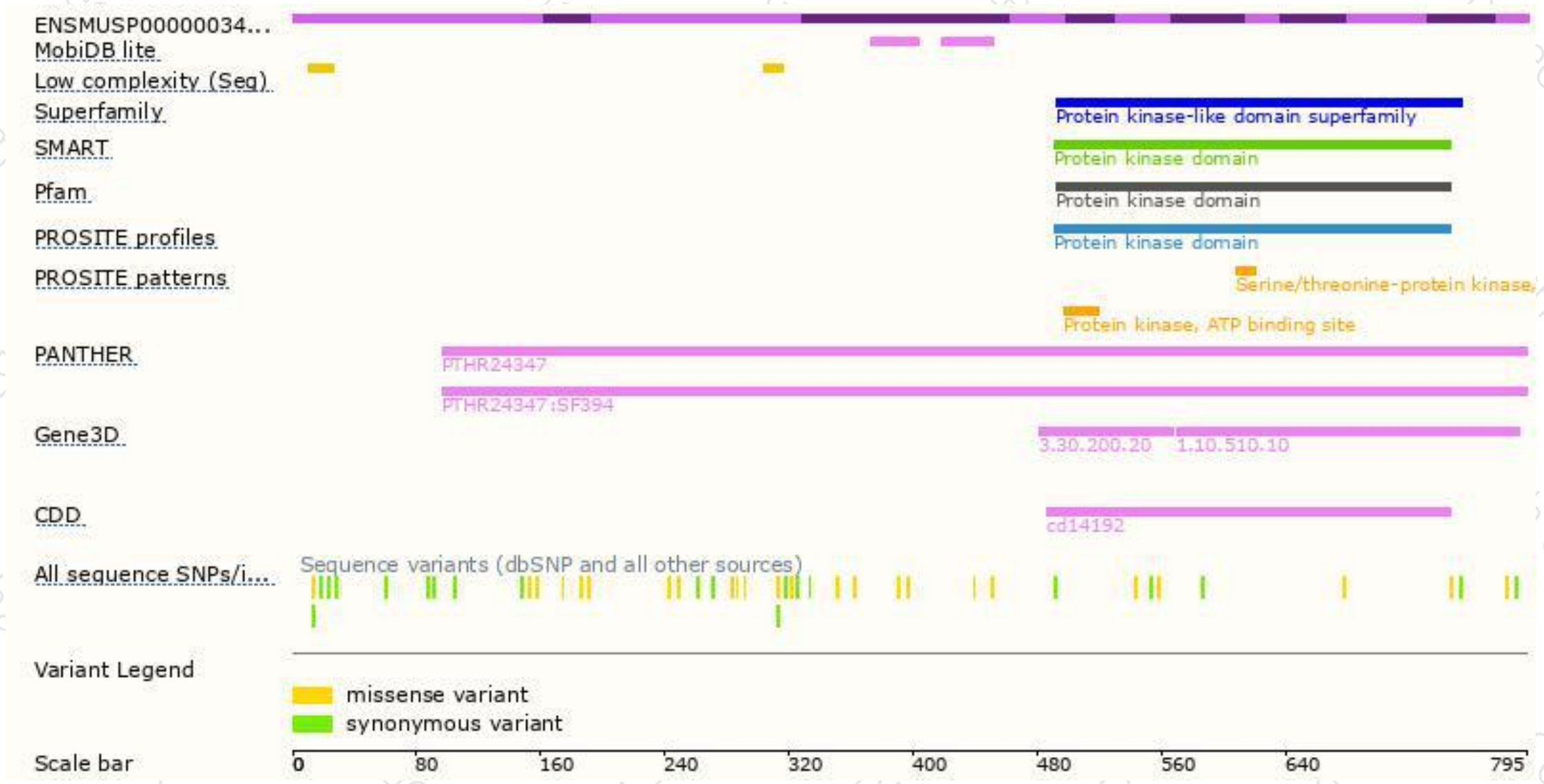
The strategy is based on the design of *Mylk3-201* transcript,the transcription is shown below:



Genomic location distribution

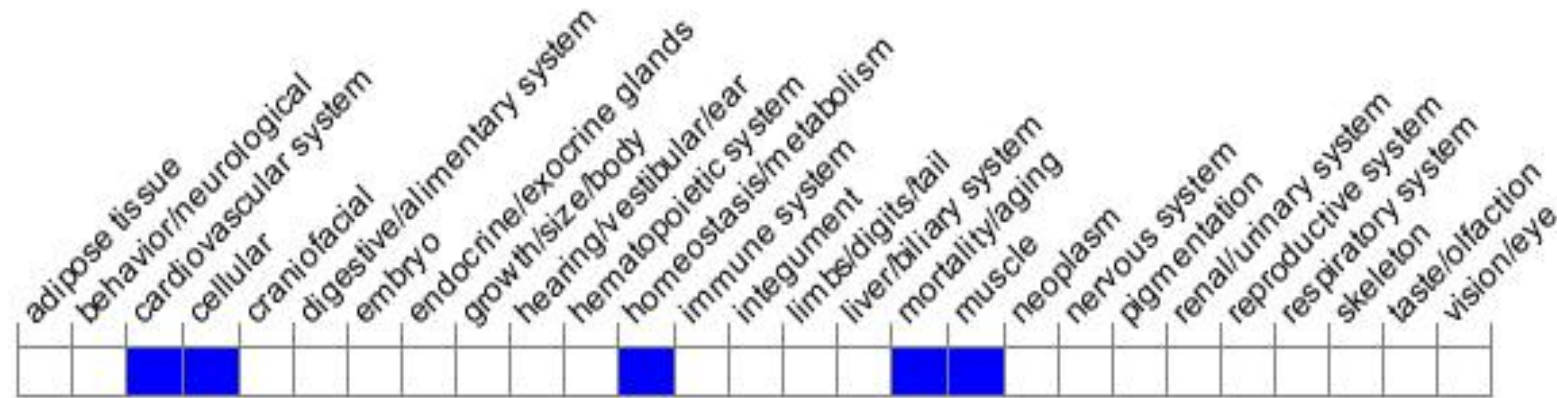


Protein domain



Mouse phenotype description(MGI)

Phenotype Overview



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 exhibit cardiac hypertrophy, reduced cardiac function and increased response of heart to induced stress.

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

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