Six2 Cas9-KO Strategy

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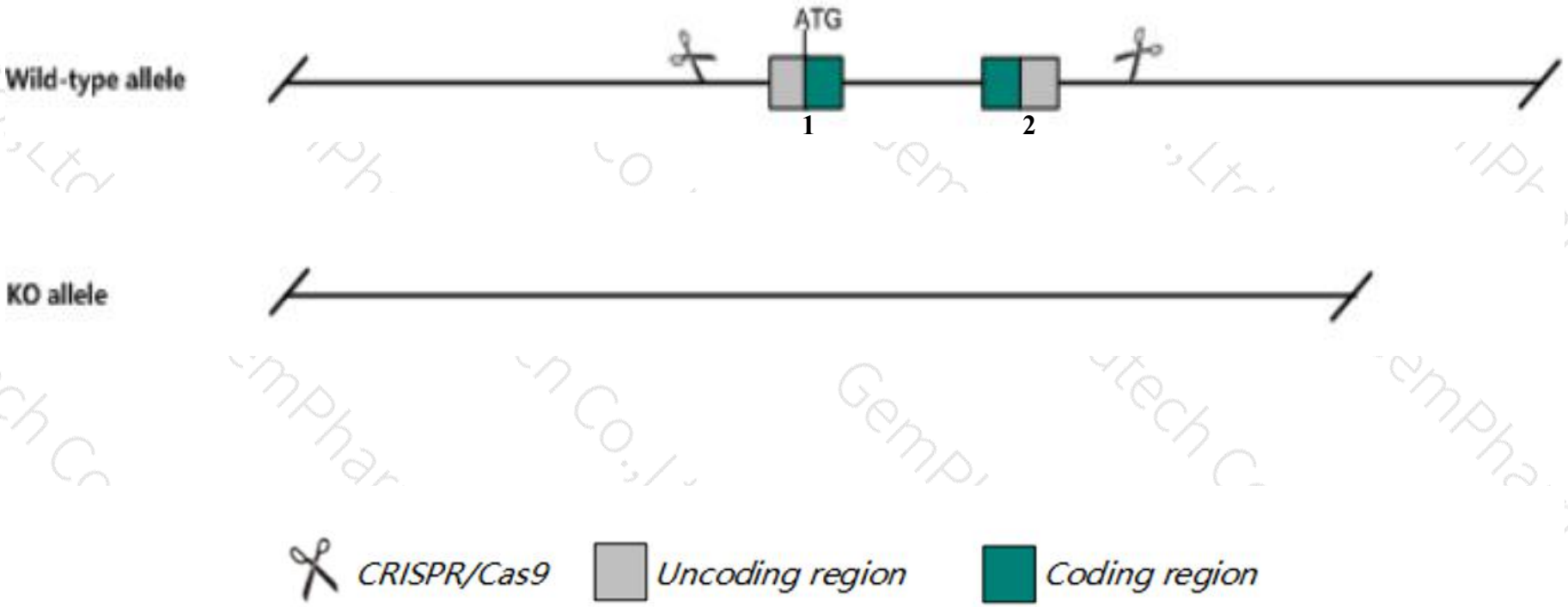
Project Overview

Project Name	***Six2***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Six2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Six2* gene has 2 transcripts. According to the structure of *Six2* gene, exon1-exon2 of *Six2-202*(ENSMUST00000163568.3) transcript is recommended as the knockout region.The region contains all of the coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Six2* 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 disruptions in this gene die shortly after birth and exhibit abnormal kidney development. Abnormalities include small kidney, lack of ureteric bud branches throughout the kidney, increased apoptosis and premature and arrested nephron development.

➢ The KO region contains functional region of the Six2 gene.Knockout the region may affect the function of CJ186046Rik gene.

➢ The *Six2* gene is located on the Chr17. 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）

集萃药康 GemPharmatech

Six2 sine oculis-related homeobox 2 [Mus musculus (house mouse)]

Gene ID: 20472, updated on 13-Mar-2020

Summary

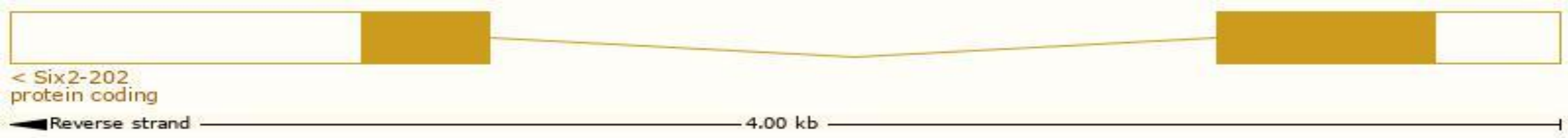
Official Symbol	Six2 provided by MGI
Official Full Name	sine oculis-related homeobox 2 provided byMGI
Primary source	MGI:MGI:102778
See related	Ensembl:ENSMUSG00000024134
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Biased expression in limb E14.5 (RPKM 11.3), CNS E11.5 (RPKM 10.4) and 6 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

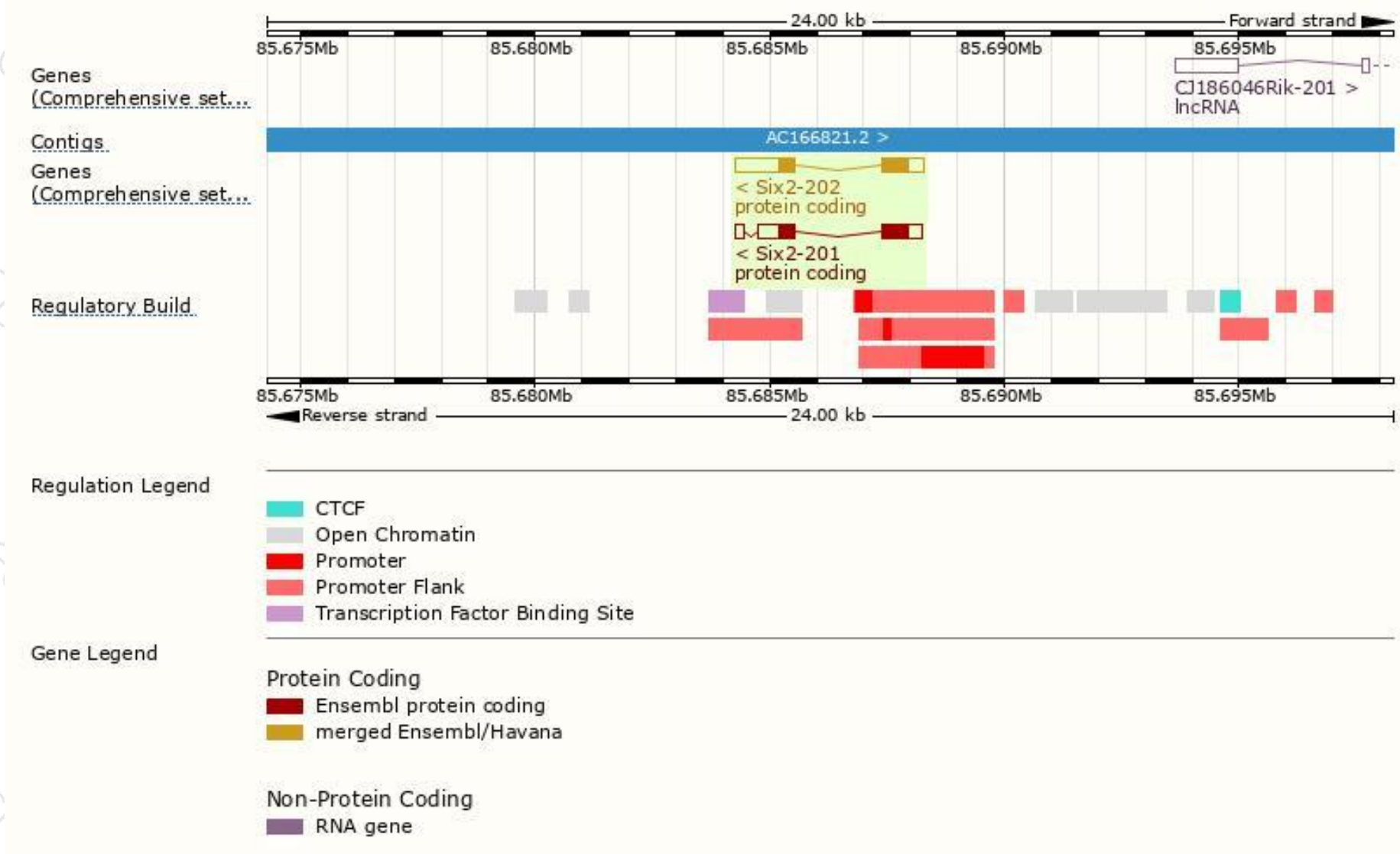
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Six2-202	ENSMUST00000163568.3	2119	296aa	Protein coding	CCDS29007	Q62232	TSL:1 GENCODE basic APPRIS P1
Six2-201	ENSMUST00000024947.7	1812	296aa	Protein coding	CCDS29007	Q62232	TSL:1 GENCODE basic APPRIS P1

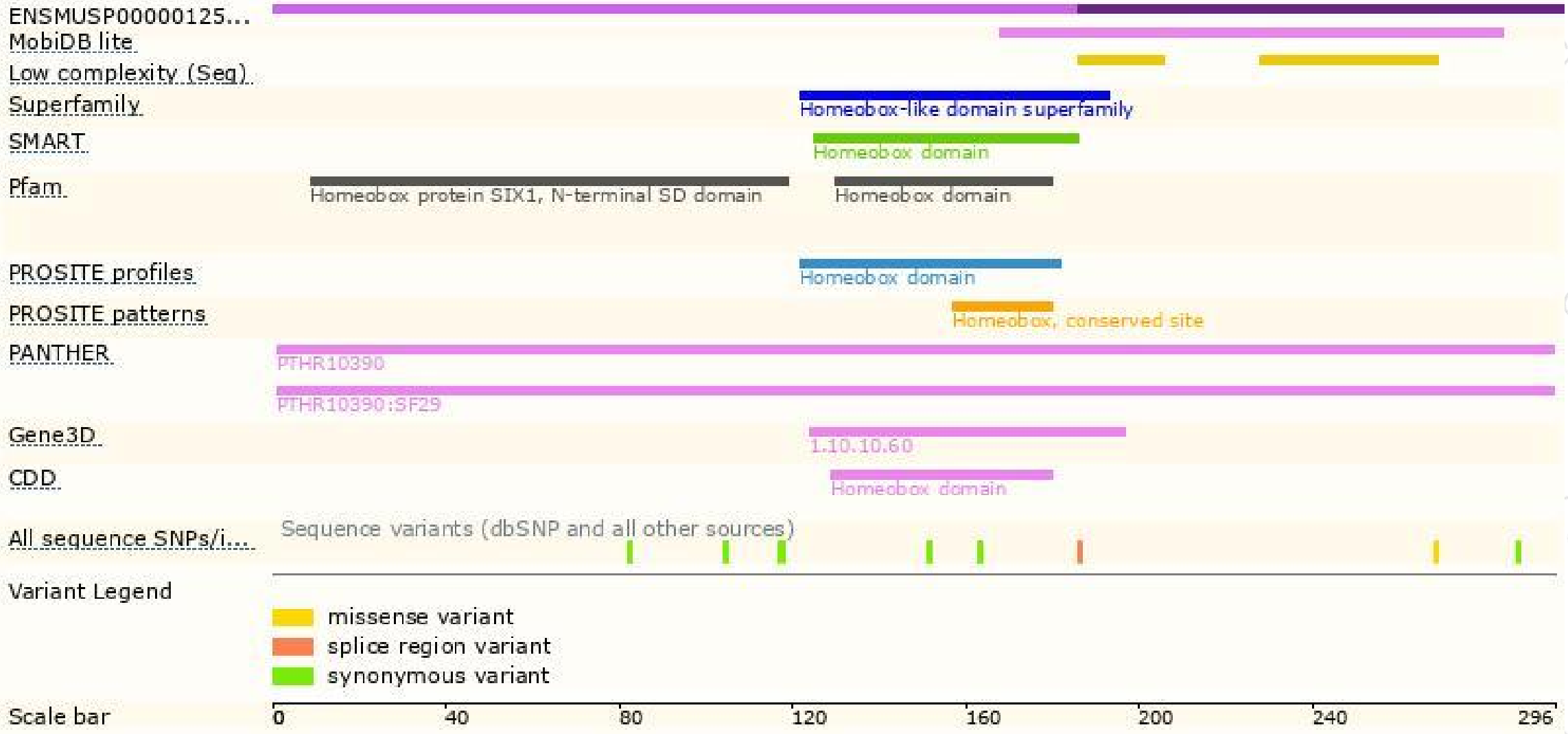
The strategy is based on the design of *Six2-202* transcript,the transcription is shown below:



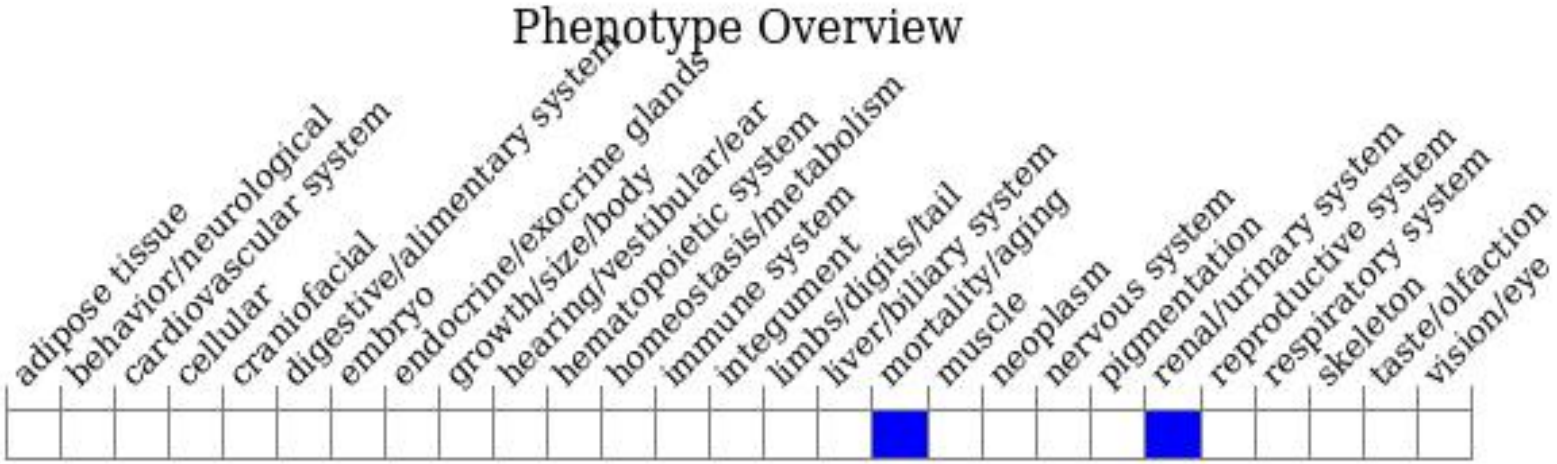
Genomic location distribution



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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