Mtm1 Cas9-CKO Strategy

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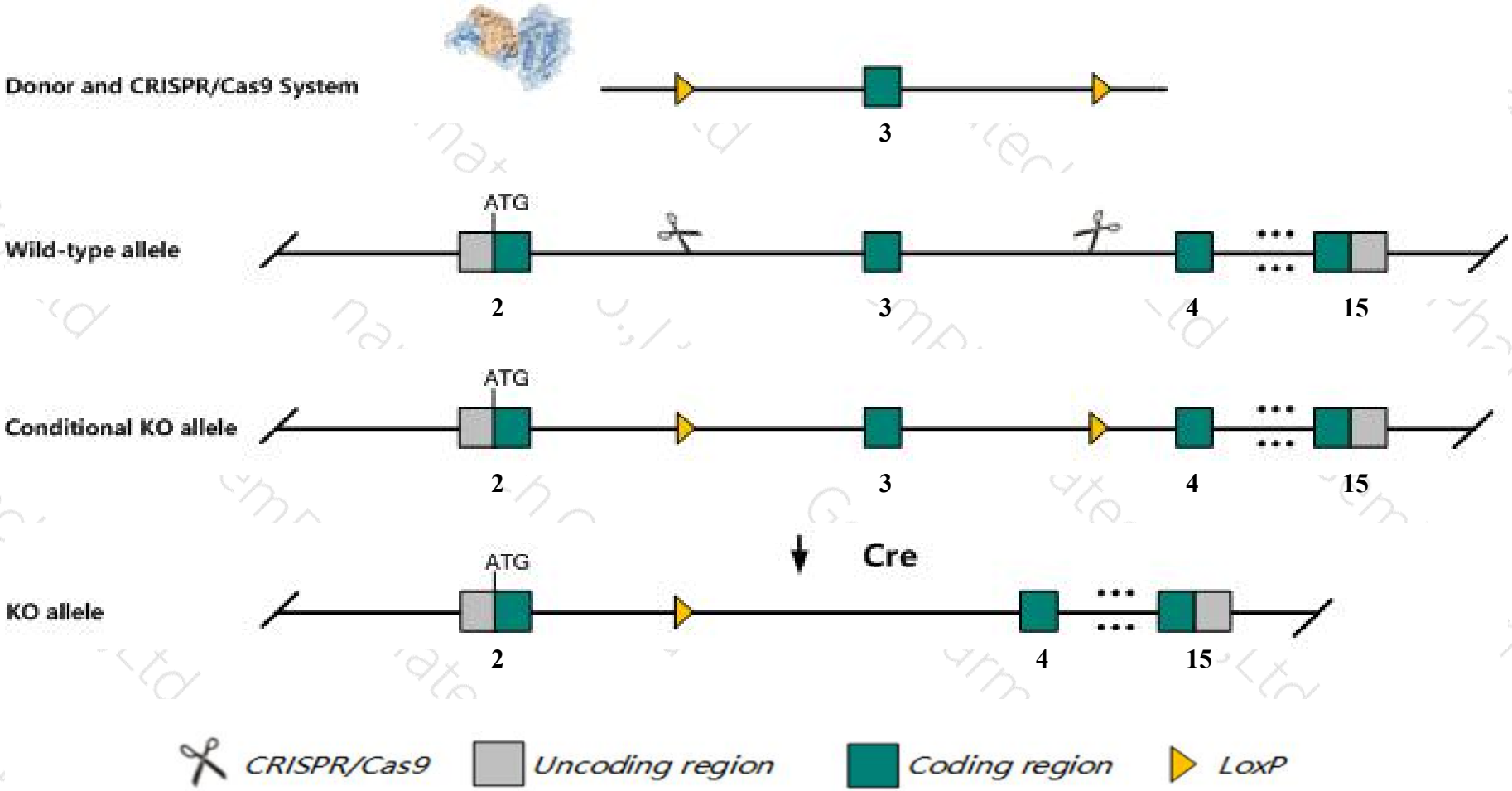
Design Date: 2020-5-9

Project Overview

Project Name	***Mtm1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6J**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mtm1* gene. The schematic diagram is as follows:



Technical routes

- The *Mtm1* gene has 13 transcripts. According to the structure of *Mtm1* gene, exon3 of *Mtm1-213* (ENSMUST00000171933.7) transcript is recommended as the knockout region.The region contains 73bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Mtm1* gene. The brief process is as follows:CRISPR/Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6J 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/6J 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,hemizygotes for targeted null mutations develop a generalized, progressive myopathy beginning around 1 month and leading to death at 6-14 weeks of age. mutant mice show amyotrophy with accumulation of central nuclei in skeletal muscle fibers.
- Transcript *Mtm1-208* may not be affected.
- The *Mtm1* gene is located on the ChrX. 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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Mtm1 X-linked myotubular myopathy gene 1 [Mus musculus (house mouse)]

Gene ID: 17772, updated on 13-Mar-2020

Summary

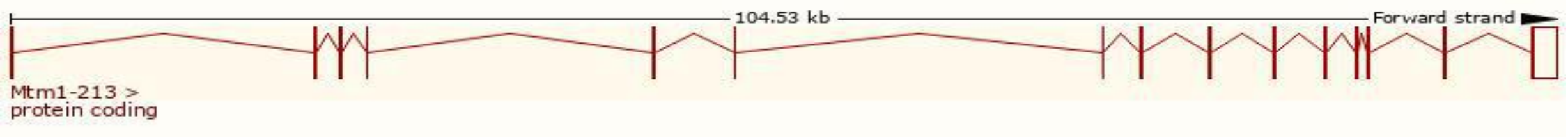
Official Symbol	Mtm1 provided by MGI
Official Full Name	X-linked myotubular myopathy gene 1 provided by MGI
Primary source	MGI:MGI:1099452
See related	Ensembl:ENSMUSG00000031337
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AF073996, Mtm, mKIAA4176
Expression	Ubiquitous expression in large intestine adult (RPKM 2.8), placenta adult (RPKM 2.7) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

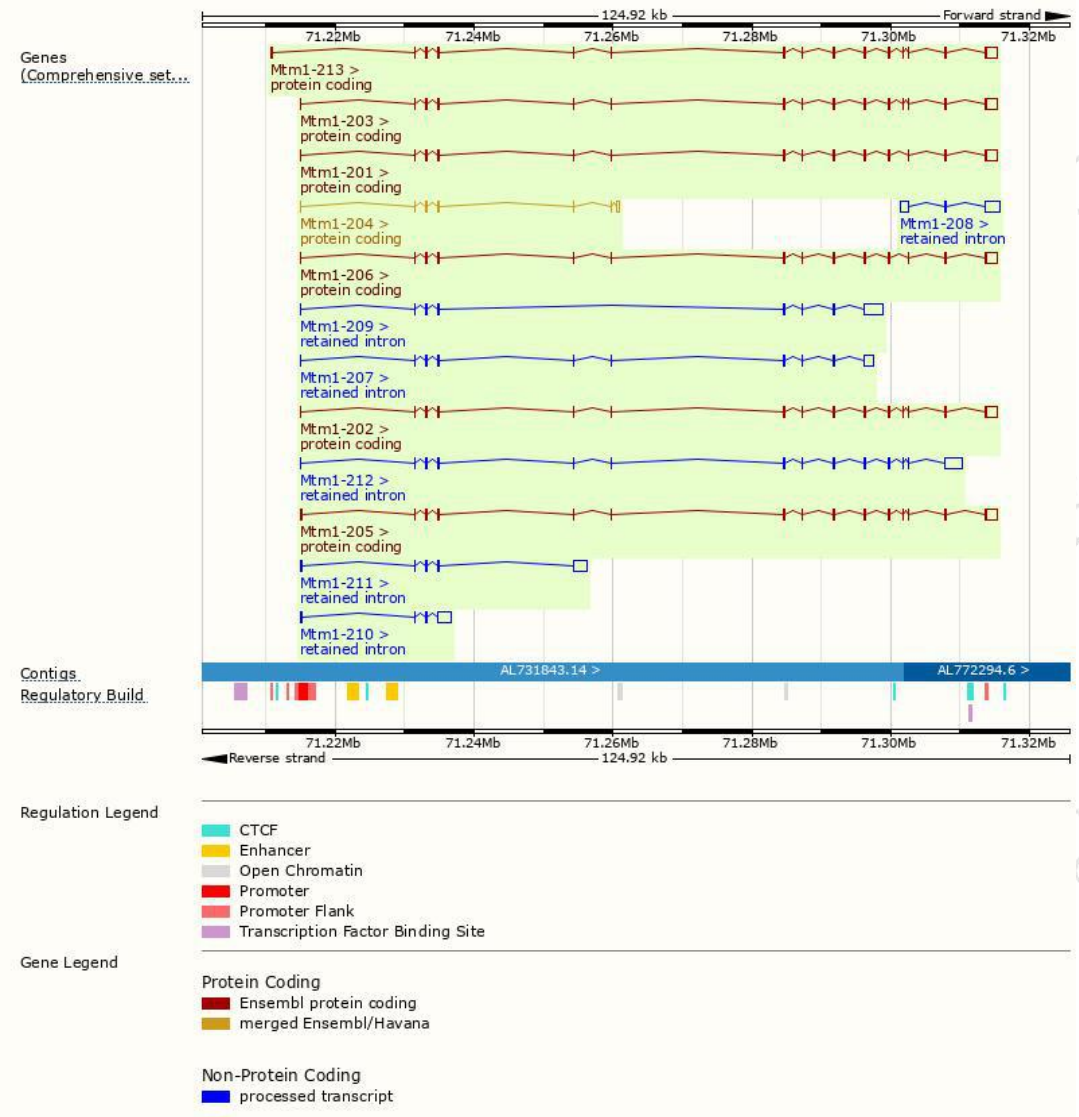
The gene has 13 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mtm1-213	ENSMUST00000171933.7	3488	603aa	Protein coding	CCDS30177	Q9Z2C5	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 P1
Mtm1-203	ENSMUST00000061970.11	3379	603aa	Protein coding	CCDS30177	Q9Z2C5	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 P1
Mtm1-201	ENSMUST00000025391.10	3286	572aa	Protein coding	CCDS53090	B1AW21	TSL:1 GENCODE basic
Mtm1-204	ENSMUST00000101501.9	770	162aa	Protein coding	CCDS53089	Q3UDN6	TSL:1 GENCODE basic
Mtm1-205	ENSMUST00000114617.1	3338	603aa	Protein coding	-	Q9Z2C5	TSL:5 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 P1
Mtm1-202	ENSMUST00000033700.11	3316	603aa	Protein coding	-	Q9Z2C5	TSL:5 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 P1
Mtm1-206	ENSMUST00000114621.7	3257	572aa	Protein coding	-	B1AW21	TSL:5 GENCODE basic
Mtm1-212	ENSMUST00000156452.7	4111	No protein	Retained intron	-	-	TSL:5
Mtm1-208	ENSMUST00000129722.1	3469	No protein	Retained intron	-	-	TSL:1
Mtm1-209	ENSMUST00000134859.7	3412	No protein	Retained intron	-	-	TSL:1
Mtm1-211	ENSMUST00000152810.1	2353	No protein	Retained intron	-	-	TSL:5
Mtm1-207	ENSMUST00000126208.7	2339	No protein	Retained intron	-	-	TSL:1
Mtm1-210	ENSMUST00000151415.7	2042	No protein	Retained intron	-	-	TSL:1

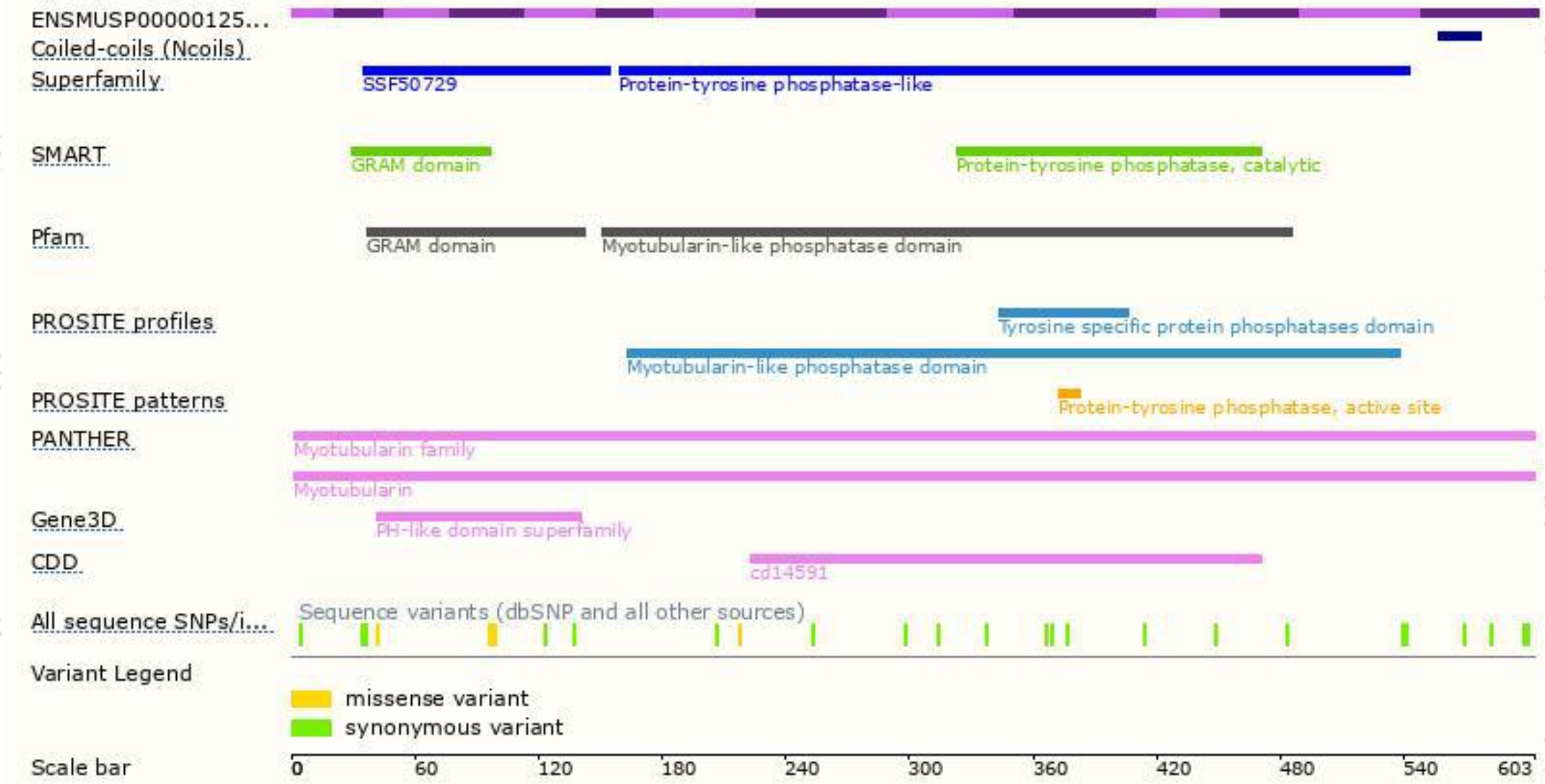
The strategy is based on the design of *Mtm1-213* transcript,the transcription is shown below:



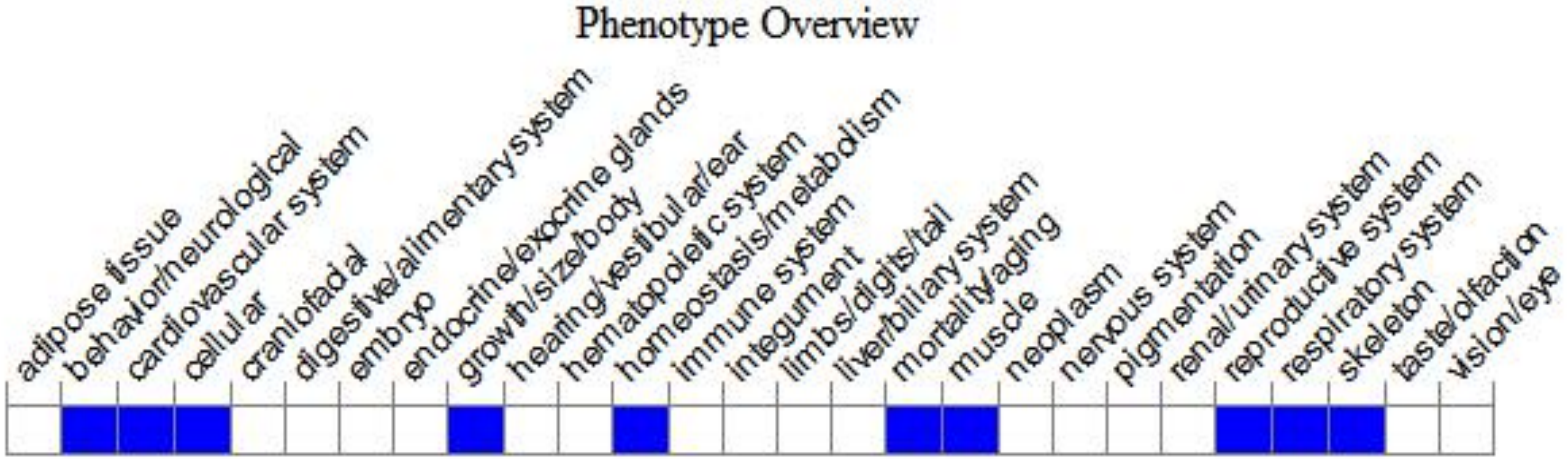
Genomic location distribution



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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