Rfwd3 Cas9-KO Strategy

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Reviewer: Daohua Xu

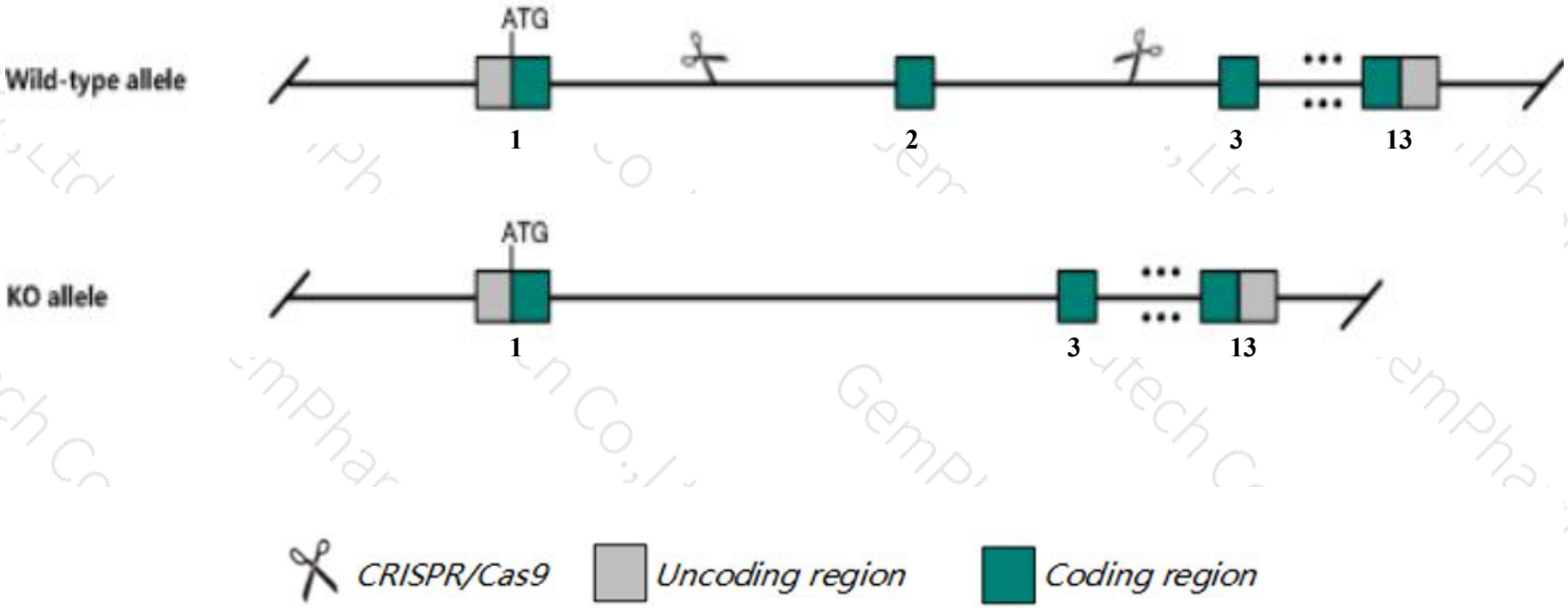
Design Date: 2020-10-13

Project Overview

Project Name	***Rfwd3***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rfwd3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Rfwd3* gene has 2 transcripts. According to the structure of *Rfwd3* gene, exon2 of *Rfwd3-201*(ENSMUST00000038739.4) transcript is recommended as the knockout region.The region contains 508bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Rfwd3* gene. The brief process is as follows: CRISPR/Cas9 system 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.

Notice

➢ According to the existing MGI data,mice homozygous for a knock-out allele exhibit female and male fertility (possibly infertility), gonad atrophy, oligospermia, failure of follicular development, increased cellular sensitivity to MMC treatment, and premature death.

➢ The *Rfwd3* gene is located on the Chr8. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Rfwd3 ring finger and WD repeat domain 3 [Mus musculus (house mouse)]

Gene ID: 234736, updated on 13-Mar-2020

Summary

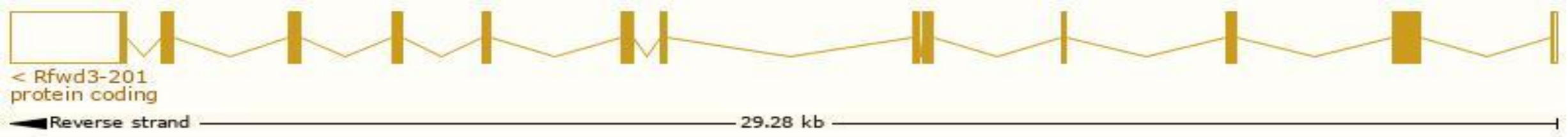
Official Symbol	Rfwd3 provided by MGI
Official Full Name	ring finger and WD repeat domain 3 provided byMGI
Primary source	MGI:MGI:2384584
See related	Ensembl:ENSMUSG00000033596
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	BC027246
Expression	Ubiquitous expression in liver E14 (RPKM 16.9), placenta adult (RPKM 16.6) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

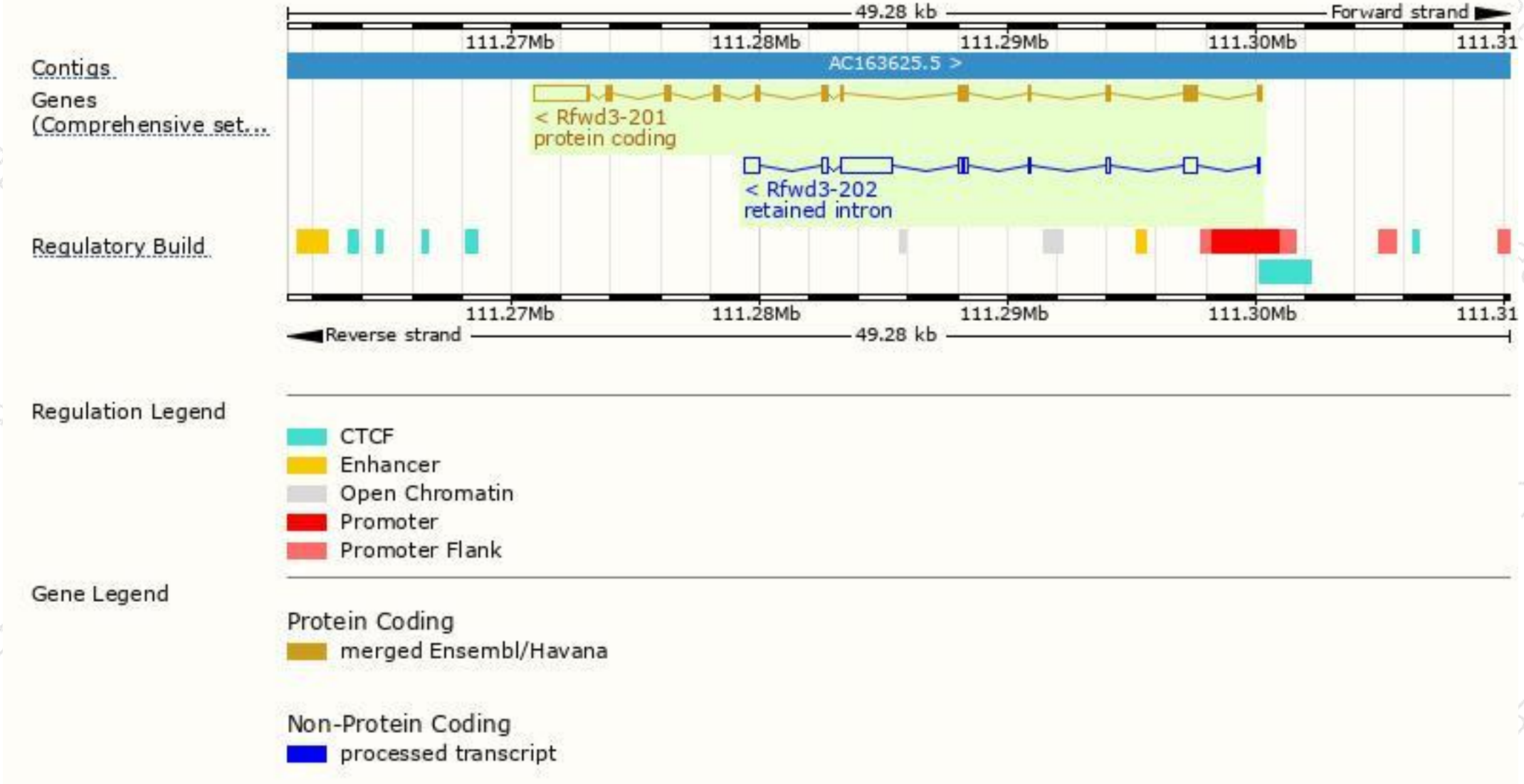
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rfwd3-201	ENSMUST00000038739.4	4443	774aa	Protein coding	CCDS22672	Q8CIK8	TSL:1 GENCODE basic APPRIS P1
Rfwd3-202	ENSMUST00000212958.1	4082	No protein	Retained intron	-	-	TSL:5

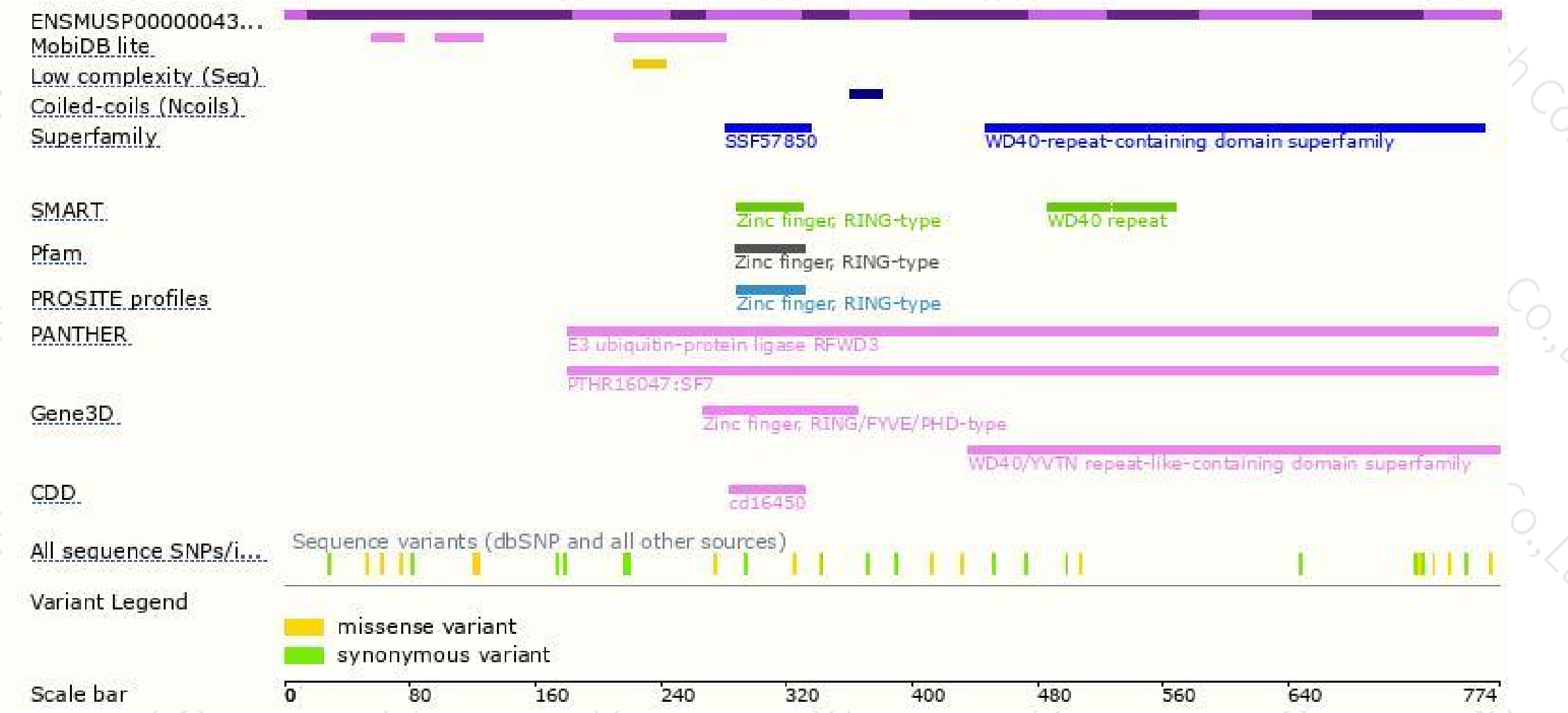
The strategy is based on the design of *Rfwd3-201* transcript,the transcription is shown below:



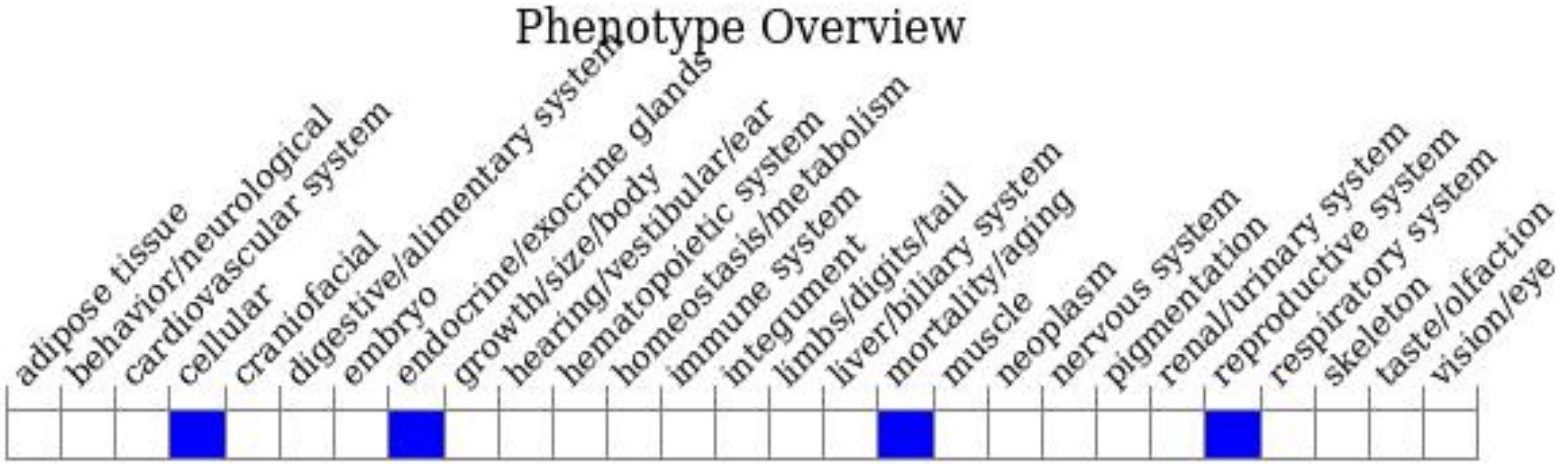
Genomic location distribution



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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