Myo5b Cas9-CKO Strategy

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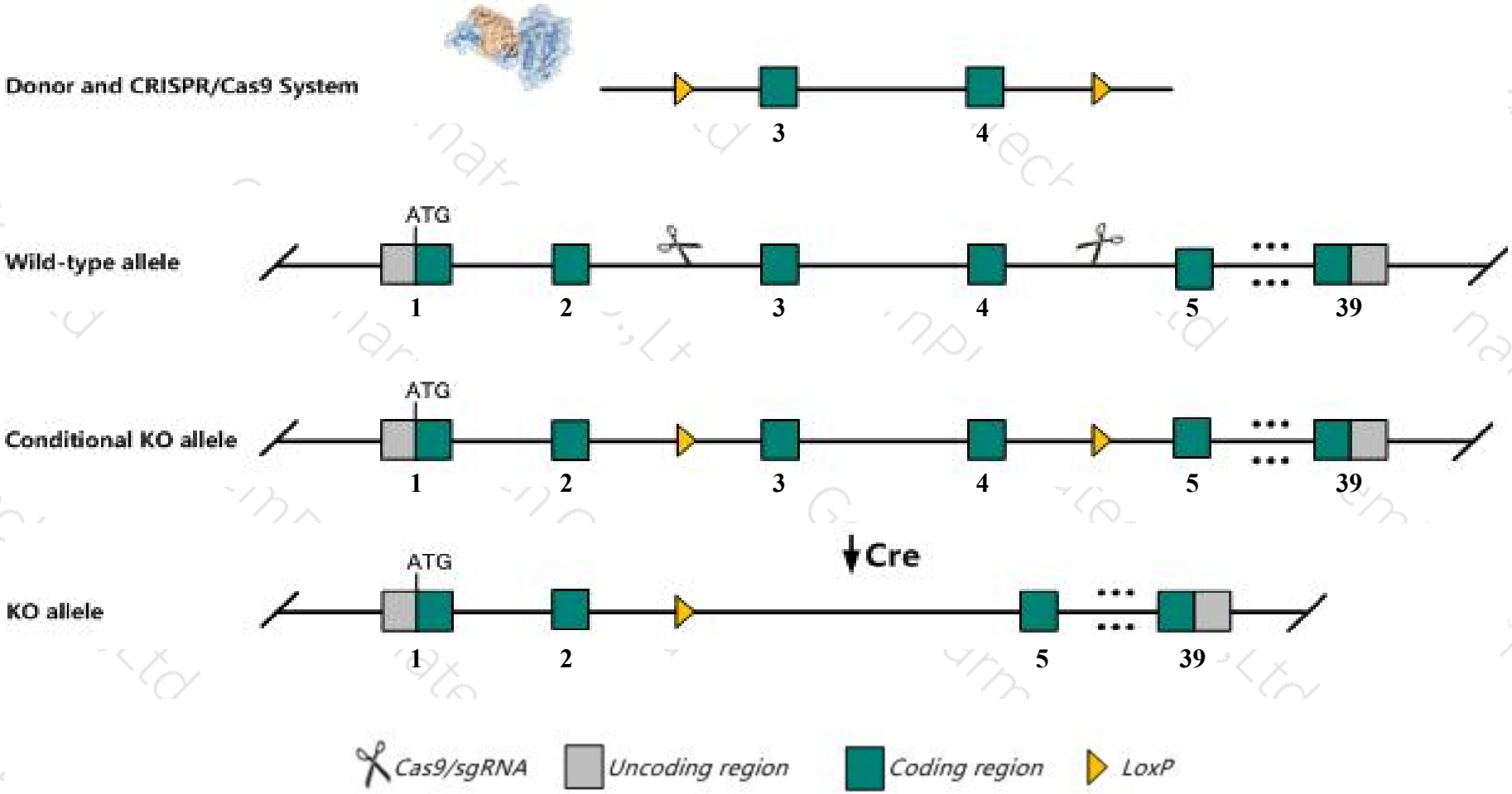
Project Overview

Project Name	***Myo5b***
Project type	**Cas9-CKO**
Strain background	**C57BL/6J**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Myo5b* gene. The schematic diagram is as follows:



Technical routes

- The *Myo5b* gene has 11 transcripts. According to the structure of *Myo5b* gene, exon3-exon4 of *Myo5b-201* (ENSMUST00000074157.12) transcript is recommended as the knockout region.The region contains 317bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Myo5b* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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,Homozygous null mice show perinatal mortality, diarrhea, intestinal microvillus atrophy and the presence of microvillus inclusion bodies, resembling phenotype of Microvillus Inclusion Disease.

- The *Myo5b* gene is located on the Chr18. 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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Myo5b myosin VB [*Mus musculus* (house mouse)]

Gene ID: 17919, updated on 12-Aug-2019

Summary

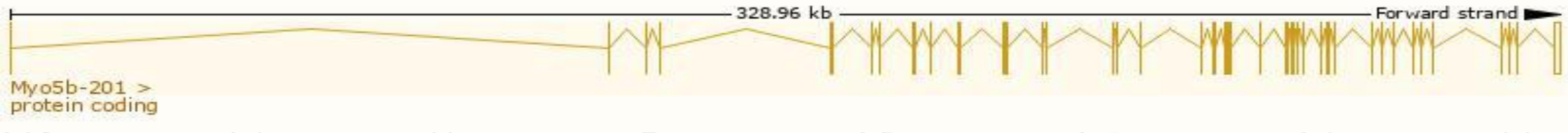
Official Symbol	Myo5b provided by MGI
Official Full Name	myosin VB provided by MGI
Primary source	MGI:MGI:106598
See related	Ensembl:ENSMUSG00000025885
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI661750; mKIAA1119
Expression	Broad expression in large intestine adult (RPKM 24.0), genital fat pad adult (RPKM 20.9) and 17 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

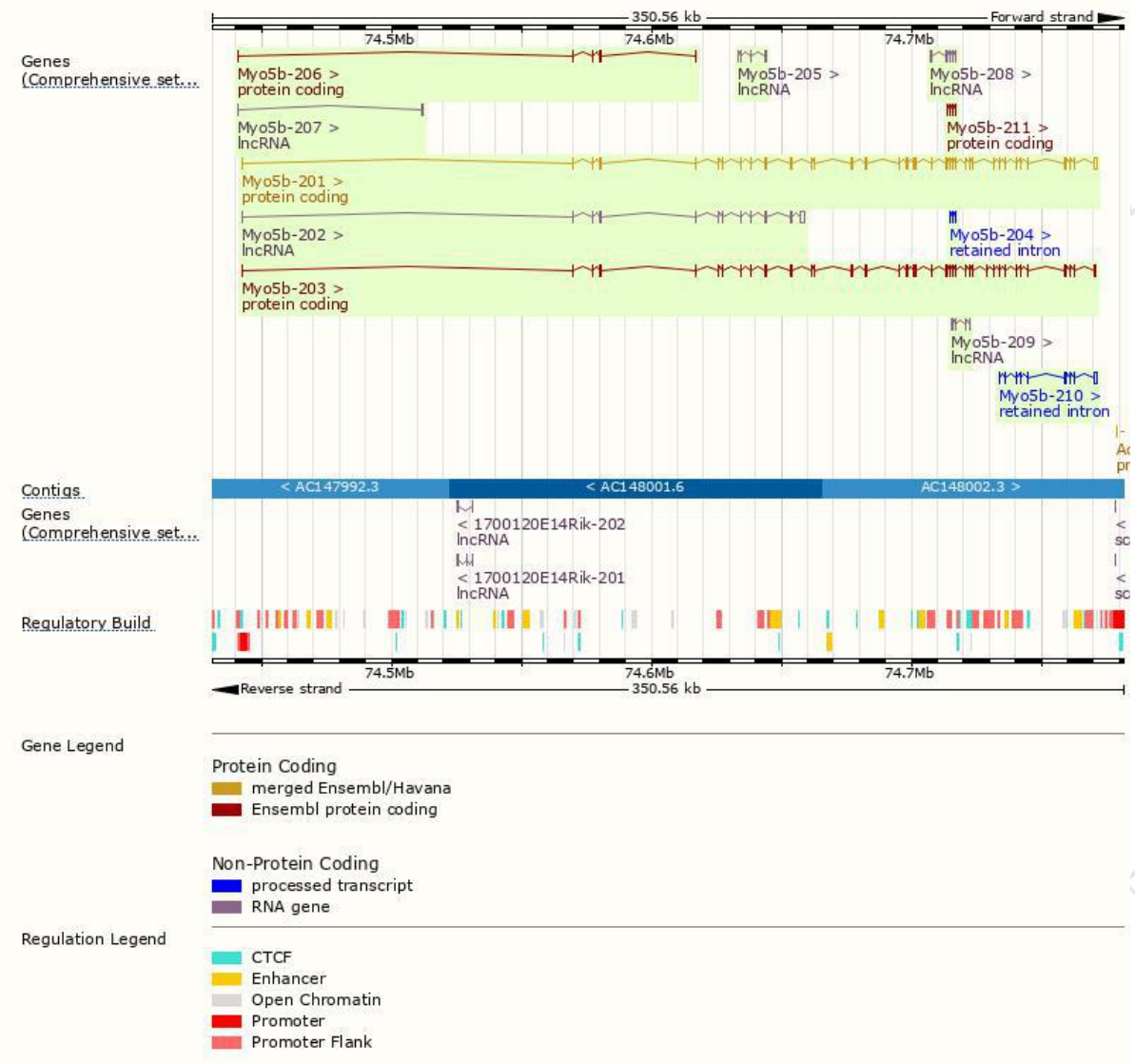
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Myo5b-201	ENSMUST00000074157.12	6745	1818aa	Protein coding	CCDS37858	G5E8G6	TSL:1 GENCODE basic APPRIS P2
Myo5b-203	ENSMUST00000121875.7	6065	1844aa	Protein coding	-	G3X9Y9	TSL:5 GENCODE basic APPRIS ALT2
Myo5b-206	ENSMUST00000125882.7	646	183aa	Protein coding	-	D3Z135	CDS 3' incomplete TSL:3
Myo5b-211	ENSMUST00000177366.1	382	128aa	Protein coding	-	H3BLK6	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Myo5b-202	ENSMUST00000120161.7	3400	No protein	Processed transcript	-	-	TSL:1
Myo5b-207	ENSMUST00000135878.1	862	No protein	Processed transcript	-	-	TSL:2
Myo5b-208	ENSMUST00000140904.7	764	No protein	Processed transcript	-	-	TSL:3
Myo5b-209	ENSMUST00000146253.1	470	No protein	Processed transcript	-	-	TSL:2
Myo5b-205	ENSMUST00000125751.1	383	No protein	Processed transcript	-	-	TSL:3
Myo5b-210	ENSMUST00000154986.1	2500	No protein	Retained intron	-	-	TSL:1
Myo5b-204	ENSMUST00000122914.7	388	No protein	Retained intron	-	-	TSL:3

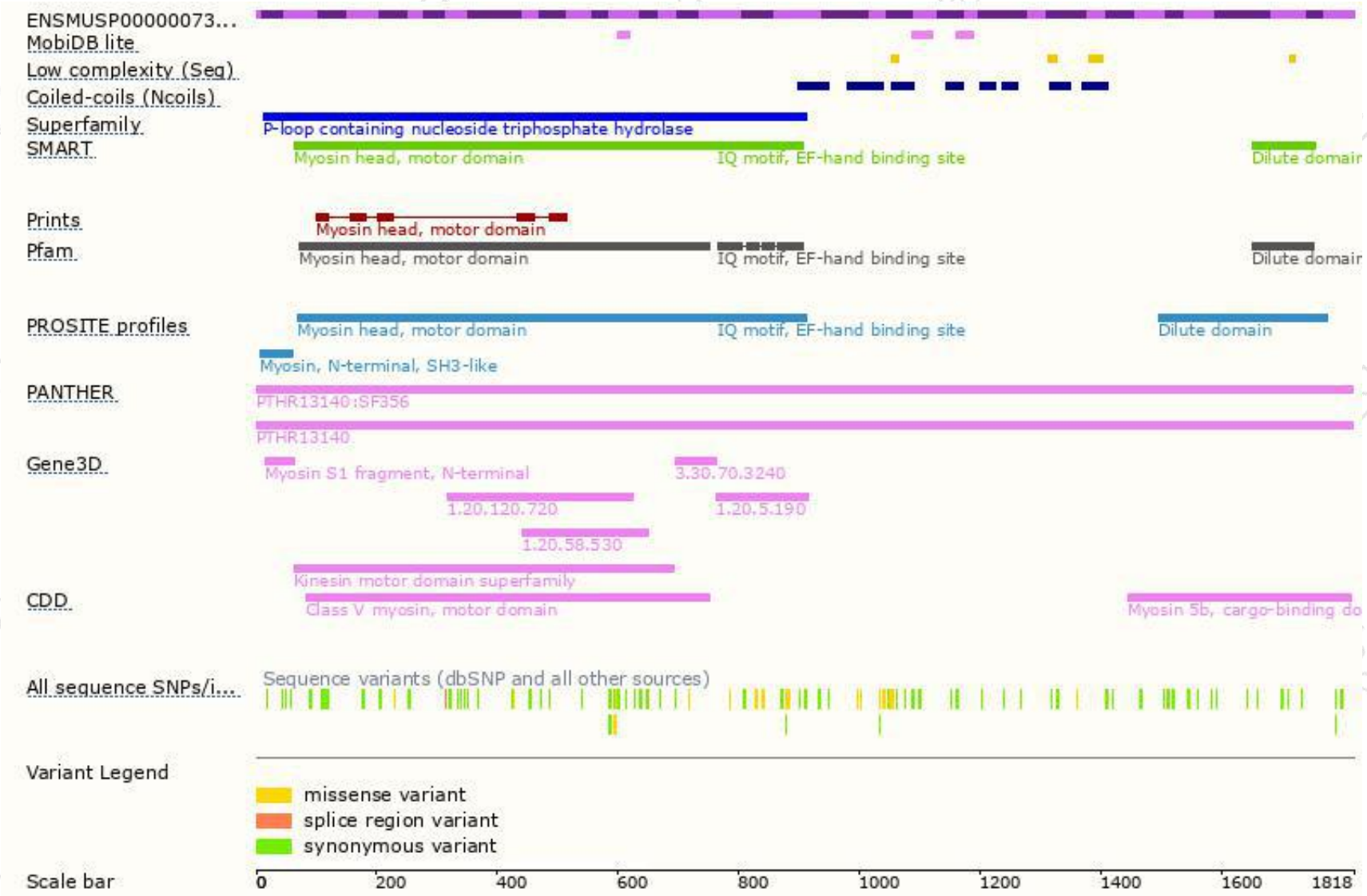
The strategy is based on the design of *Myo5b-201* transcript,The transcription is shown below



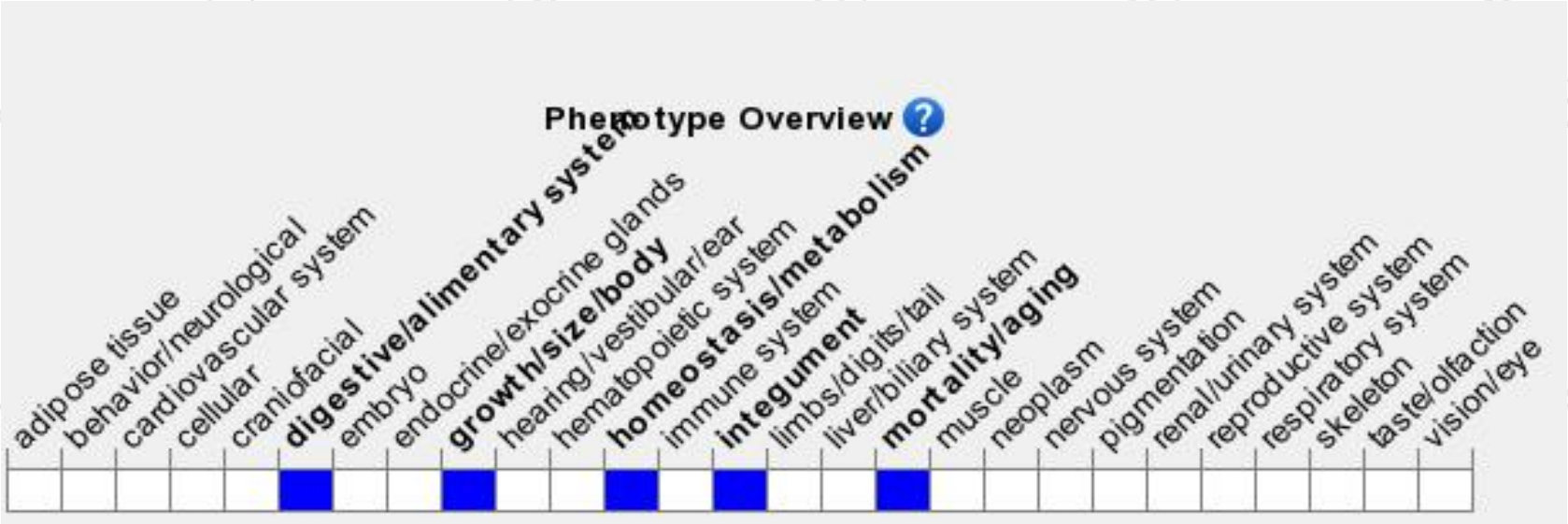
Genomic location distribution



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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