

Map4k4 Cas9-CKO Strategy

Designer:

Huan Fan

Design Date:

2019-7-25

Project Overview

Project Name

Map4k4

Project type

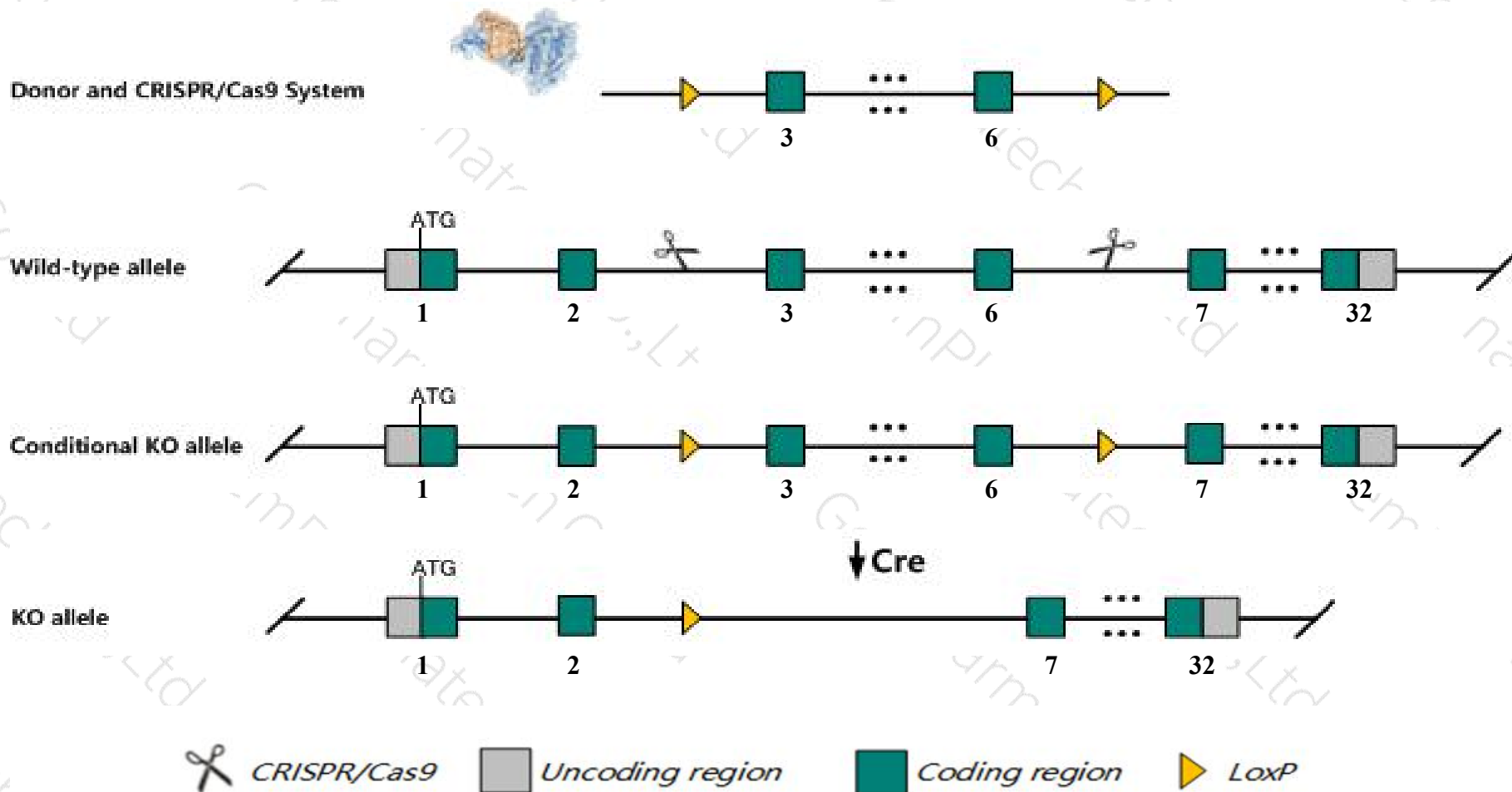
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Map4k4* gene. The schematic diagram is as follows:



- The *Map4k4* gene has 18 transcripts. According to the structure of *Map4k4* gene, exon3-exon6 of *Map4k4-218* (ENSMUST00000195860.5) transcript is recommended as the knockout region. The region contains 385bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Map4k4* 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.

- According to the existing MGI data, Mice homozygous for disruptions in this gene die as embryos around day E9.5-10.5.
- Transcript *Map4k4-205,206,208* may not be affected.
- The *Map4k4* gene is located on the Chr1. 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)

Map4k4 mitogen-activated protein kinase kinase kinase kinase 4 [Mus musculus (house mouse)]

Gene ID: 26921, updated on 7-Apr-2019

Summary



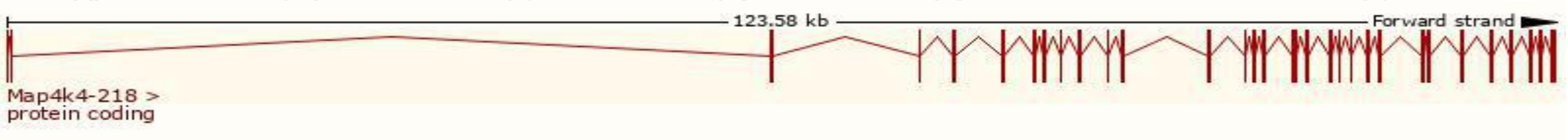
Official Symbol	Map4k4 provided by MGI
Official Full Name	mitogen-activated protein kinase kinase kinase kinase 4 provided by MGI
Primary source	MGI:MGI:1349394
See related	Ensembl:ENSMUSG00000026074
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	9430080K19Rik, AU043147, AU045934, AW046177, HGK, Nik
Expression	Broad expression in CNS E14 (RPKM 64.6), CNS E11.5 (RPKM 64.4) and 27 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

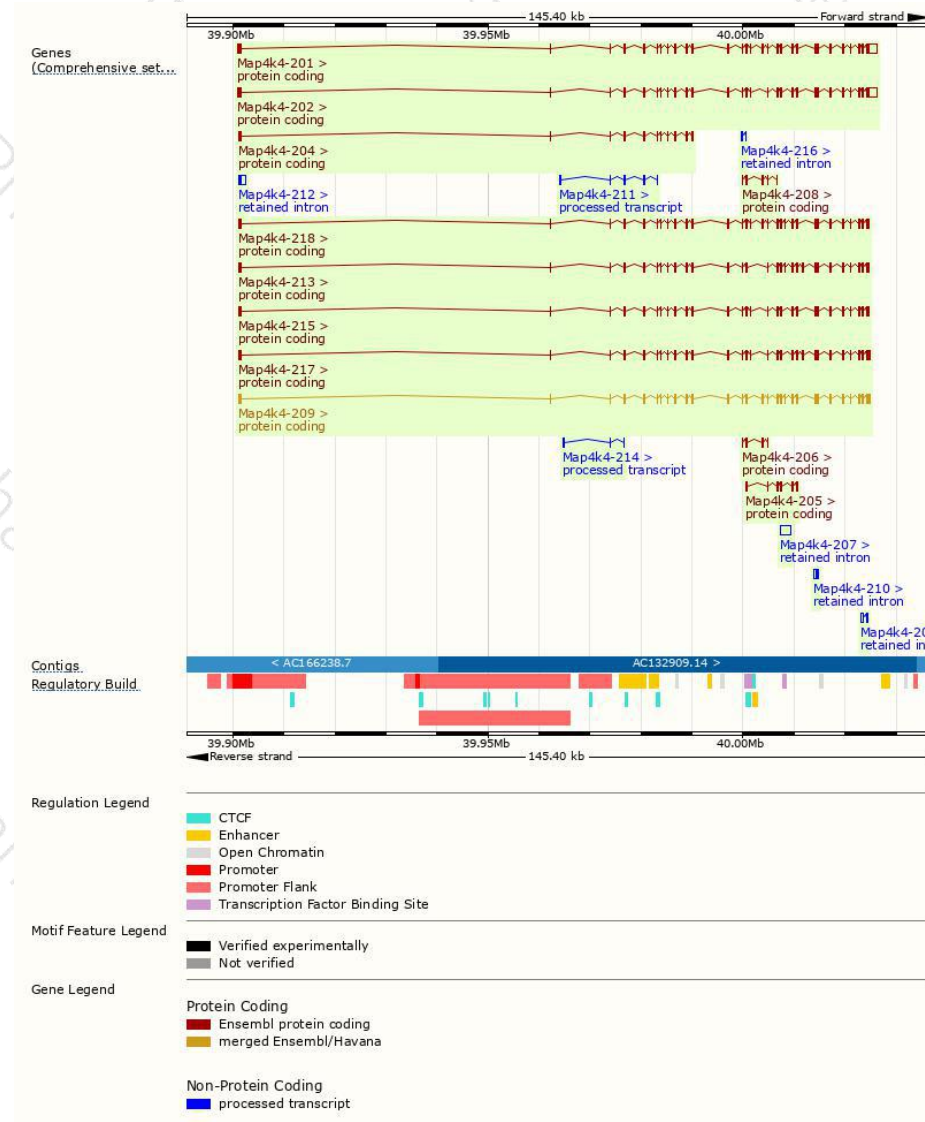
The gene has 18 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Map4k4-218	ENSMUST00000195860.5	3960	1288aa	Protein coding	CCDS78573	A0A0A6YW53	TSL:1 GENCODE basic APPRIS ALT2
Map4k4-209	ENSMUST00000192509.5	3918	1234aa	Protein coding	CCDS35546	A0A0A6YWR8	TSL:2 GENCODE basic APPRIS P3
Map4k4-213	ENSMUST00000193682.5	3759	1221aa	Protein coding	CCDS78576	B2RUE8	TSL:1 GENCODE basic APPRIS ALT2
Map4k4-215	ENSMUST00000195259.5	3719	1208aa	Protein coding	CCDS78575	B7ZNR9	TSL:1 GENCODE basic APPRIS ALT2
Map4k4-201	ENSMUST00000163854.8	5782	1288aa	Protein coding	-	E9PVG7	TSL:5 GENCODE basic APPRIS ALT2
Map4k4-202	ENSMUST00000168431.6	5632	1238aa	Protein coding	-	F8VPL5	TSL:5 GENCODE basic APPRIS ALT2
Map4k4-217	ENSMUST00000195636.5	4082	1272aa	Protein coding	-	A0A0A6YWM8	TSL:5 GENCODE basic APPRIS ALT2
Map4k4-204	ENSMUST00000191761.5	1272	404aa	Protein coding	-	A0A0A6YVZ8	CDS 3' incomplete TSL:2
Map4k4-208	ENSMUST00000192355.5	711	237aa	Protein coding	-	A0A0A6YXE5	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Map4k4-205	ENSMUST00000191865.1	707	236aa	Protein coding	-	A0A0A6YWJ2	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Map4k4-206	ENSMUST00000191964.1	629	209aa	Protein coding	-	A0A0A6YVR7	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Map4k4-211	ENSMUST00000193514.6	384	No protein	Processed transcript	-	-	TSL:5
Map4k4-214	ENSMUST00000194163.1	218	No protein	Processed transcript	-	-	TSL:5
Map4k4-207	ENSMUST00000192194.1	1937	No protein	Retained intron	-	-	TSL:NA
Map4k4-212	ENSMUST00000193661.1	1065	No protein	Retained intron	-	-	TSL:1
Map4k4-210	ENSMUST00000192884.1	544	No protein	Retained intron	-	-	TSL:5
Map4k4-203	ENSMUST00000191657.1	426	No protein	Retained intron	-	-	TSL:3
Map4k4-216	ENSMUST00000195356.1	411	No protein	Retained intron	-	-	TSL:2

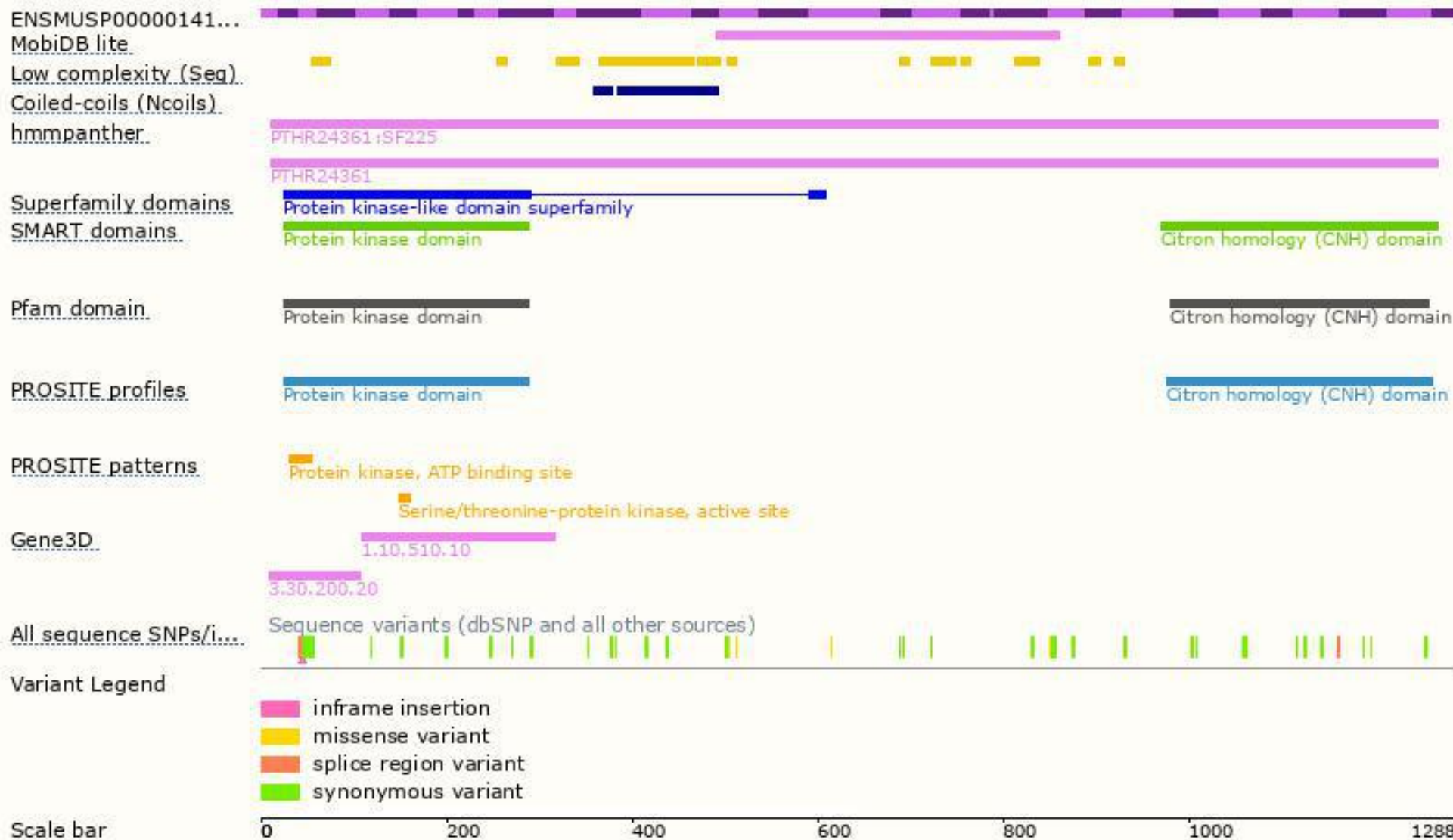
The strategy is based on the design of *Map4k4-218* transcript,The transcription is shown below



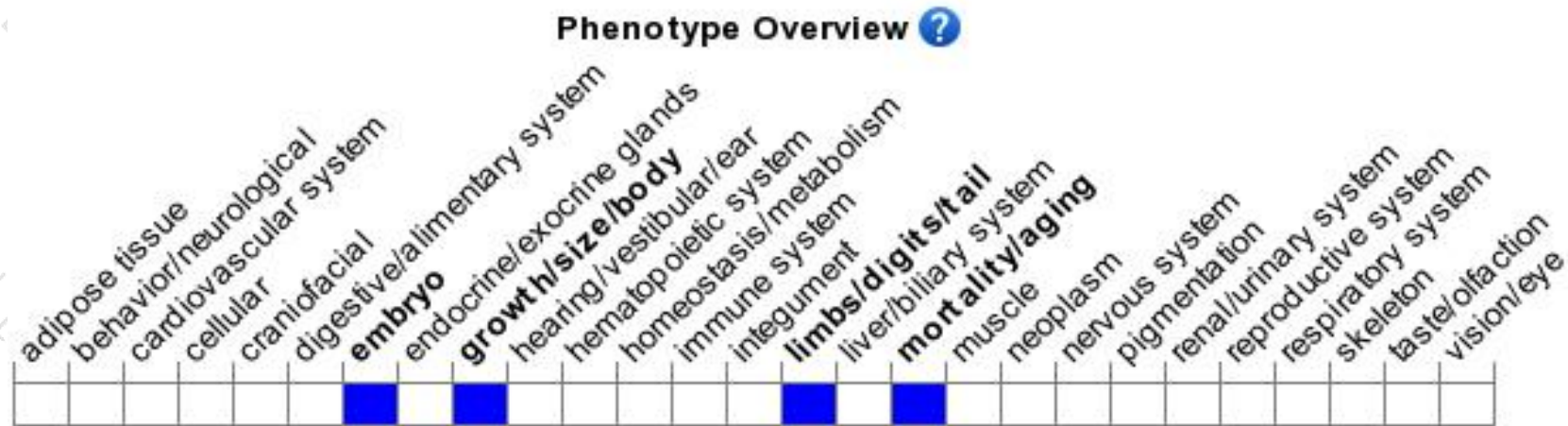
Genomic location distribution



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, Mice homozygous for disruptions in this gene die as embryos around day E9.5-10.5.

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

Tel: 400-9660890

