

Usp5 Cas9-CKO Strategy

Designer:

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Design Date:

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Project Overview

Project Name

Usp5

Project type

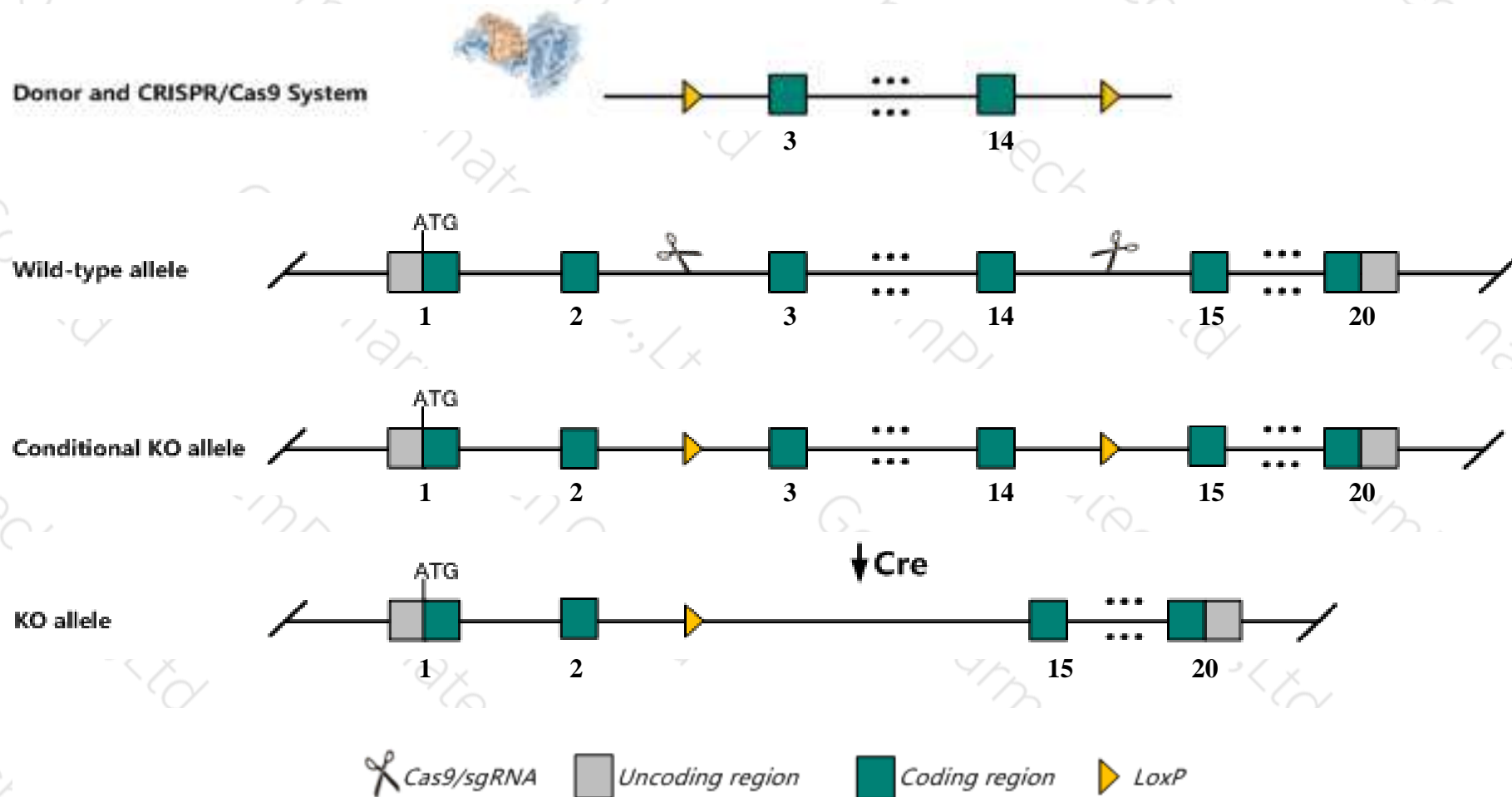
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Usp5* gene. The schematic diagram is as follows:



Technical routes

- The *Usp5* gene has 9 transcripts. According to the structure of *Usp5* gene, exon3-exon14 of *Usp5-201* (ENSMUST00000047510.9) transcript is recommended as the knockout region. The region contains 1525bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Usp5* 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/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 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, Mice homozygous for a transgenic gene disruption exhibit embryonic lethality at E7.
- The *Usp5* 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)

Usp5 ubiquitin specific peptidase 5 (isopeptidase T) [*Mus musculus* (house mouse)]

Gene ID: 22225, updated on 12-Aug-2019

Summary

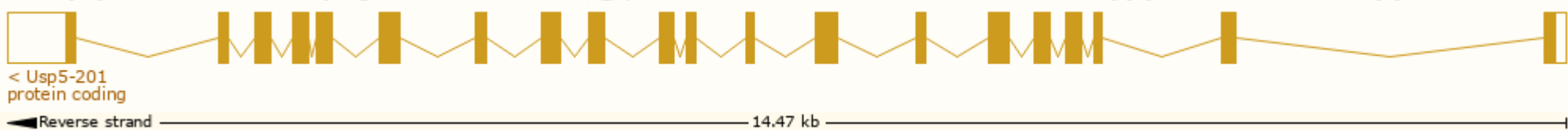
Official Symbol	Usp5 provided by MGI
Official Full Name	ubiquitin specific peptidase 5 (isopeptidase T) provided by MGI
Primary source	MGI:MGI:1347343
See related	Ensembl:ENSMUSG00000038429
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	ISOT; Ucht; ISOT-1; AA407472
Expression	Ubiquitous expression in ovary adult (RPKM 37.6), whole brain E14.5 (RPKM 37.3) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

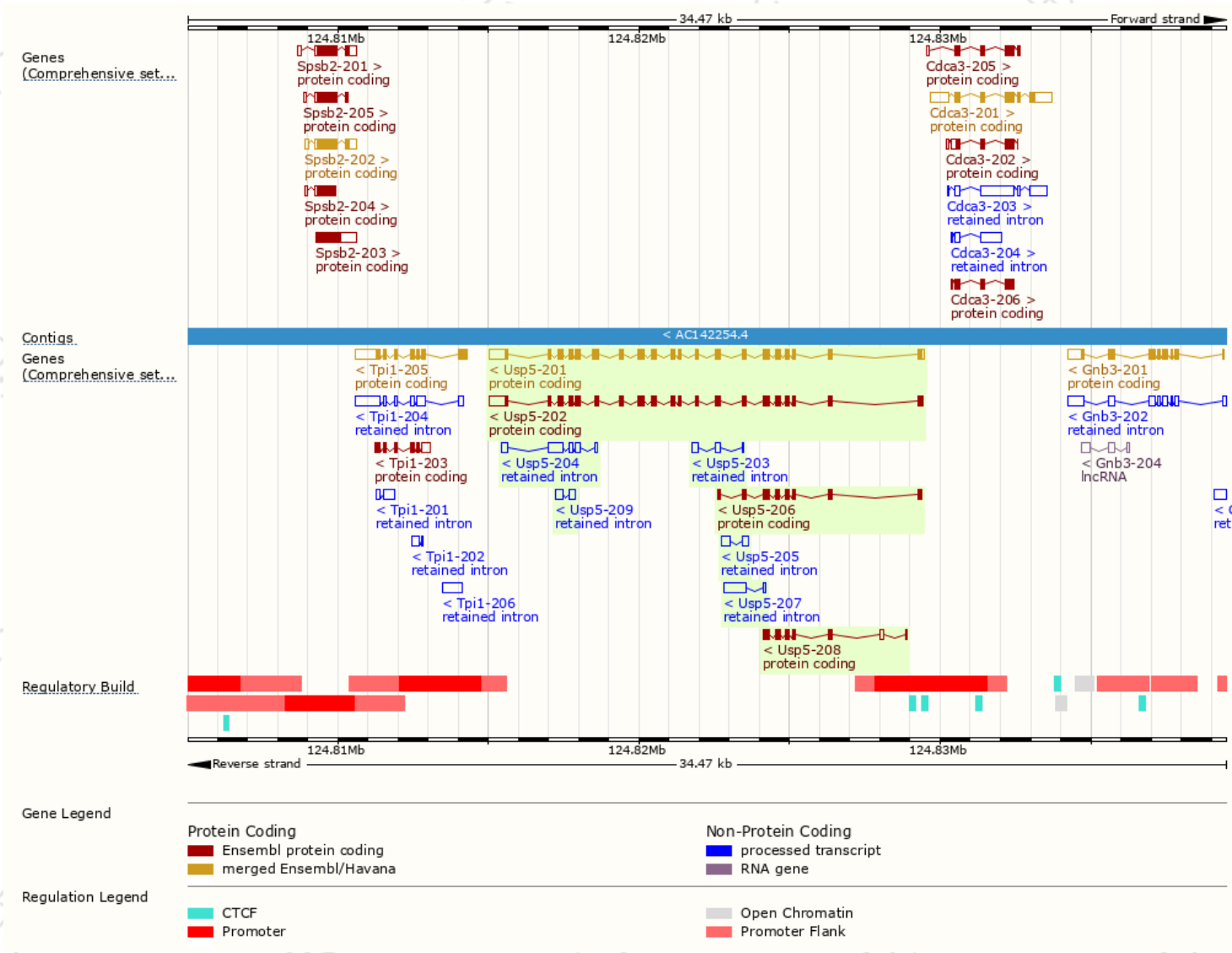
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Usp5-201	ENSMUST00000047510.9	3213	858aa	Protein coding	CCDS20531	P56399	TSL:1 GENCODE basic APPRIS P3
Usp5-202	ENSMUST00000122110.7	3076	835aa	Protein coding	CCDS85157	Q3U4W8	TSL:1 GENCODE basic APPRIS ALT1
Usp5-206	ENSMUST00000142058.7	924	303aa	Protein coding	-	D3Z4K7	CDS 3' incomplete TSL:3
Usp5-208	ENSMUST00000153306.1	825	209aa	Protein coding	-	D3YYA5	CDS 3' incomplete TSL:5
Usp5-204	ENSMUST00000131805.1	1112	No protein	Retained intron	-	-	TSL:5
Usp5-207	ENSMUST00000146098.1	774	No protein	Retained intron	-	-	TSL:3
Usp5-205	ENSMUST00000141042.1	464	No protein	Retained intron	-	-	TSL:3
Usp5-209	ENSMUST00000154189.1	453	No protein	Retained intron	-	-	TSL:3
Usp5-203	ENSMUST00000129159.1	394	No protein	Retained intron	-	-	TSL:5

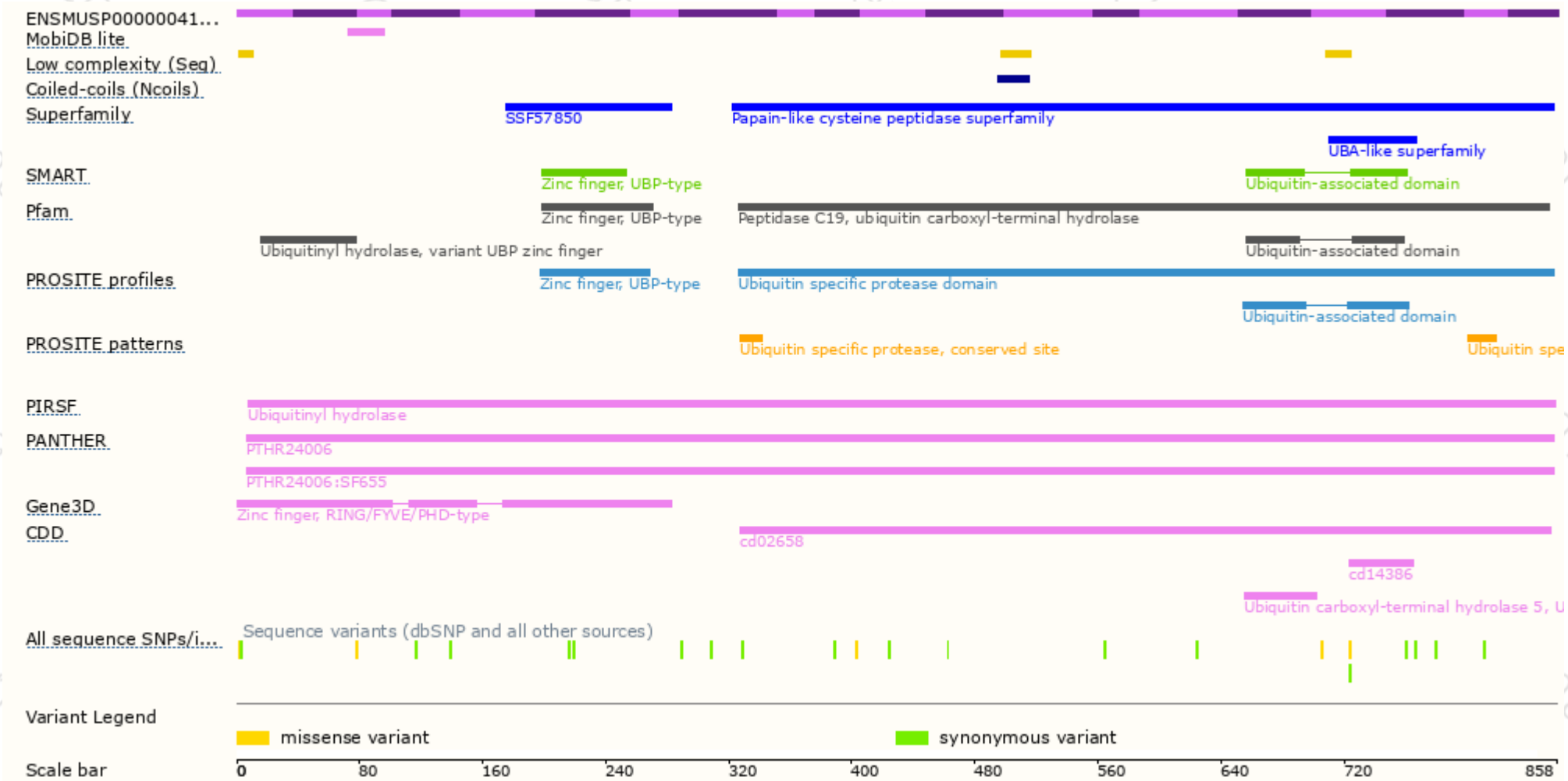
The strategy is based on the design of *Usp5-201* transcript,The transcription is shown below



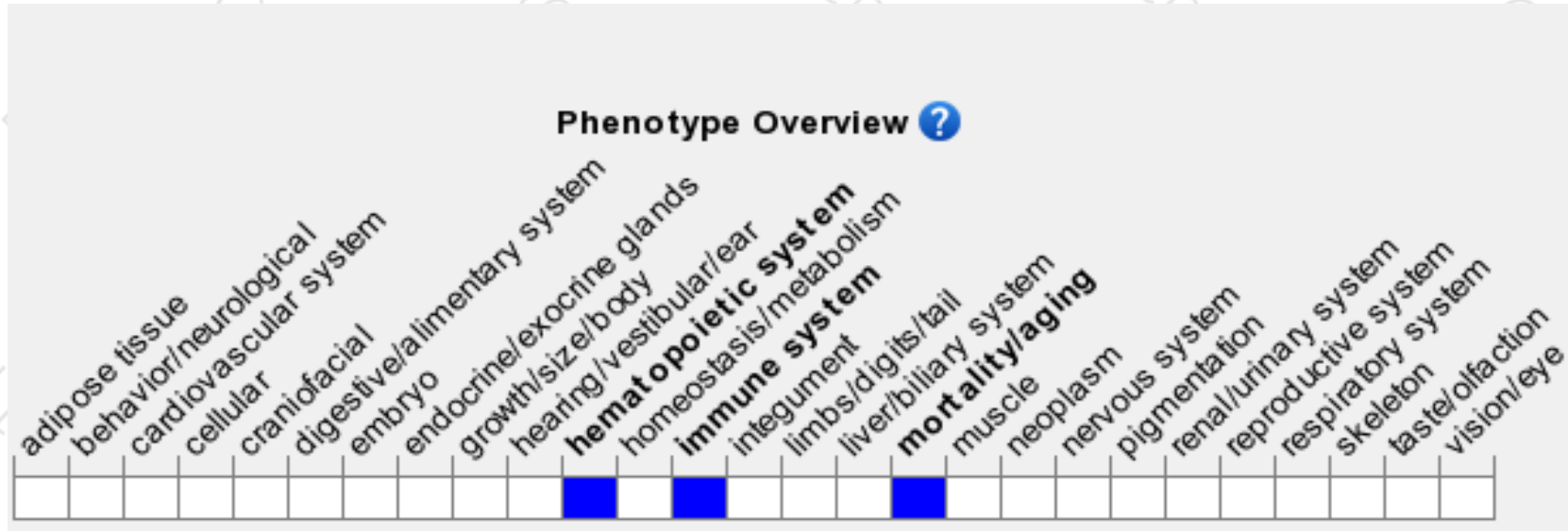
Genomic location distribution



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, Mice homozygous for a transgenic gene disruption exhibit embryonic lethality at E7.

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

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