Rpa1 Cas9-CKO Strategy

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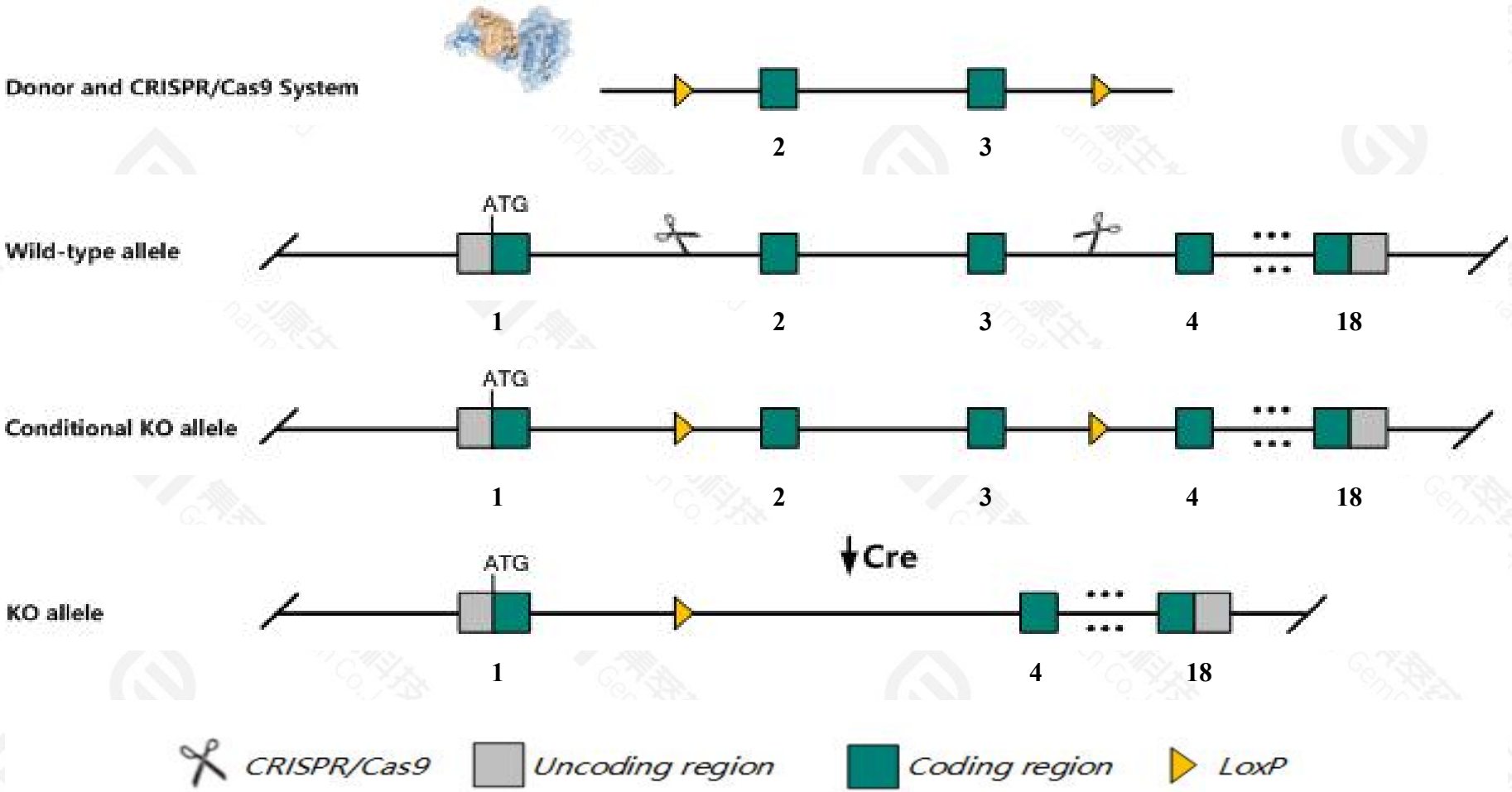
Project Overview

Project Name	***Rpa1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rpa1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Rpa1* gene has 5 transcripts. According to the structure of *Rpa1* gene, exon2-exon3 of *Rpa1-201*(ENSMUST00000000767.6) transcript is recommended as the knockout region.The region contains 130bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Rpa1* 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,homozygous null mice display embryonic lethality before implantation and impaired cell proliferation. Heterozygous null mice display decreased survival, chromosomal instability, impaired double strand break repair, and develop lymphomas.

➢ The *Rpa1* gene is located on the Chr11. 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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Rpa1 replication protein A1 [Mus musculus (house mouse)]

Gene ID: 68275, updated on 17-Dec-2020

Summary

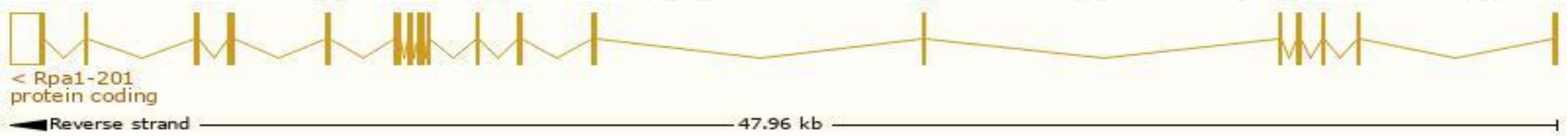
Official Symbol	Rpa1 provided by MGI
Official Full Name	replication protein A1 provided byMGI
Primary source	MGI:MGI:1915525
See related	Ensembl:ENSMUSG00000000751
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	5031405K23Rik, 70kD, 70kDa, AA589576, AW557552, R, RF, RF-A, RP-A, Rpa
Expression	Ubiquitous expression in liver E14 (RPKM 24.0), liver E14.5 (RPKM 21.1) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

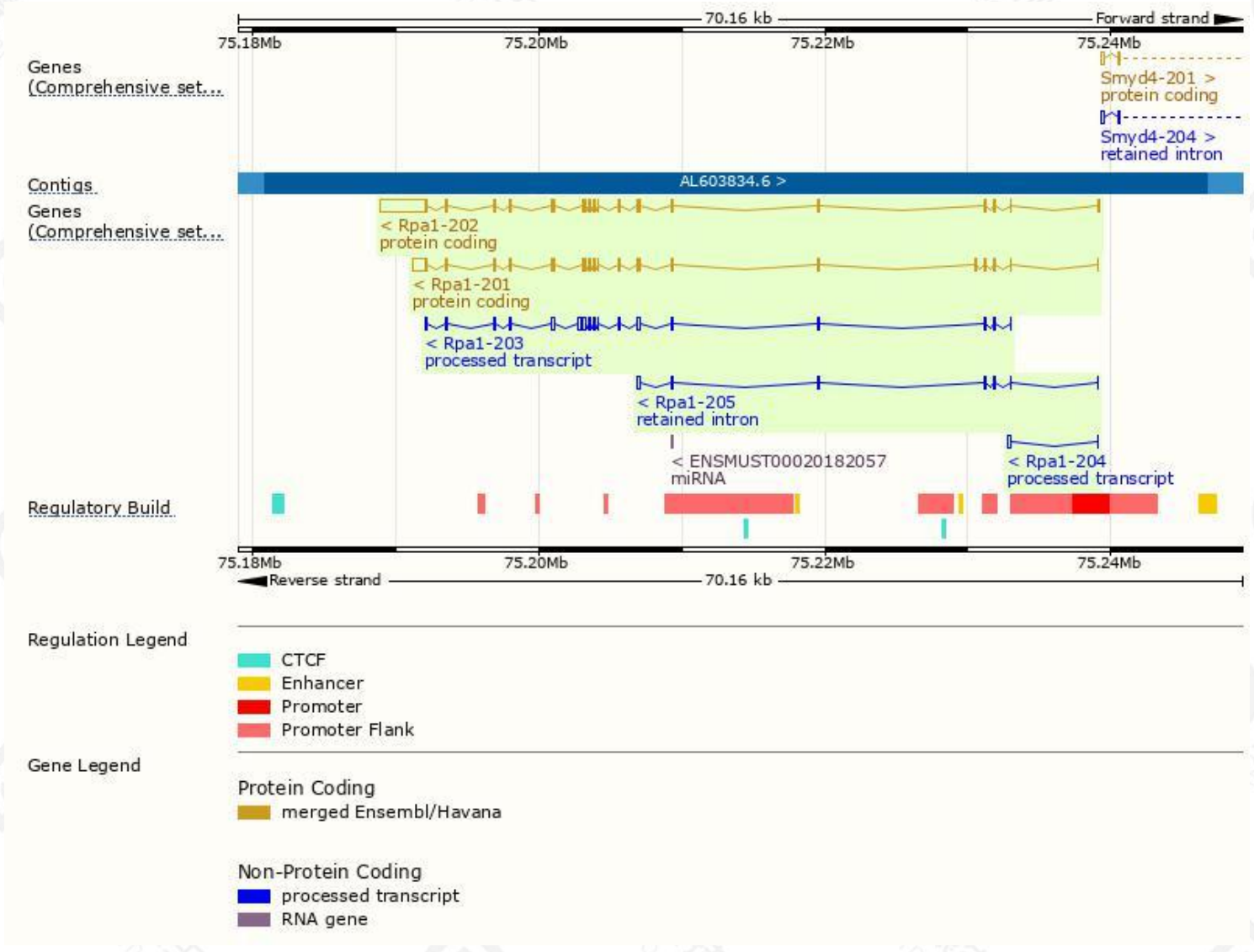
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rpa1-202	ENSMUST00000092907.12	5077	623aa	Protein coding	CCDS25044		TSL:1 , GENCODE basic , APPRIS P1 ,
Rpa1-201	ENSMUST00000000767.6	2942	644aa	Protein coding	CCDS48847		TSL:1 , GENCODE basic ,
Rpa1-203	ENSMUST00000135770.8	2040	No protein	Processed transcript	-		TSL:5 ,
Rpa1-204	ENSMUST00000149365.2	285	No protein	Processed transcript	-		TSL:2 ,
Rpa1-205	ENSMUST00000154894.2	716	No protein	Retained intron	-		TSL:2 ,

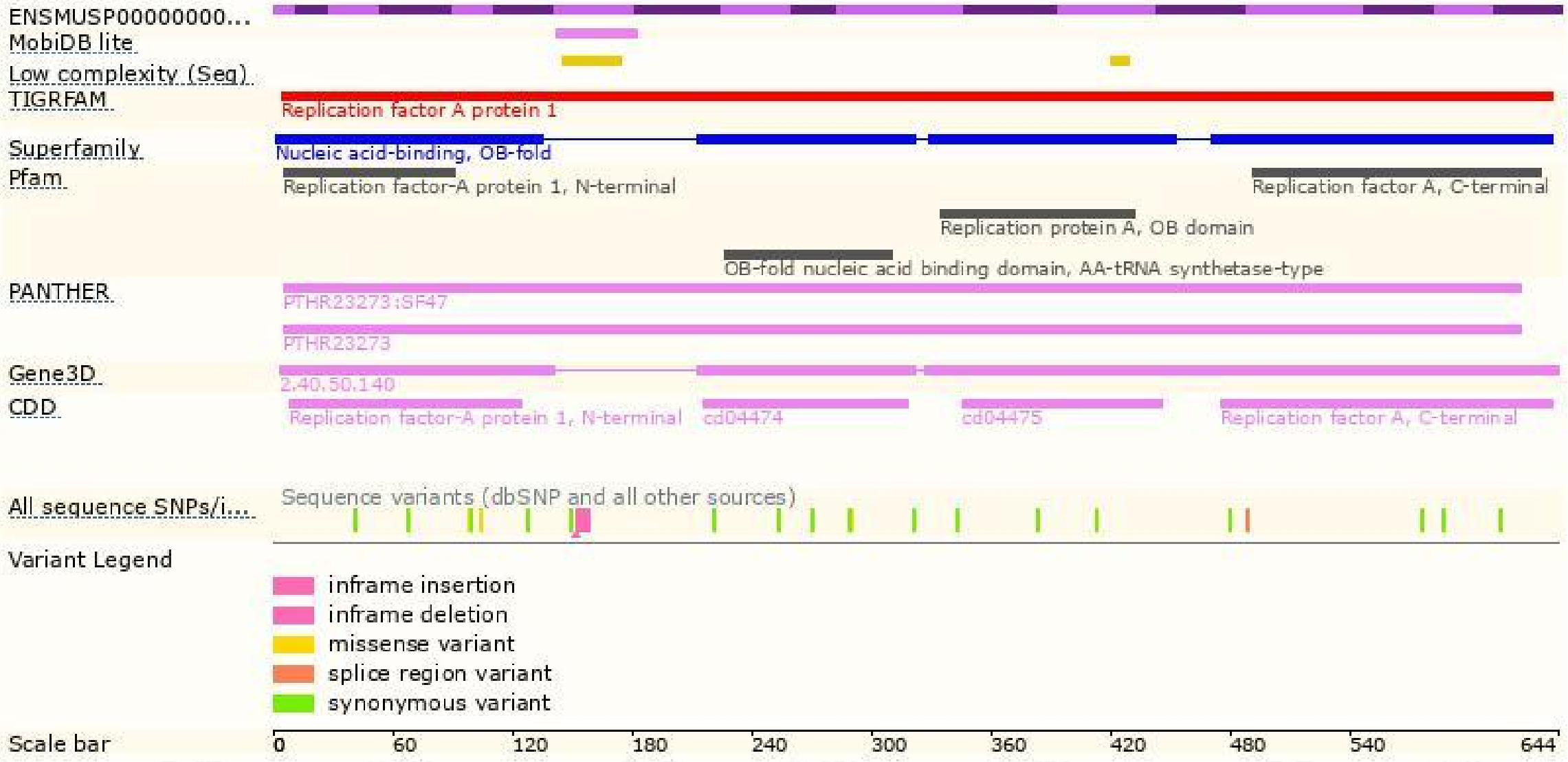
The strategy is based on the design of *Rpa1-201* transcript,the transcription is shown below:



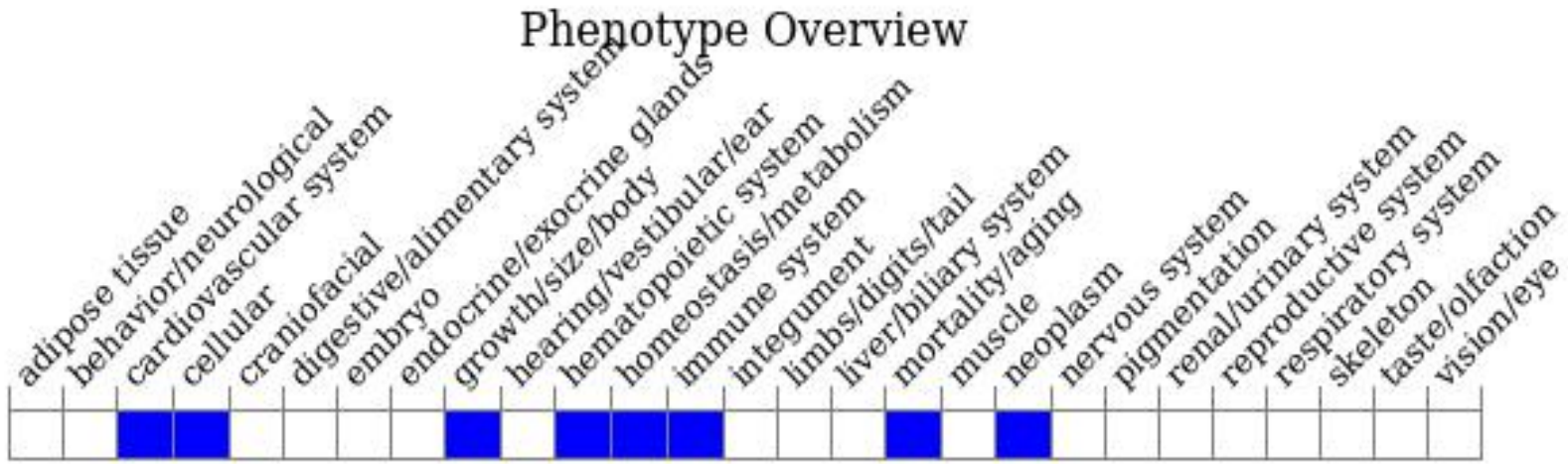
Genomic location distribution



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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