

Pfkfb3 Cas9-CKO Strategy

Designer: Huimin Su

Reviewer: Ruirui Zhang

Date: 2019/9/25

Project Overview

Project Name

Pfkfb3

Project type

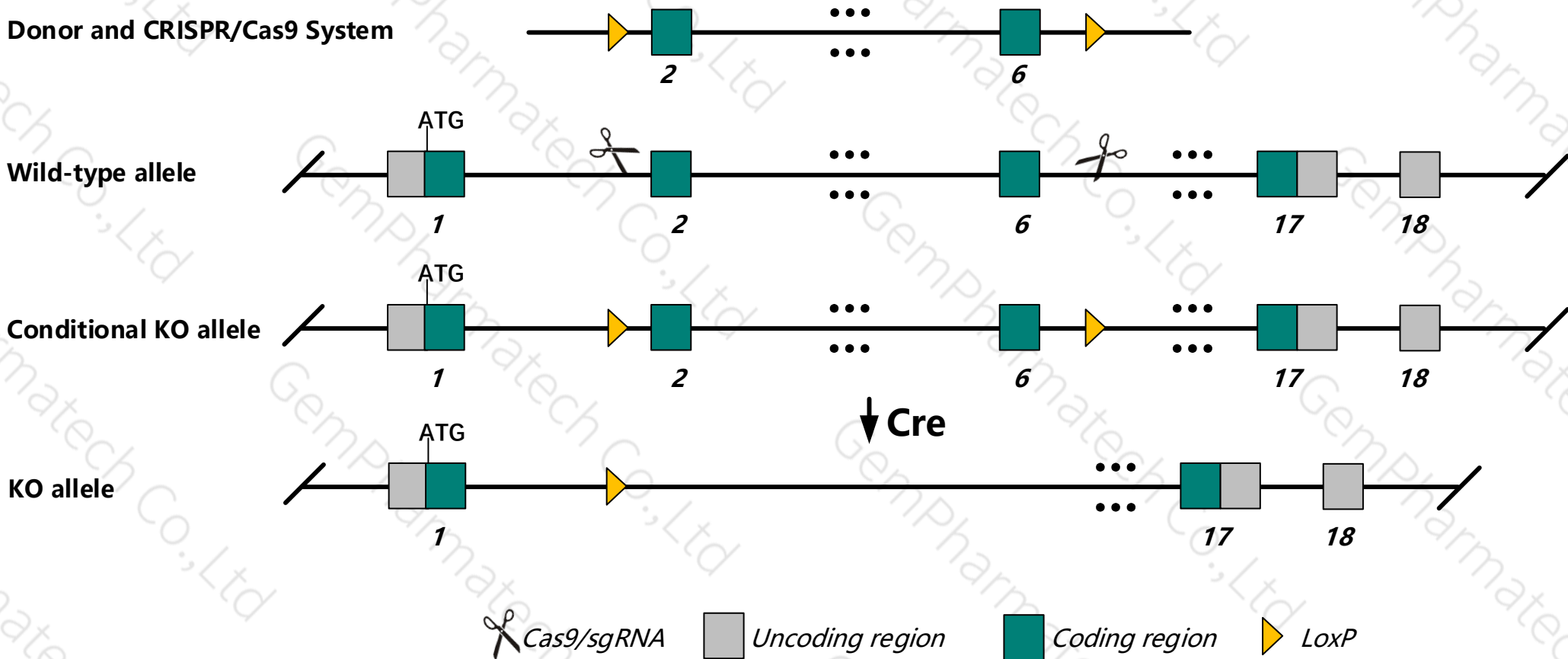
Cas9-CKO

Strain background

C57BL/6J

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pfkfb3* gene. The schematic diagram is as follows:



- The *Pfkfb3* gene has 21 transcripts. According to the structure of *Pfkfb3* gene, exon2-exon6 of *Pfkfb3*-216 (ENSMUST00000191668.5) transcript is recommended as the knockout region. The region contains 422bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Pfkfb3* 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.

- According to the existing MGI data, Homozygous null mice display embryonic lethality before E8
- The *Pfkfb3* gene is located on the Chr2. 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)

Pfkfb3 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 [Mus musculus (house mouse)]

Gene ID: 170768, updated on 3-Feb-2019

Summary



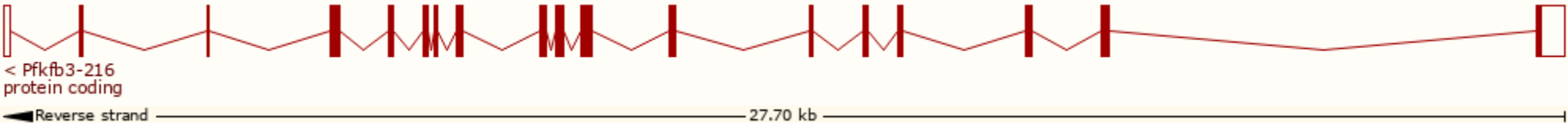
Official Symbol	Pfkfb3 provided by MGI
Official Full Name	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 provided by MGI
Primary source	MGI:MGI:2181202
See related	Ensembl:ENSMUSG00000026773
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	E330010H22Rik, iPFK-2, uPFK-2
Expression	Broad expression in subcutaneous fat pad adult (RPKM 57.0), genital fat pad adult (RPKM 44.9) and 22 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

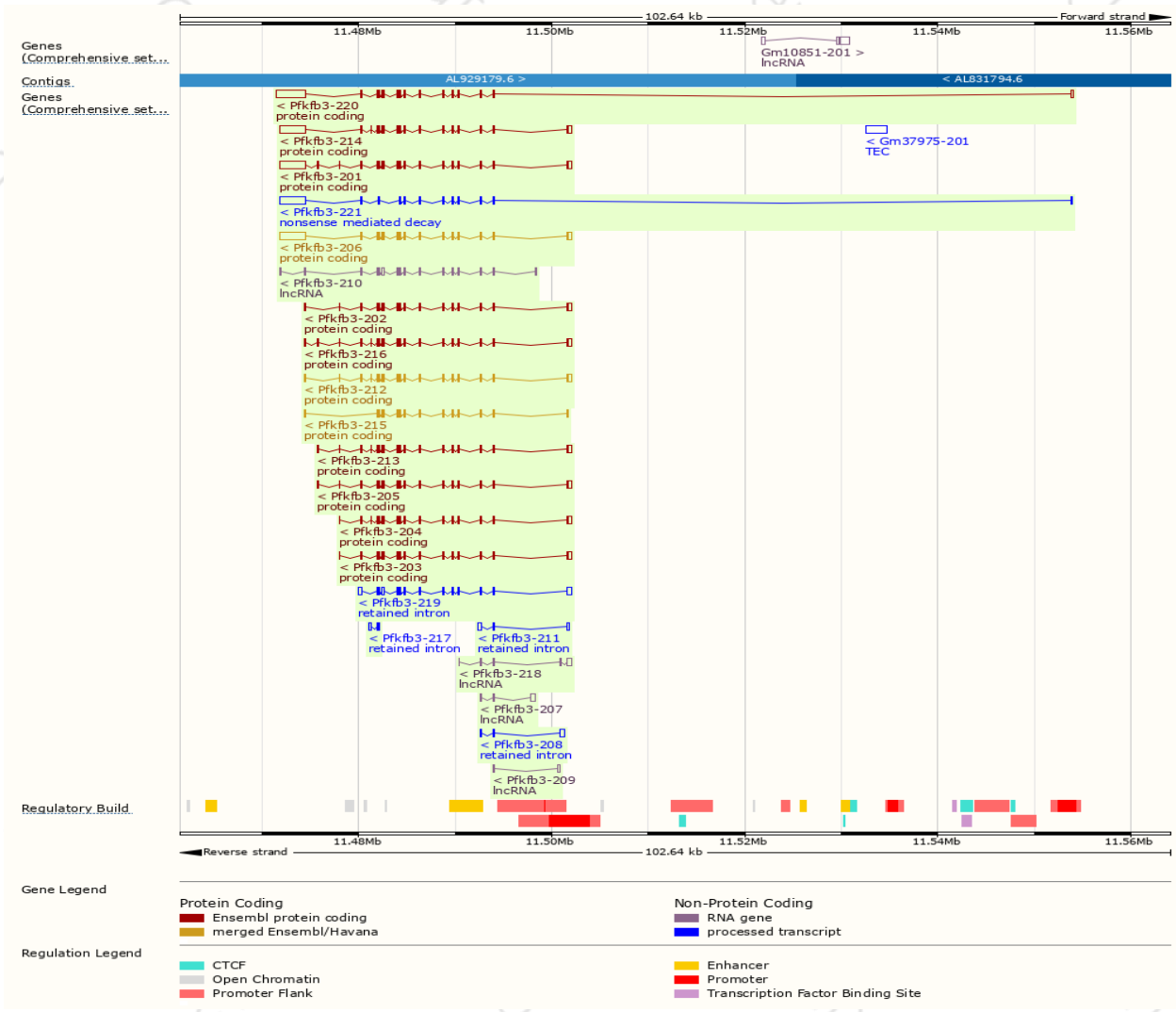
The gene has 21 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pfkfb3-220	ENSMUST00000192949.5	4801	500aa	Protein coding	CCDS79733	A0A0A6YY64	TSL:1 GENCODE basic
Pfkfb3-214	ENSMUST00000179584.7	4756	549aa	Protein coding	CCDS79732	Q3U3S6	TSL:1 GENCODE basic
Pfkfb3-201	ENSMUST00000028114.12	4712	526aa	Protein coding	CCDS0499	Q7TS91	TSL:1 GENCODE basic
Pfkfb3-206	ENSMUST00000114846.8	4634	520aa	Protein coding	CCDS15683	A2AUP1	TSL:1 GENCODE basic APPRIS P3
Pfkfb3-216	ENSMUST00000191668.5	2216	555aa	Protein coding	CCDS50501	A7UAK5	TSL:1 GENCODE basic
Pfkfb3-212	ENSMUST00000170196.8	2163	543aa	Protein coding	CCDS50500	A7UAK8	TSL:1 GENCODE basic
Pfkfb3-202	ENSMUST00000049849.11	2076	514aa	Protein coding	CCDS79731	A2AUP5	TSL:1 GENCODE basic APPRIS ALT1
Pfkfb3-213	ENSMUST00000171188.8	2058	555aa	Protein coding	CCDS50501	A7UAK5	TSL:5 GENCODE basic
Pfkfb3-205	ENSMUST00000114845.9	1971	526aa	Protein coding	CCDS50499	Q7TS91	TSL:5 GENCODE basic
Pfkfb3-215	ENSMUST00000183869.7	1478	462aa	Protein coding	CCDS50498	A7UAK4	TSL:1 GENCODE basic APPRIS ALT1
Pfkfb3-204	ENSMUST00000114844.7	2107	542aa	Protein coding	-	A2AUP4	TSL:5 GENCODE basic
Pfkfb3-203	ENSMUST00000100411.3	2008	513aa	Protein coding	-	A2AUP3	TSL:5 GENCODE basic APPRIS ALT1
Pfkfb3-221	ENSMUST00000195779.5	4001	327aa	Nonsense mediated decay	-	A0A0A6YW89	TSL:5
Pfkfb3-210	ENSMUST00000150086.6	1736	No protein	Processed transcript	-	-	TSL:1
Pfkfb3-218	ENSMUST00000192827.5	775	No protein	Processed transcript	-	-	TSL:5
Pfkfb3-207	ENSMUST00000140580.7	666	No protein	Processed transcript	-	-	TSL:5
Pfkfb3-209	ENSMUST00000145970.2	369	No protein	Processed transcript	-	-	TSL:2
Pfkfb3-219	ENSMUST00000192844.5	2188	No protein	Retained intron	-	-	TSL:1
Pfkfb3-211	ENSMUST00000155167.7	646	No protein	Retained intron	-	-	TSL:2
Pfkfb3-208	ENSMUST00000144921.2	614	No protein	Retained intron	-	-	TSL:5
Pfkfb3-217	ENSMUST00000191726.1	447	No protein	Retained intron	-	-	TSL:3

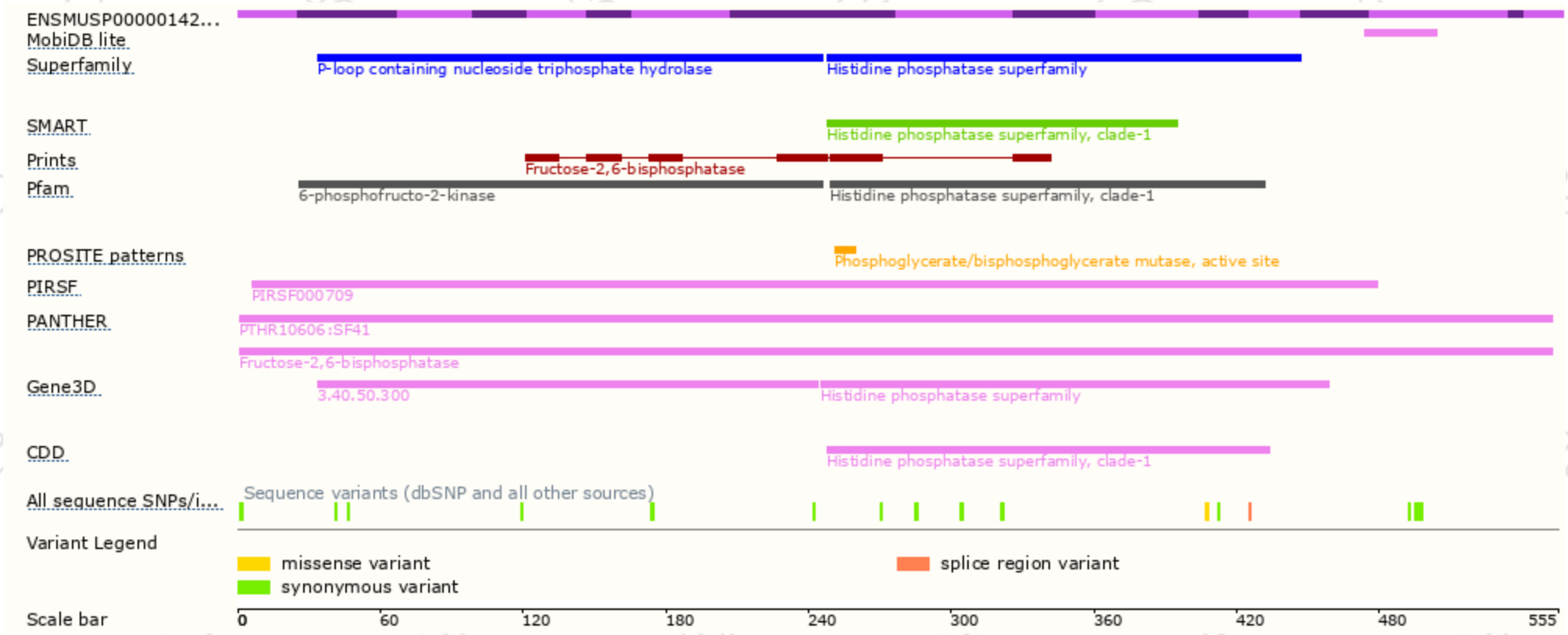
The strategy is based on the design of *Pfkfb3-216* transcript,The transcription is shown below



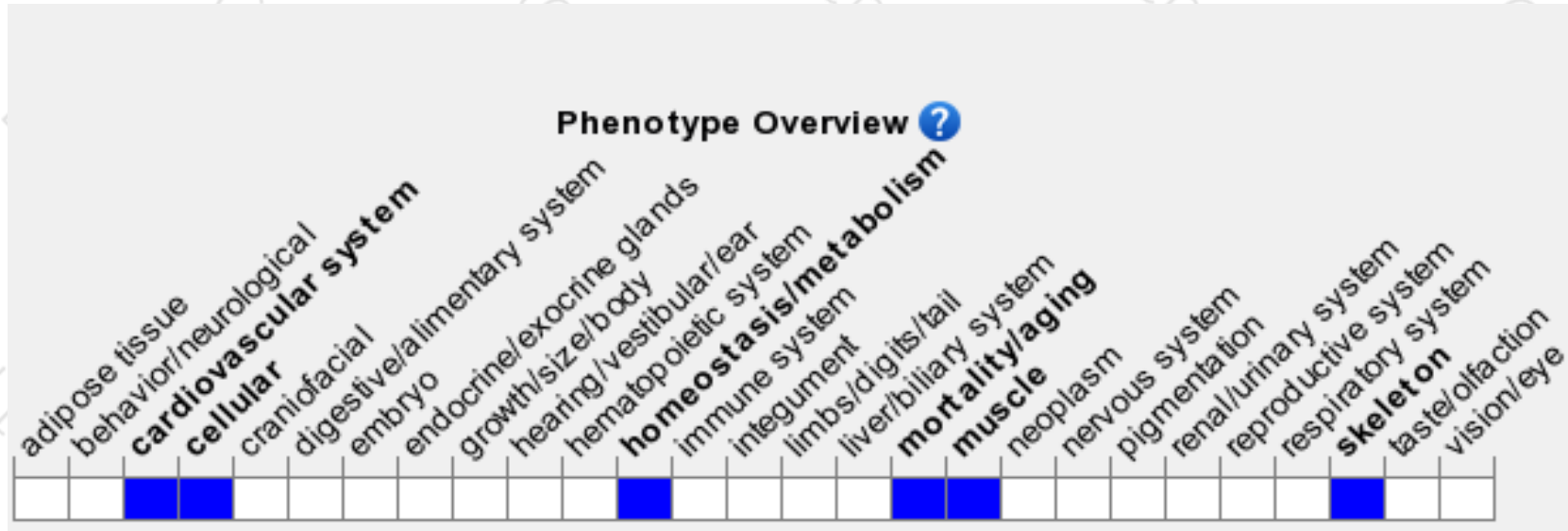
Genomic location distribution



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, Homozygous null mice display embryonic lethality before E8

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

Tel: 025-5864 1534

