Apod Cas9-CKO Strategy

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Reviewer:	**Xueting Zhang**
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Project Overview

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

Conditional Knockout strategy

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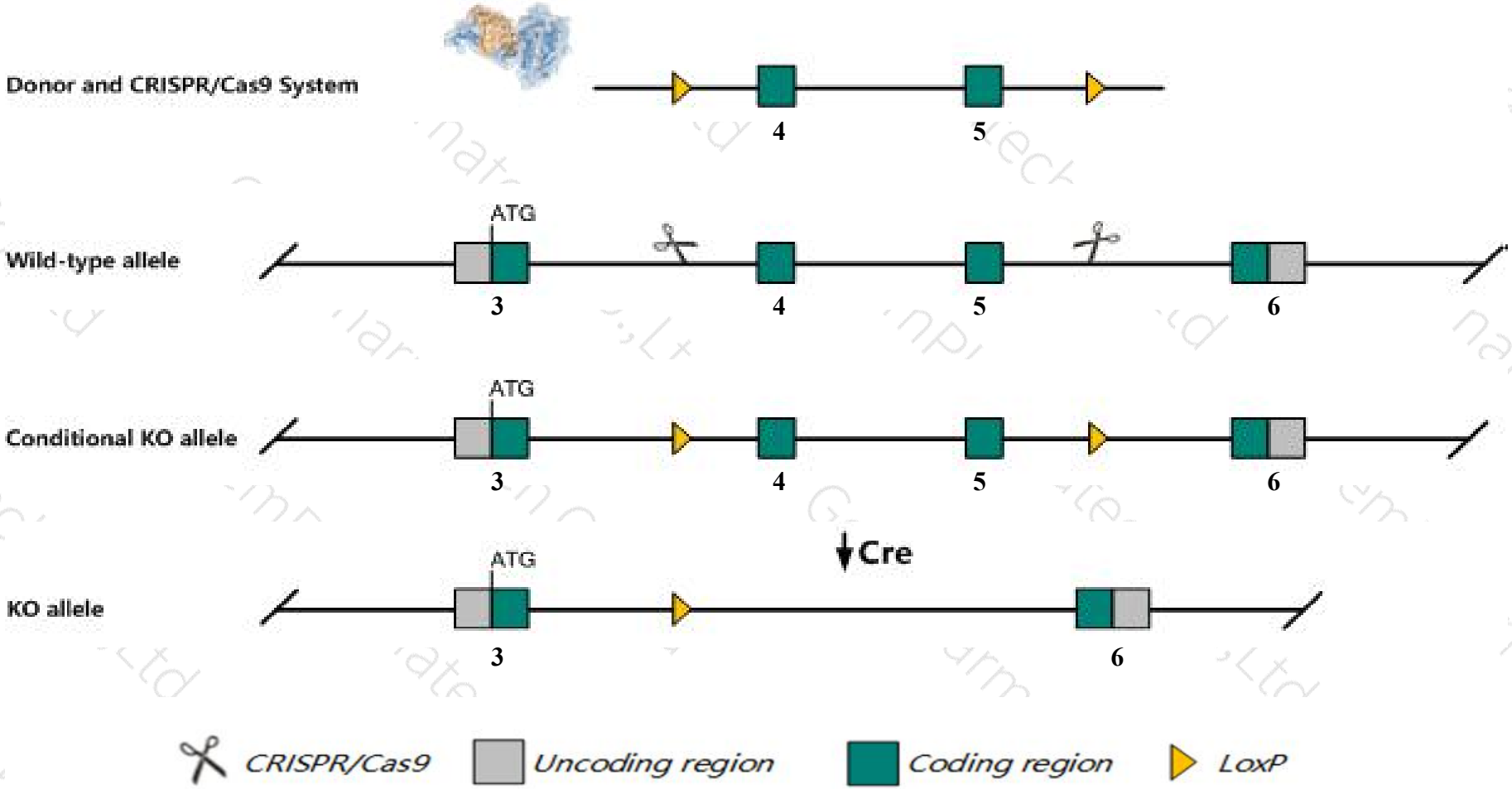
This model will use CRISPR/Cas9 technology to edit the *Apod* gene. The schematic diagram is as follows:



Technical routes

- The *Apod* gene has 6 transcripts. According to the structure of *Apod* gene, exon4-exon5 of *Apod-203* (ENSMUST00000130560.7) transcript is recommended as the knockout region.The region contains 211bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Apod* 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 null allele display increased sensitivity to reactive oxygen species, impaired motor and spatial learning, and decreased vertical and horizontal activity.

- The *Apod* gene is located on the Chr16. 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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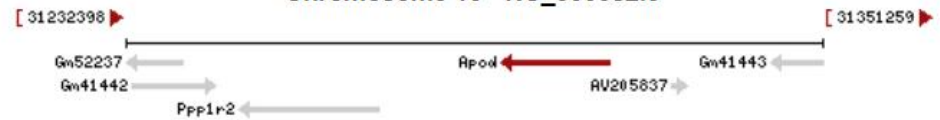
Apod apolipoprotein D [*Mus musculus* (house mouse)]

Gene ID: 11815, updated on 8-Oct-2019

Summary

Official Symbol	Apod provided by MGI
Official Full Name	apolipoprotein D provided by MGI
Primary source	MGI:MGI:88056
See related	Ensembl:ENSMUSG00000022548
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
Summary	The protein encoded by this gene is a component of high-density lipoprotein (HDL), but is unique in that it shares greater structural similarity to lipocalin than to other members of the apolipoprotein family, and has a wider tissue expression pattern. The encoded protein is involved in lipid metabolism, and ablation of this gene results in defects in triglyceride metabolism. Elevated levels of this gene product have been observed in multiple tissues of Niemann-Pick disease mouse models, as well as in some tumors. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Aug 2014]
Expression	Biased expression in frontal lobe adult (RPKM 55.7), cerebellum adult (RPKM 53.8) and 9 other tissues See more
Orthologs	human all

Chromosome 16 - NC_000082.6

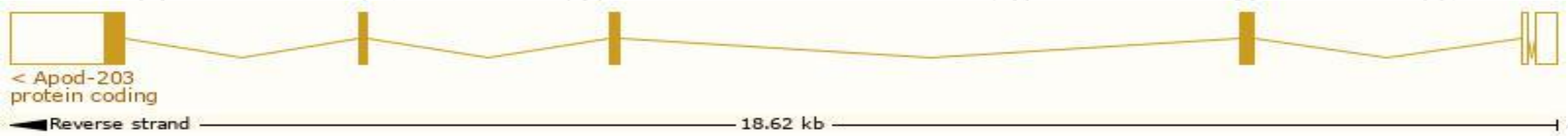


Transcript information（Ensembl）

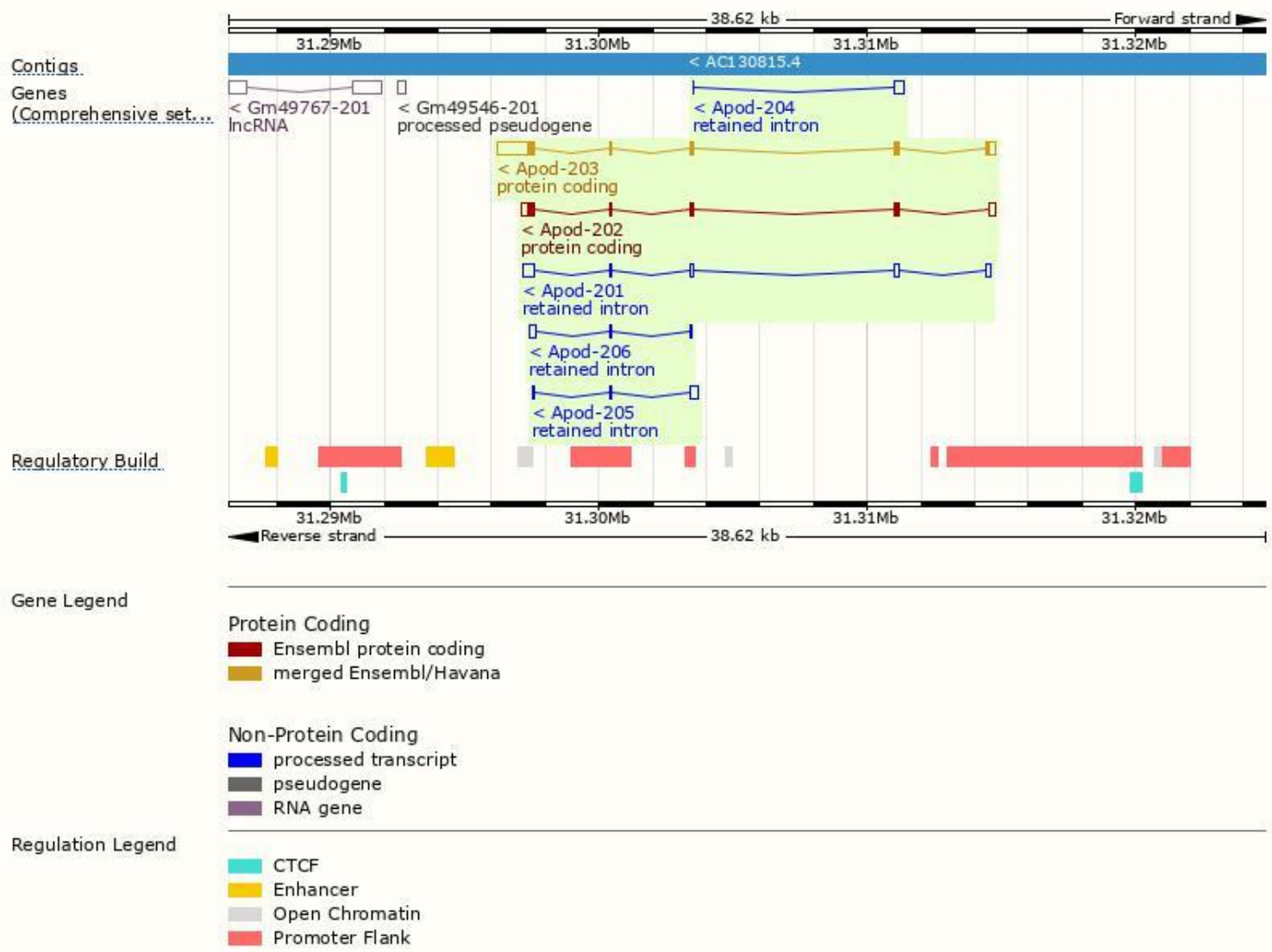
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Apod-203	ENSMUST00000130560.7	2074	189aa	ENSMUSP00000119827.1	Protein coding	CCDS28105	P51910	TSL:1 GENCODE basic APPRIS P1
Apod-202	ENSMUST00000115230.1	1070	189aa	ENSMUSP00000110885.1	Protein coding	CCDS28105	P51910	TSL:1 GENCODE basic APPRIS P1
Apod-201	ENSMUST00000023207.14	979	No protein	-	Retained intron	-	-	TSL:1
Apod-205	ENSMUST00000155682.1	422	No protein	-	Retained intron	-	-	TSL:2
Apod-206	ENSMUST00000156456.7	371	No protein	-	Retained intron	-	-	TSL:3
Apod-204	ENSMUST00000145837.1	362	No protein	-	Retained intron	-	-	TSL:3

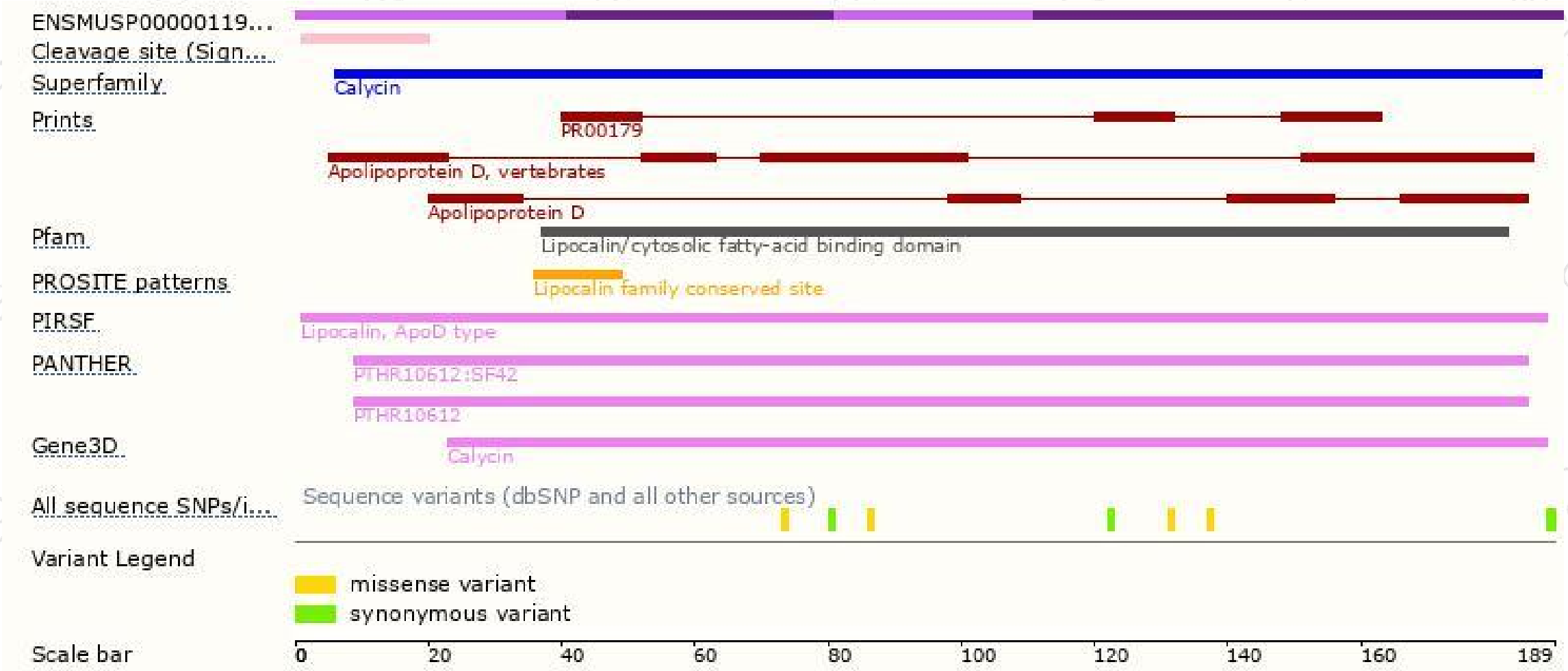
The strategy is based on the design of *Apod-203* transcript,The transcription is shown below



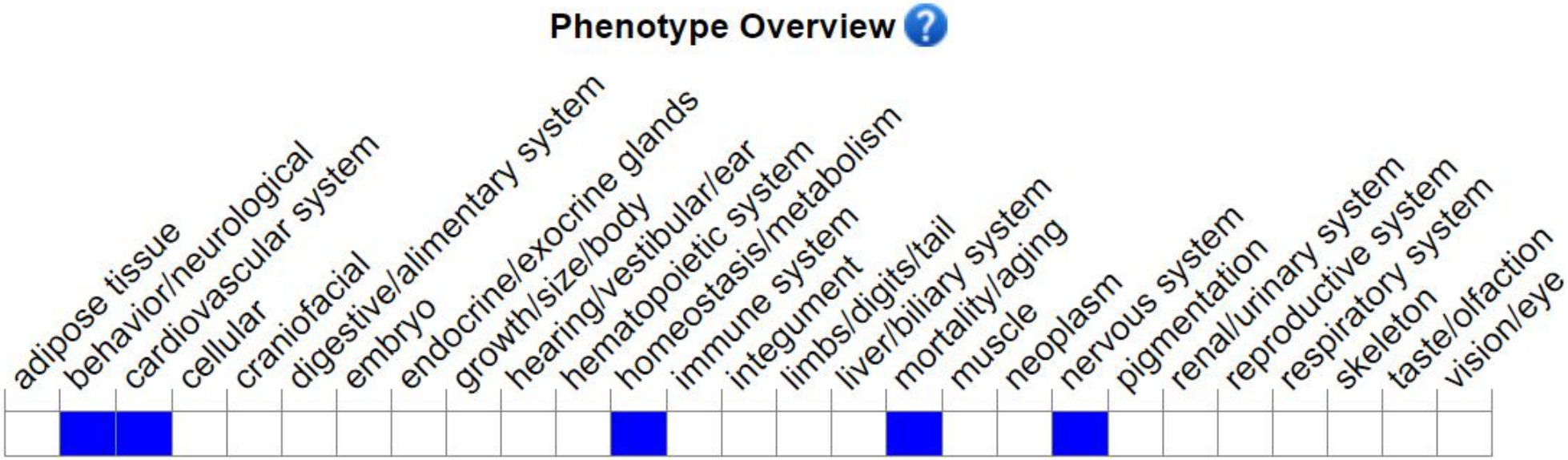
Genomic location distribution



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 one null allele display increased sensitivity to reactive oxygen species, impaired motor and spatial learning, and decreased vertical and horizontal activity.

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

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