Myof Cas9-CKO Strategy

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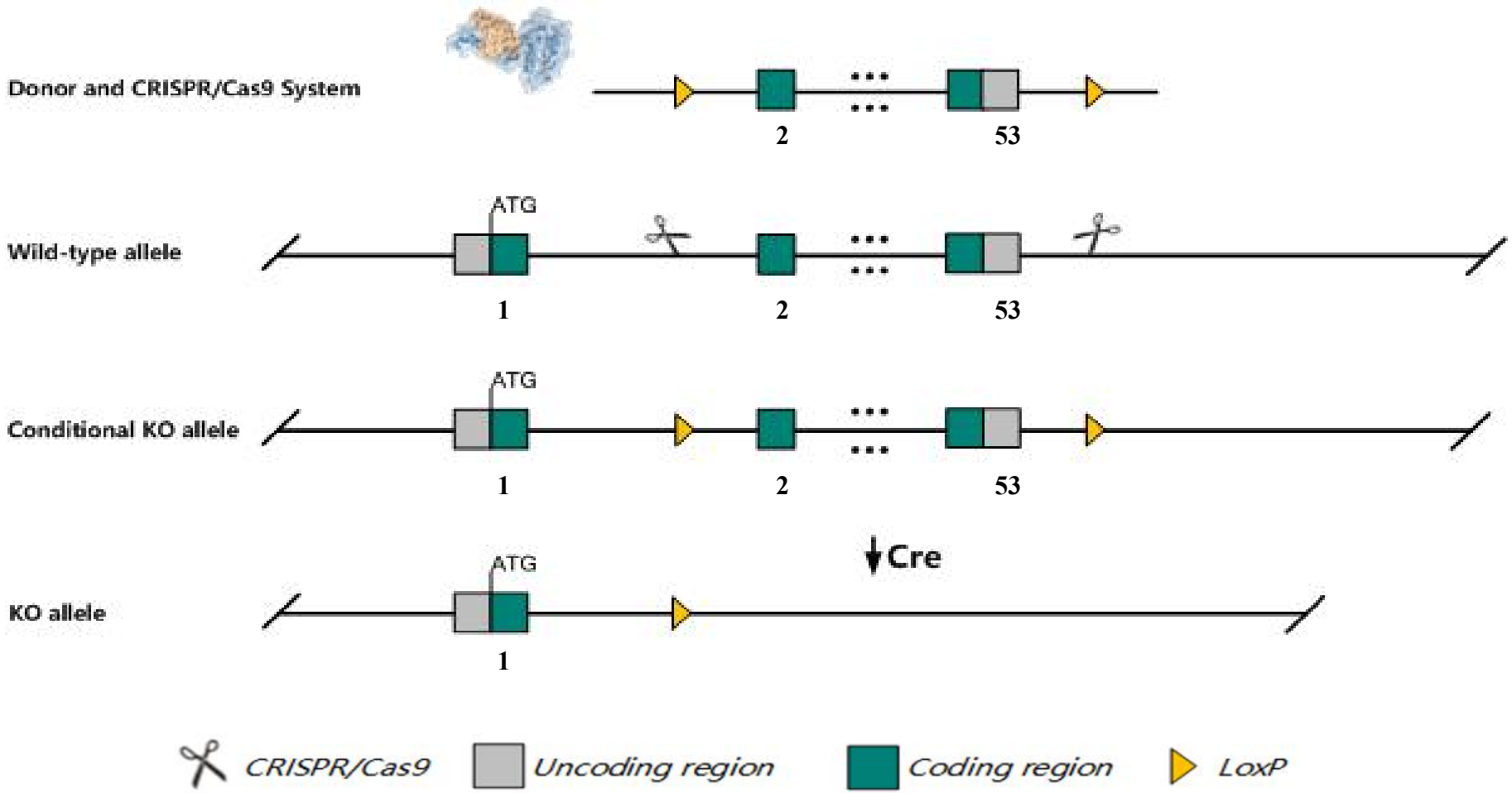
Project Overview

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

Conditional Knockout strategy

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



Technical routes

The *Myof* gene has 12 transcripts. According to the structure of *Myof* gene, exon2-exon53 of *Myof-201*(ENSMUST00000041475.15) transcript is recommended as the knockout region.The region contains 6059bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Myof* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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 a knock-out allele exhibit decreased body size, impaired myogenesis, lack of large diameter myofibers, abnormal skeletal muscle regeneration after injury, and decreased vascular permeability.

The *Myof* gene is located on the Chr19. 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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Myof myoferlin [Mus musculus (house mouse)]

Gene ID: 226101, updated on 13-Mar-2020

Summary

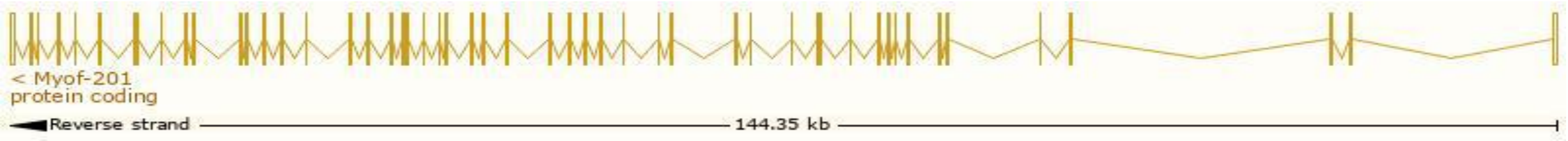
Official Symbol	Myof provided by MGI
Official Full Name	myoferlin provided by MGI
Primary source	MGI:MGI:1919192
See related	Ensembl:ENSMUSG00000048612
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2310004N10Rik, 2310051D19Rik, E030042N20Rik, Fer1, Fer1l3
Summary	The protein encoded by this gene is a member of the ferlin family of proteins, which have been implicated in fusion events in muscle tissue. Members of this family have a carboxy-terminal single pass transmembrane domain and multiple C2 domains, which bind negatively charged phospholipids in the presence of calcium ions. This gene is expressed at high levels in myoblasts and upregulated in damaged skeletal muscle. Mice deficient in this protein display defects in myoblast fusion, muscle regeneration, and angiogenesis. Alternative splicing results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Oct 2014]
Expression	Biased expression in bladder adult (RPKM 56.9), placenta adult (RPKM 16.0) and 7 other tissuesSee more
Orthologs	human all

Transcript information Ensembl

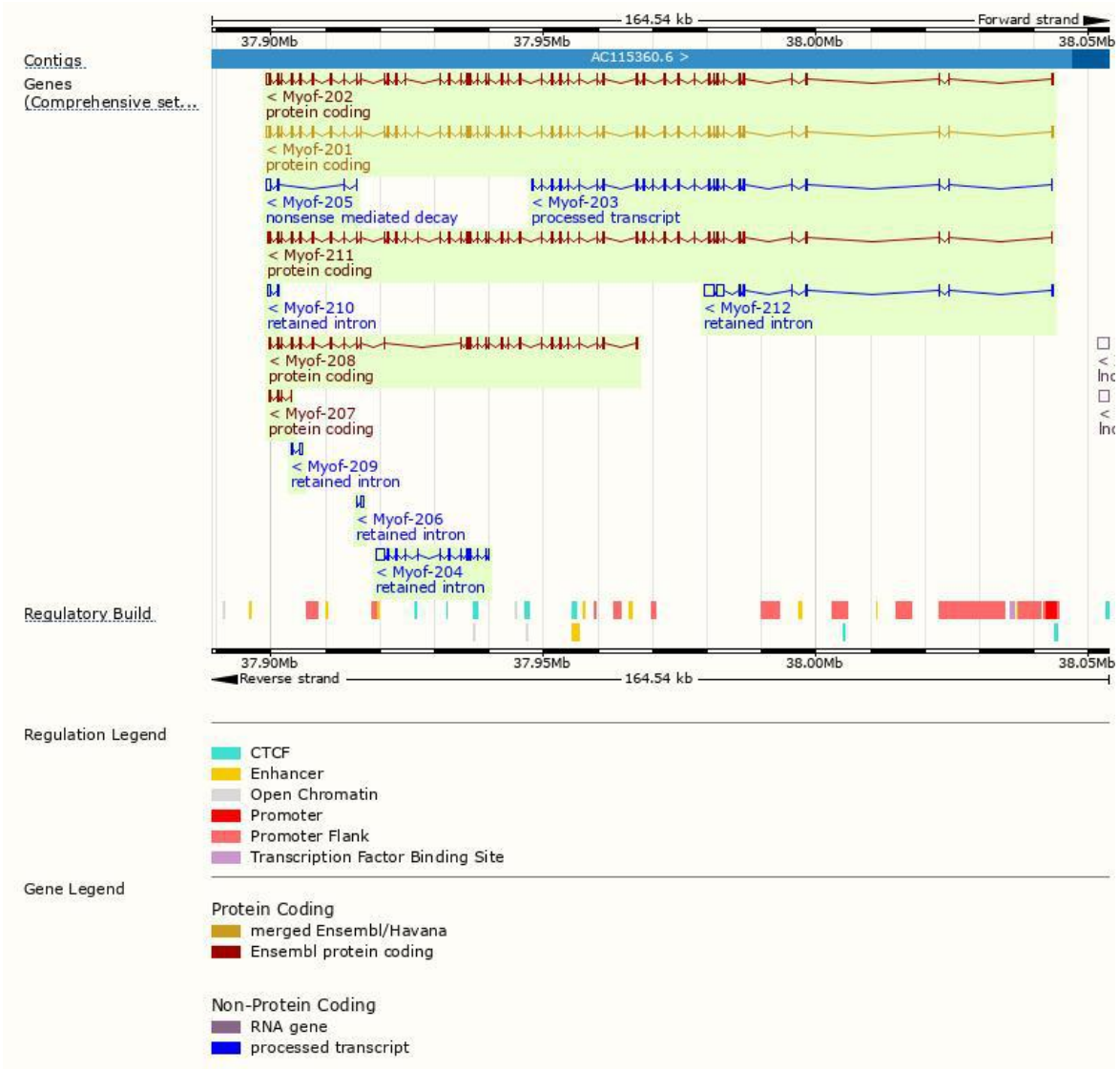
The gene has 12 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Myof-201	ENSMUST00000041475.15	6902	2048aa	Protein coding	CCDS37970	Q69ZN7	TSL:2 GENCODE basic APPRIS P2
Myof-202	ENSMUST00000172095.2	7094	2048aa	Protein coding	-	E9Q390	TSL:5 GENCODE basic
Myof-211	ENSMUST00000226068.1	6633	2061aa	Protein coding	-	A0A286YDF5	GENCODE basic APPRIS ALT1
Myof-208	ENSMUST00000225159.1	4129	1307aa	Protein coding	-	A0A286YCZ3	CDS 5' incomplete
Myof-207	ENSMUST00000224900.1	631	186aa	Protein coding	-	A0A286YDV5	CDS 5' incomplete
Myof-205	ENSMUST00000224560.1	863	85aa	Nonsense mediated decay	-	A0A286YE65	CDS 5' incomplete
Myof-203	ENSMUST00000223650.1	3066	No protein	Processed transcript	-	-	
Myof-212	ENSMUST00000226084.1	3926	No protein	Retained intron	-	-	
Myof-204	ENSMUST00000224518.1	3030	No protein	Retained intron	-	-	
Myof-209	ENSMUST00000225287.1	718	No protein	Retained intron	-	-	
Myof-206	ENSMUST00000224580.1	662	No protein	Retained intron	-	-	
Myof-210	ENSMUST00000225435.1	567	No protein	Retained intron	-	-	

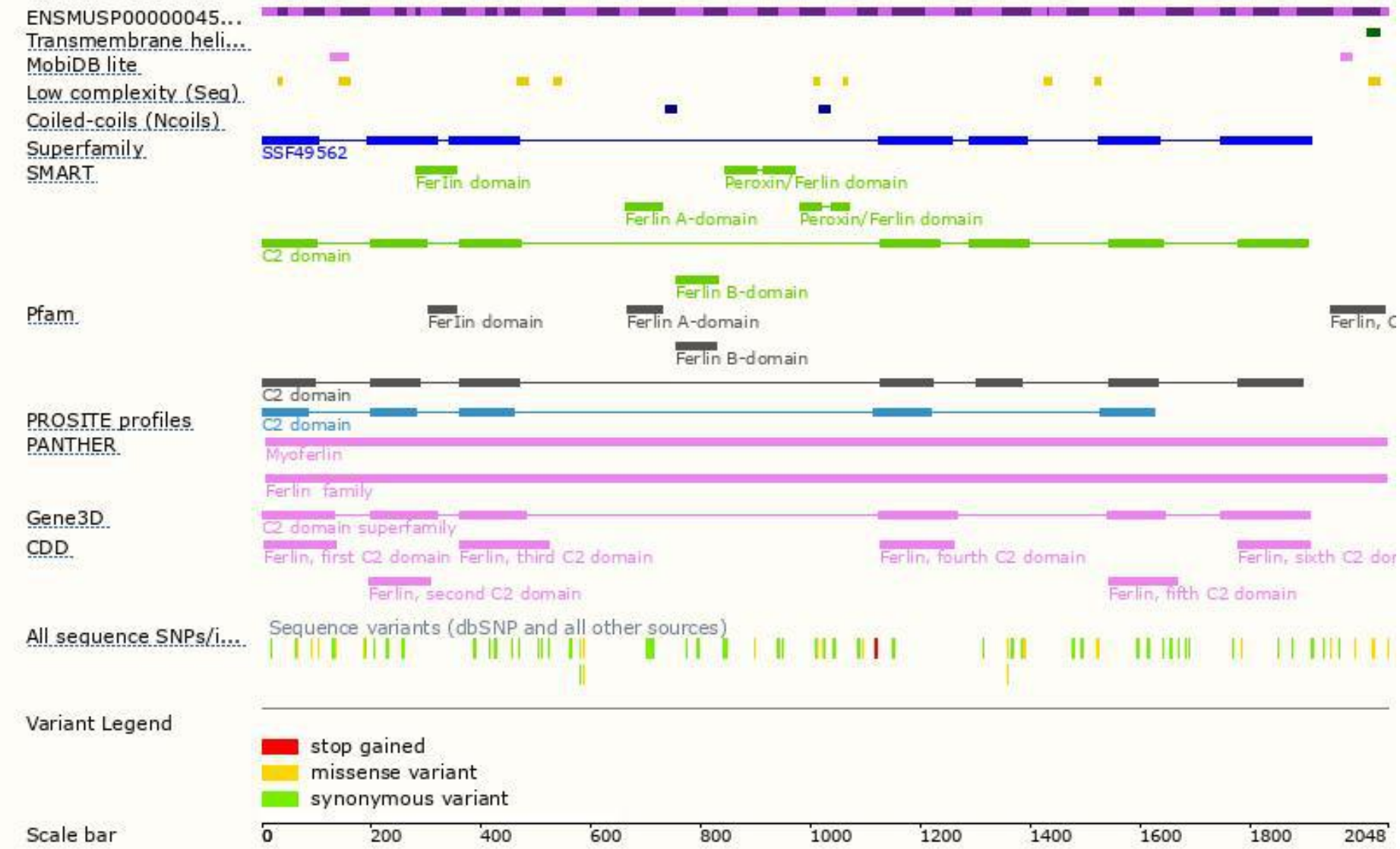
The strategy is based on the design of *Myof-201* transcript,the transcription is shown below:



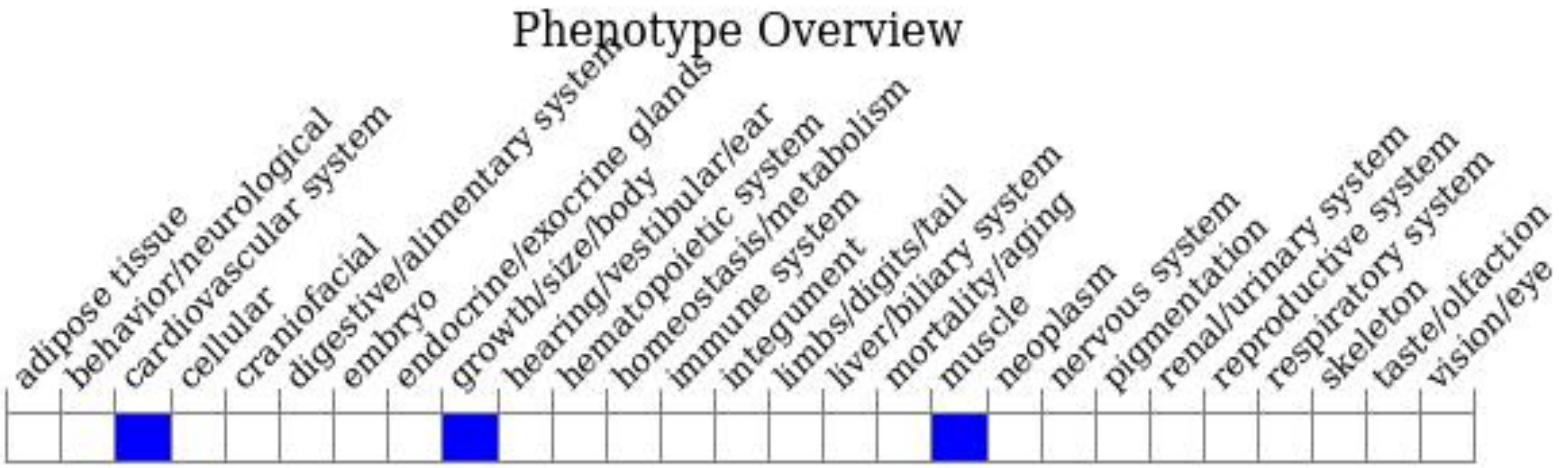
Genomic location distribution



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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