Macroh2a1 Cas9-CKO Strategy

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Reviewer: Ruirui Zhang

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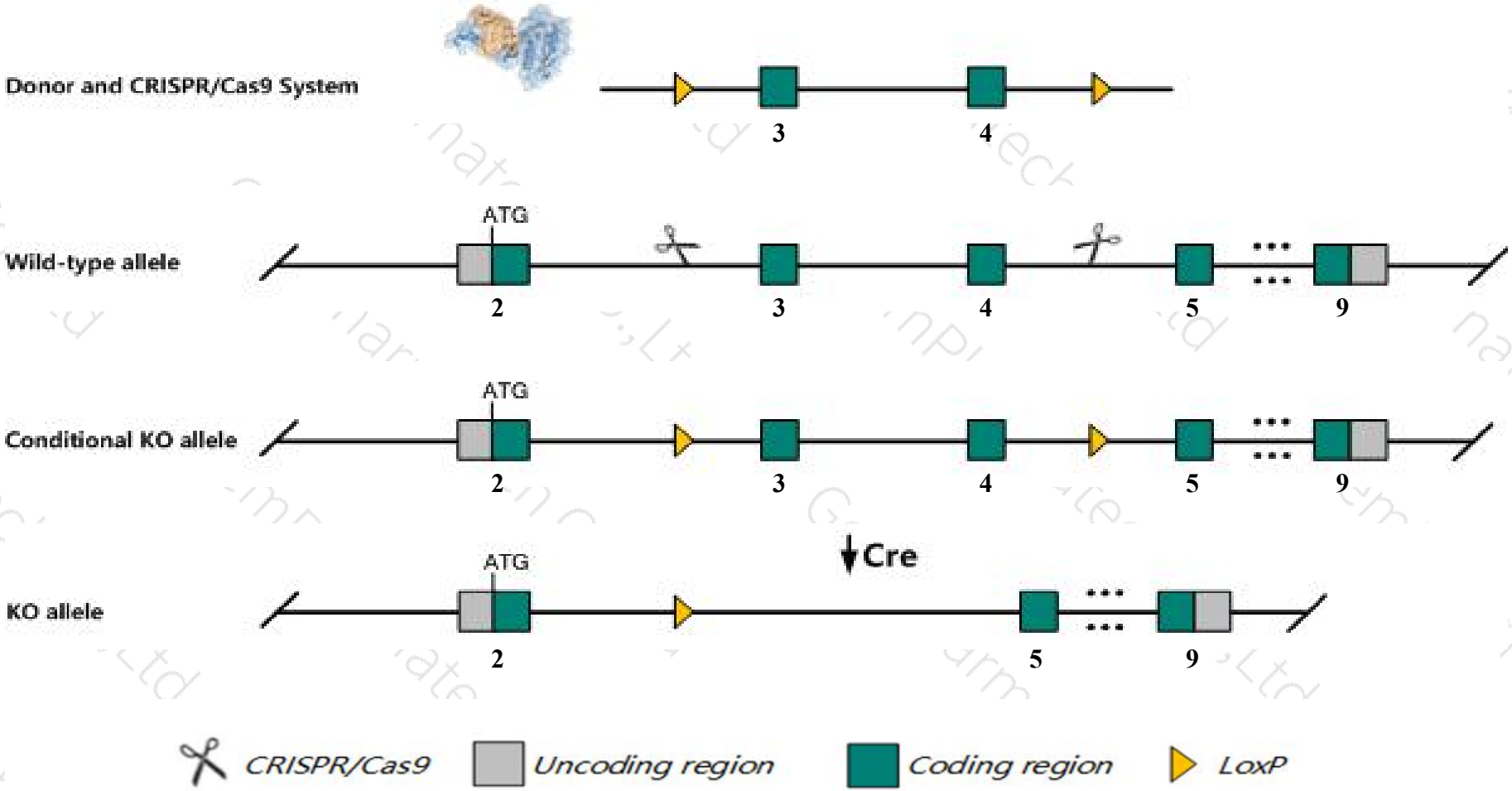
Project Overview

Project Name	***Macroh2a1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Macroh2a1* gene. The schematic diagram is as follows:



Technical routes

- The *Macroh2a1* gene has 8 transcripts. According to the structure of *Macroh2a1* gene, exon3-exon4 of *Macroh2a1-201* (ENSMUST00000016081.12) transcript is recommended as the knockout region.The region contains 305bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Macroh2a1* 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,Mice homozygous for one knock-out allele are viable and fertile and display no gross phenotypic abnormalities. Mice homozygous for a different knock-out allele exhibit female-specific hepatic steatosis.

➢ The *Macroh2a1* gene is located on the Chr13. 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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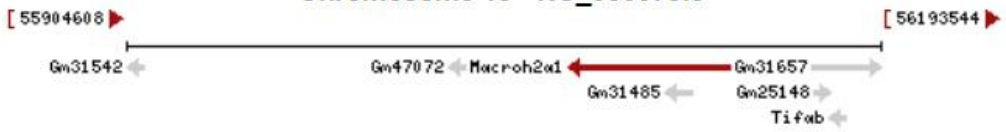
Macroh2a1 macroH2A.1 histone [*Mus musculus* (house mouse)]

Gene ID: 26914, updated on 10-Oct-2019

Summary

Official Symbol	Macroh2a1 provided by MGI
Official Full Name	macroH2A.1 histone provided by MGI
Primary source	MGI:MGI:1349392
See related	Ensembl:ENSMUSG00000015937
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	H2afy; mH2a1; H2AF12M
Summary	Histones are basic nuclear proteins that are responsible for the nucleosome structure of the chromosomal fiber in eukaryotes. Nucleosomes consist of approximately 146 bp of DNA wrapped around a histone octamer composed of pairs of each of the four core histones (H2A, H2B, H3, and H4). The chromatin fiber is further compacted through the interaction of a linker histone, H1, with the DNA between the nucleosomes to form higher order chromatin structures. This gene encodes a replication-independent histone that is a member of the histone H2A family. It replaces conventional H2A histones in a subset of nucleosomes where it represses transcription and participates in stable X chromosome inactivation. Alternative splicing results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Nov 2015]
Expression	Ubiquitous expression in thymus adult (RPKM 36.0), CNS E11.5 (RPKM 35.6) and 28 other tissues See more
Orthologs	human all

Chromosome 13 - NC_000079.6

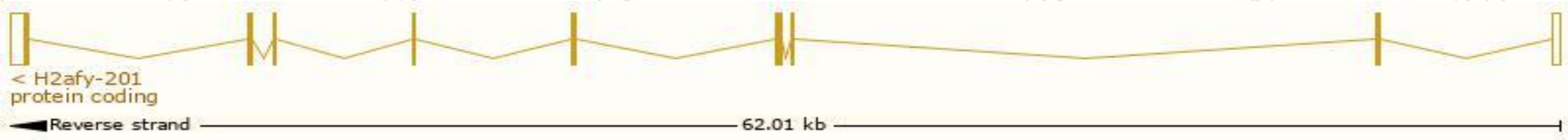


Transcript information（Ensembl）

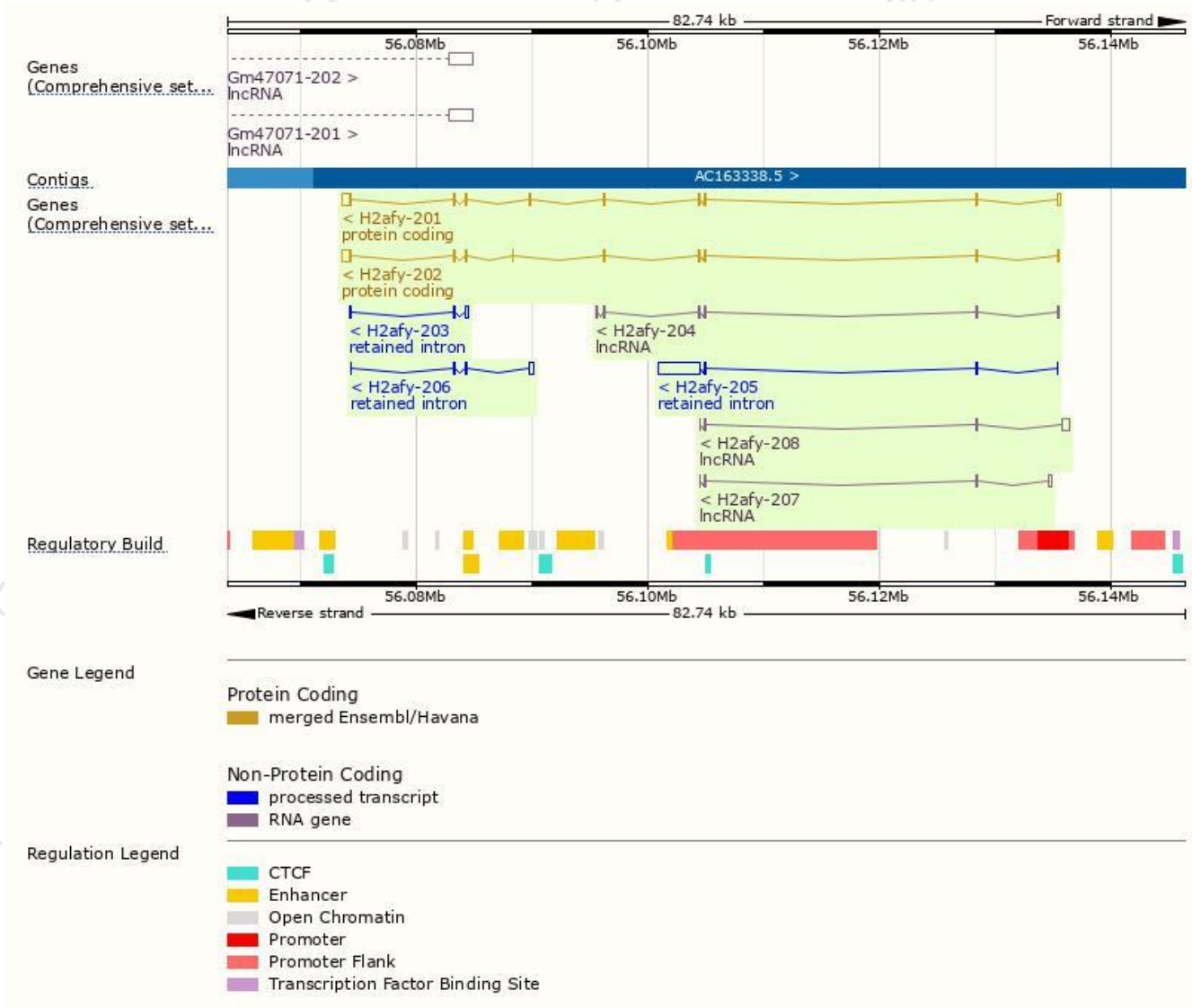
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
H2afy-201	ENSMUST00000016081.12	2046	372aa	ENSMUSP00000016081.6	Protein coding	CCDS26557	Q9QZQ8	TSL:1 GENCODE basic APPRIS P3
H2afy-202	ENSMUST00000045788.8	1933	369aa	ENSMUSP00000038221.7	Protein coding	CCDS49278	Q9QZQ8	TSL:1 GENCODE basic APPRIS ALT1
H2afy-205	ENSMUST00000141031.7	4018	No protein	-	Retained intron	-	-	TSL:1
H2afy-203	ENSMUST00000137835.7	728	No protein	-	Retained intron	-	-	TSL:2
H2afy-206	ENSMUST00000141589.1	600	No protein	-	Retained intron	-	-	TSL:2
H2afy-208	ENSMUST00000154778.7	942	No protein	-	lncRNA	-	-	TSL:3
H2afy-204	ENSMUST00000139511.7	889	No protein	-	lncRNA	-	-	TSL:1
H2afy-207	ENSMUST00000154564.1	547	No protein	-	lncRNA	-	-	TSL:3

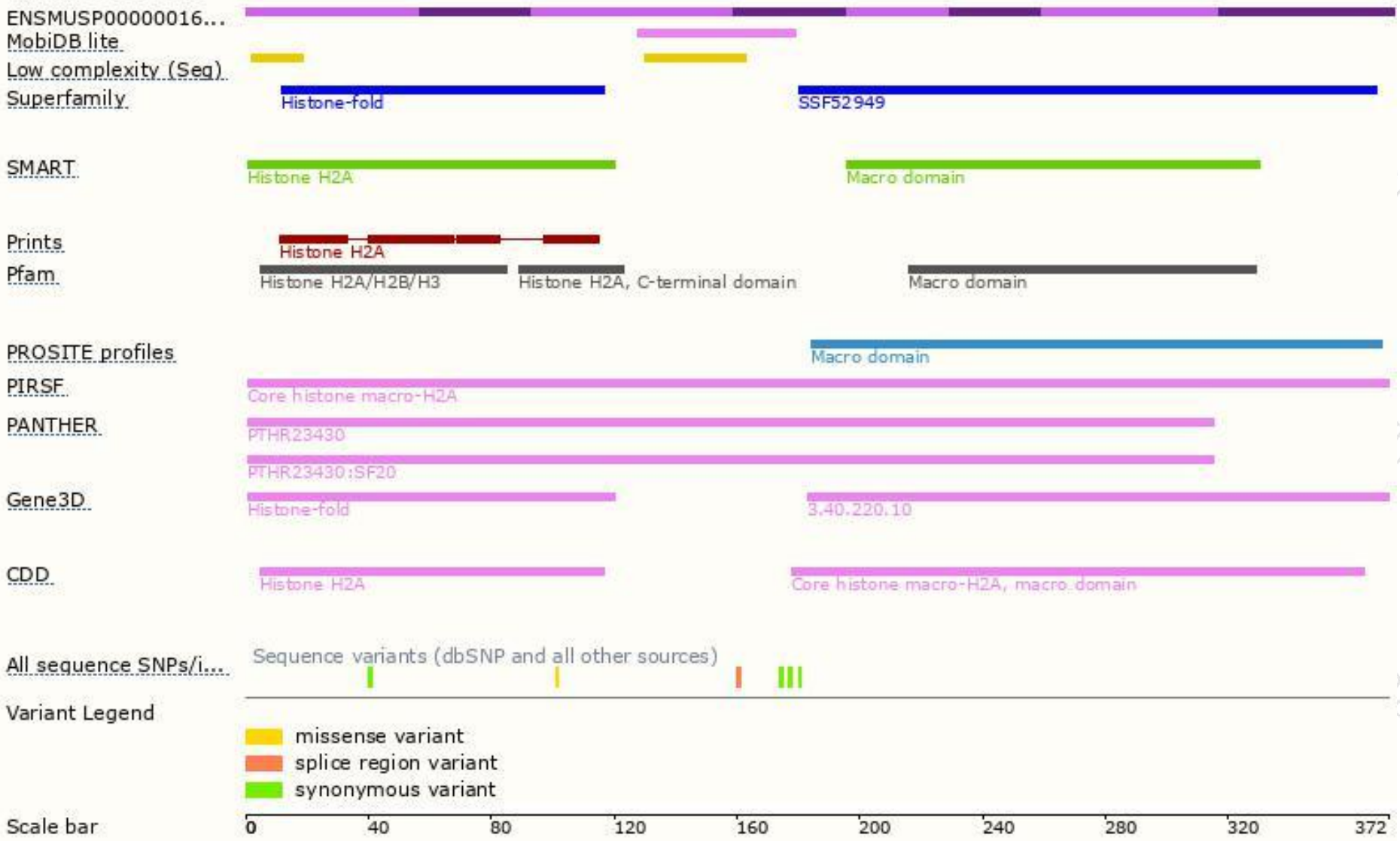
The strategy is based on the design of *Macroh2a1-201* transcript,The transcription is shown below



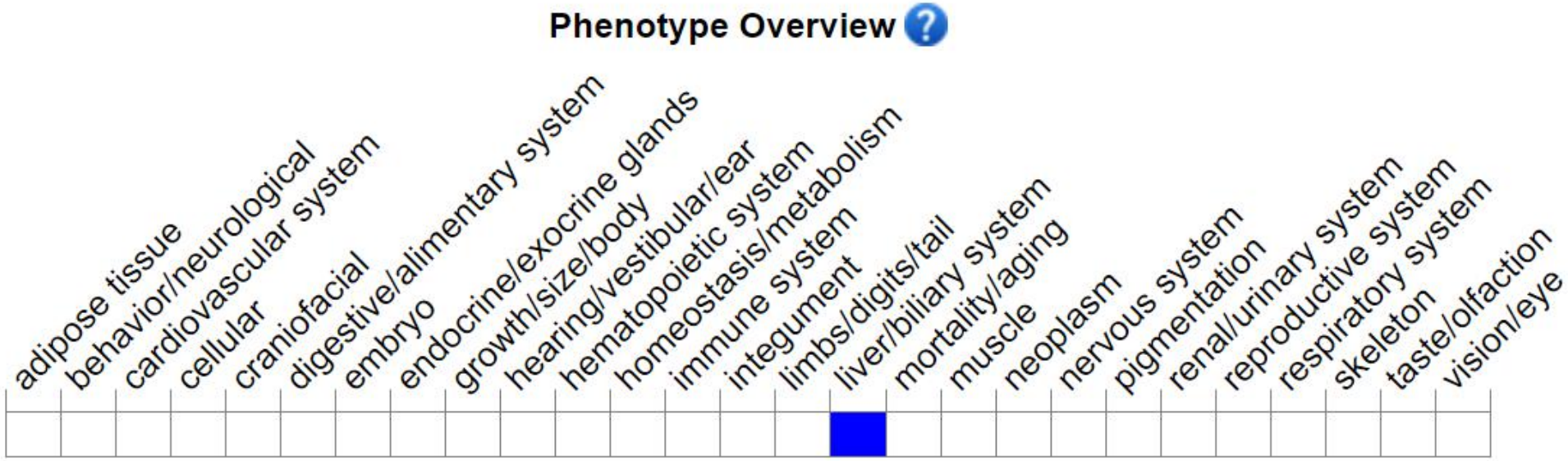
Genomic location distribution



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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