Dysf Cas9-CKO Strategy

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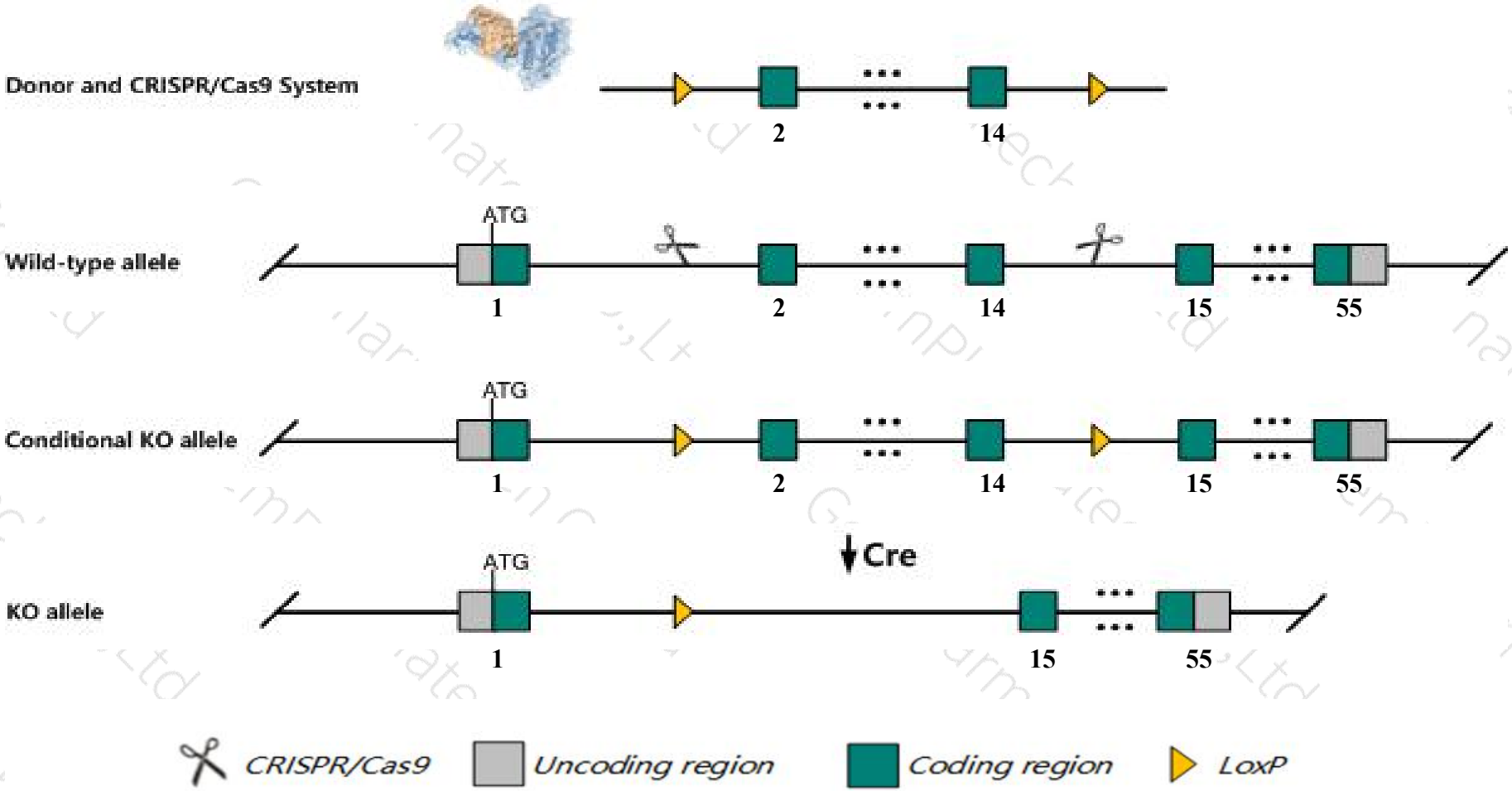
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Dysf* gene has 16 transcripts. According to the structure of *Dysf* gene, exon2-exon14 of *Dysf-201* (ENSMUST00000081904.6) transcript is recommended as the knockout region.The region contains 1295bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Dysf* 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.

Notice

➢ According to the existing MGI data,Homozygotes display dystrophic muscle changes and progressive muscle weakness developing over time.

➢Transcript *Dysf*-206&207&208&209 may not be affected.

➢ The *Dysf* gene is located on the Chr6. 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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Dysf dysferlin [*Mus musculus* (house mouse)]

Gene ID: 26903, updated on 10-Oct-2019

Summary

Official Symbol	Dysf provided by MGI
Official Full Name	dysferlin provided by MGI
Primary source	MGI:MGI:1349385
See related	Ensembl:ENSMUSG00000033788
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	D6Pas3; AI604795; 2310004N10Rik
Expression	Broad expression in genital fat pad adult (RPKM 9.2), heart adult (RPKM 8.9) and 24 other tissues See more
Orthologs	human all

Genomic context

Location: 6 C3; 6 36.14 cM

See Dysf in Genome Data Viewer

Exon count: 64

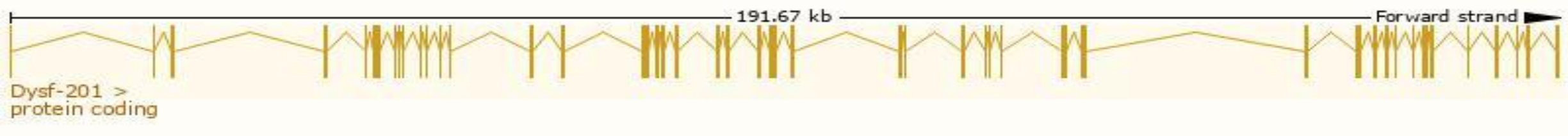
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	6	NC_000072.6 (84008361..84211060)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	6	NC_000072.5 (83958584..84161036)

Transcript information（Ensembl）

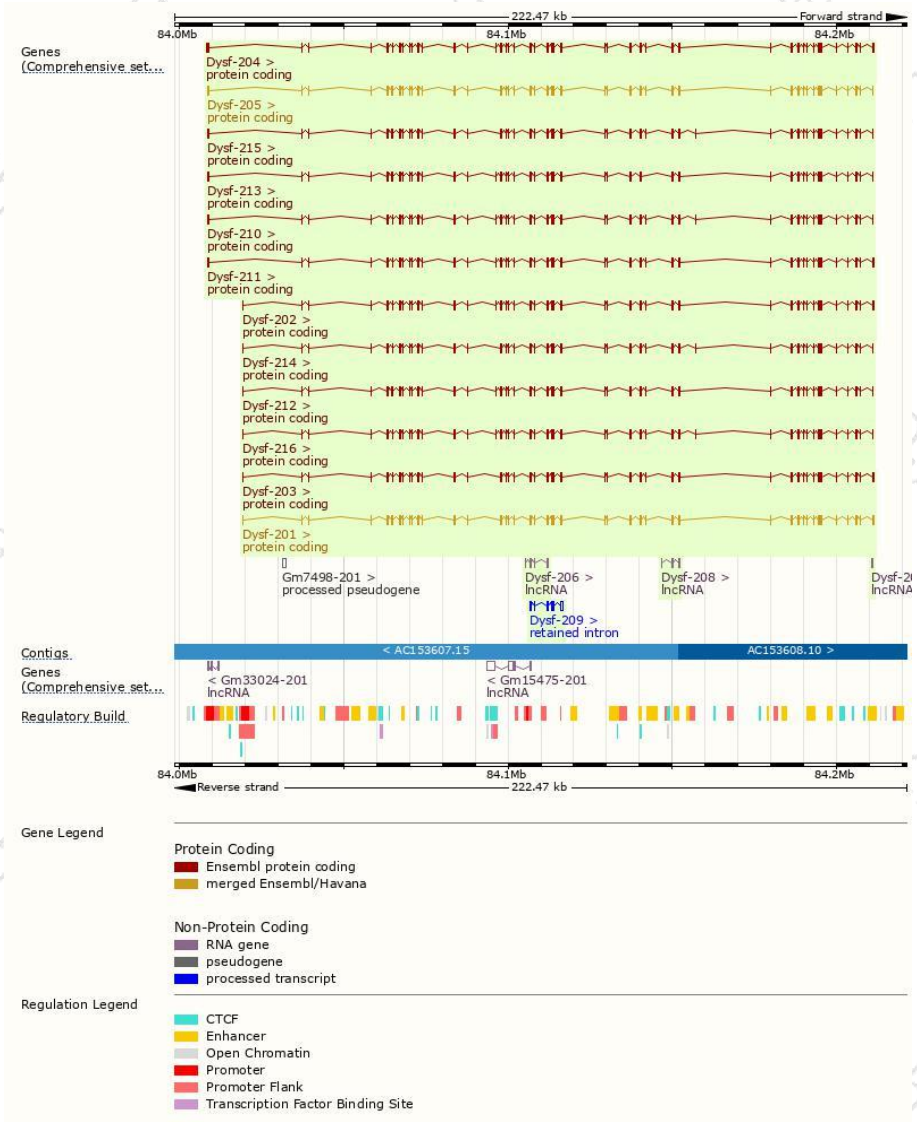
The gene has 16 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dysf-201	ENSMUST00000081904.6	6660	2100aa	Protein coding	CCDS39536	Q9ESD7	TSL:1 GENCODE basic APPRIS P4
Dysf-205	ENSMUST00000113823.7	6578	2099aa	Protein coding	CCDS39535	E9Q423	TSL:5 GENCODE basic APPRIS ALT1
Dysf-203	ENSMUST00000113818.7	6571	2069aa	Protein coding	CCDS85081	Q9ESD7	TSL:1 GENCODE basic APPRIS ALT1
Dysf-204	ENSMUST00000113821.7	6803	2082aa	Protein coding	-	E9QL12	TSL:5 GENCODE basic APPRIS ALT1
Dysf-215	ENSMUST00000204591.2	6641	2120aa	Protein coding	-	A0A0N4SV63	TSL:5 GENCODE basic APPRIS ALT1
Dysf-202	ENSMUST00000089595.11	6613	2083aa	Protein coding	-	E9PXU9	TSL:5 GENCODE basic APPRIS ALT1
Dysf-213	ENSMUST00000203803.2	6590	2103aa	Protein coding	-	A0A0N4SWG7	TSL:5 GENCODE basic
Dysf-210	ENSMUST00000153860.3	6525	2103aa	Protein coding	-	A0A0N4SWH3	TSL:5 GENCODE basic APPRIS ALT1
Dysf-212	ENSMUST00000203695.2	6504	2114aa	Protein coding	-	A0A0N4SVX9	TSL:5 GENCODE basic APPRIS ALT1
Dysf-211	ENSMUST00000168387.7	6501	2090aa	Protein coding	-	Q9ESD7	TSL:5 GENCODE basic
Dysf-216	ENSMUST00000204987.2	6474	2104aa	Protein coding	-	A0A0N4SUN3	TSL:5 GENCODE basic APPRIS ALT1
Dysf-214	ENSMUST00000204354.2	6432	2090aa	Protein coding	-	A0A0N4SUJ2	TSL:5 GENCODE basic APPRIS ALT1
Dysf-209	ENSMUST00000152646.1	1991	No protein	Retained intron	-	-	TSL:1
Dysf-206	ENSMUST00000127459.7	733	No protein	lncRNA	-	-	TSL:3
Dysf-208	ENSMUST00000142711.5	549	No protein	lncRNA	-	-	TSL:3
Dysf-207	ENSMUST00000128965.1	404	No protein	lncRNA	-	-	TSL:2

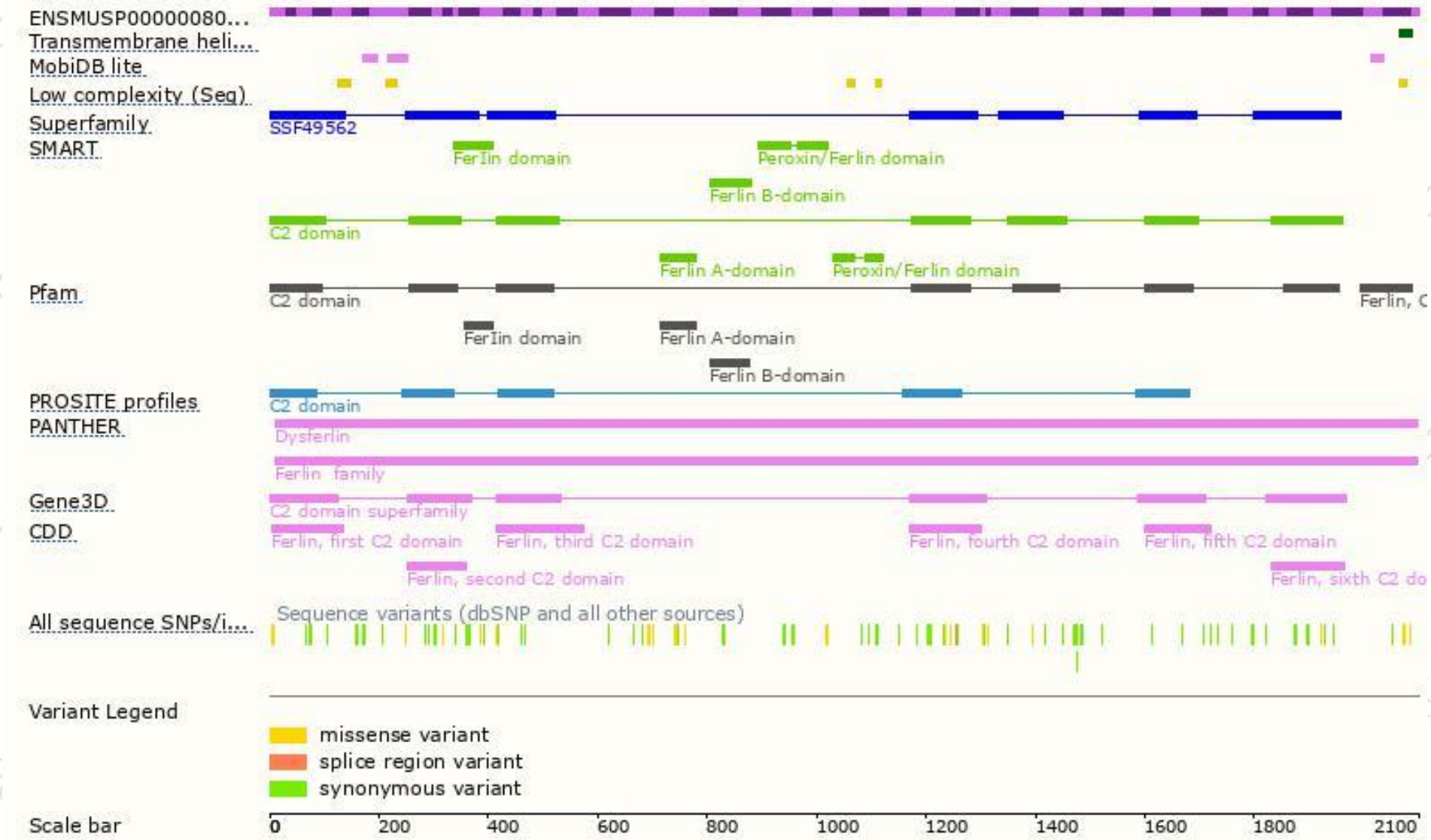
The strategy is based on the design of *Dysf-201* transcript,The transcription is shown below



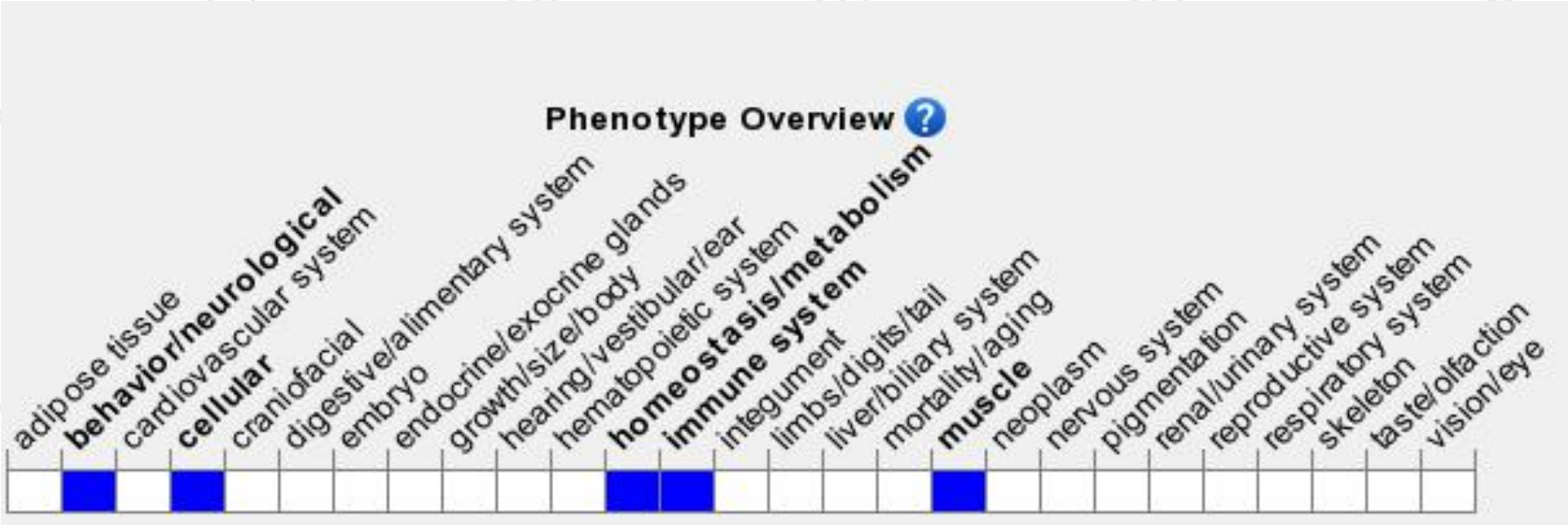
Genomic location distribution



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,Homozygotes display dystrophic muscle changes and progressive muscle weakness developing over time.

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

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