Sik3 Cas9-CKO Strategy

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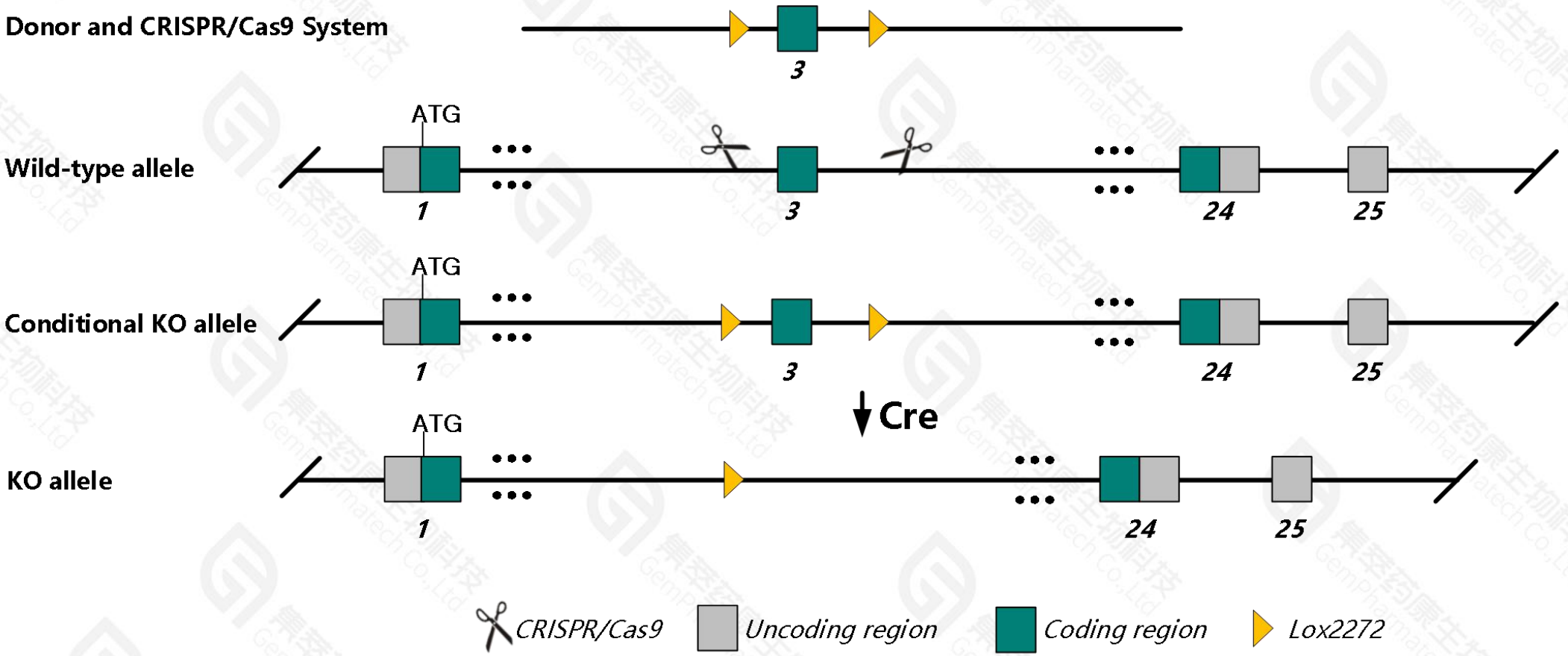
Project Overview

Project Name	***Sik3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Sik3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Sik3* gene has 7 transcripts. According to the structure of *Sik3* gene, exon3 of *Sik3-204*(ENSMUST00000126865.8) transcript is recommended as the knockout region.The region contains 64bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Sik3* 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 a knock-out allele exhibit impaired chondrocyte hypertrophy during development, neonatal lethality and reduced size. Mice homozygous for a gain of function ENU mutation exhibit decreased total wake time, owing to an increase in inherent sleep need.

➢ The *Sik3* gene is located on the Chr9. 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）

Sik3 SIK family kinase 3 [Mus musculus (house mouse)]

Gene ID: 70661, updated on 2-Mar-2021

Summary

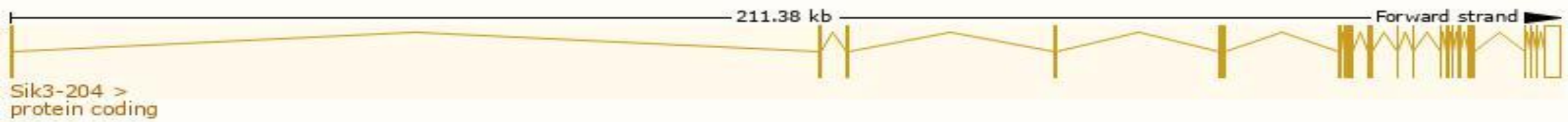
Official Symbol	Sik3 provided by MGI
Official Full Name	SIK family kinase 3 provided byMGI
Primary source	MGI:MGI:2446296
See related	Ensembl:ENSMUSG00000034135
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	5730525O22Rik, 9030204A07Rik, AI447252, AI846254, AI849445, Qsk, SIK-3, mKIAA0999
Expression	Ubiquitous expression in cerebellum adult (RPKM 12.2), adrenal adult (RPKM 10.1) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

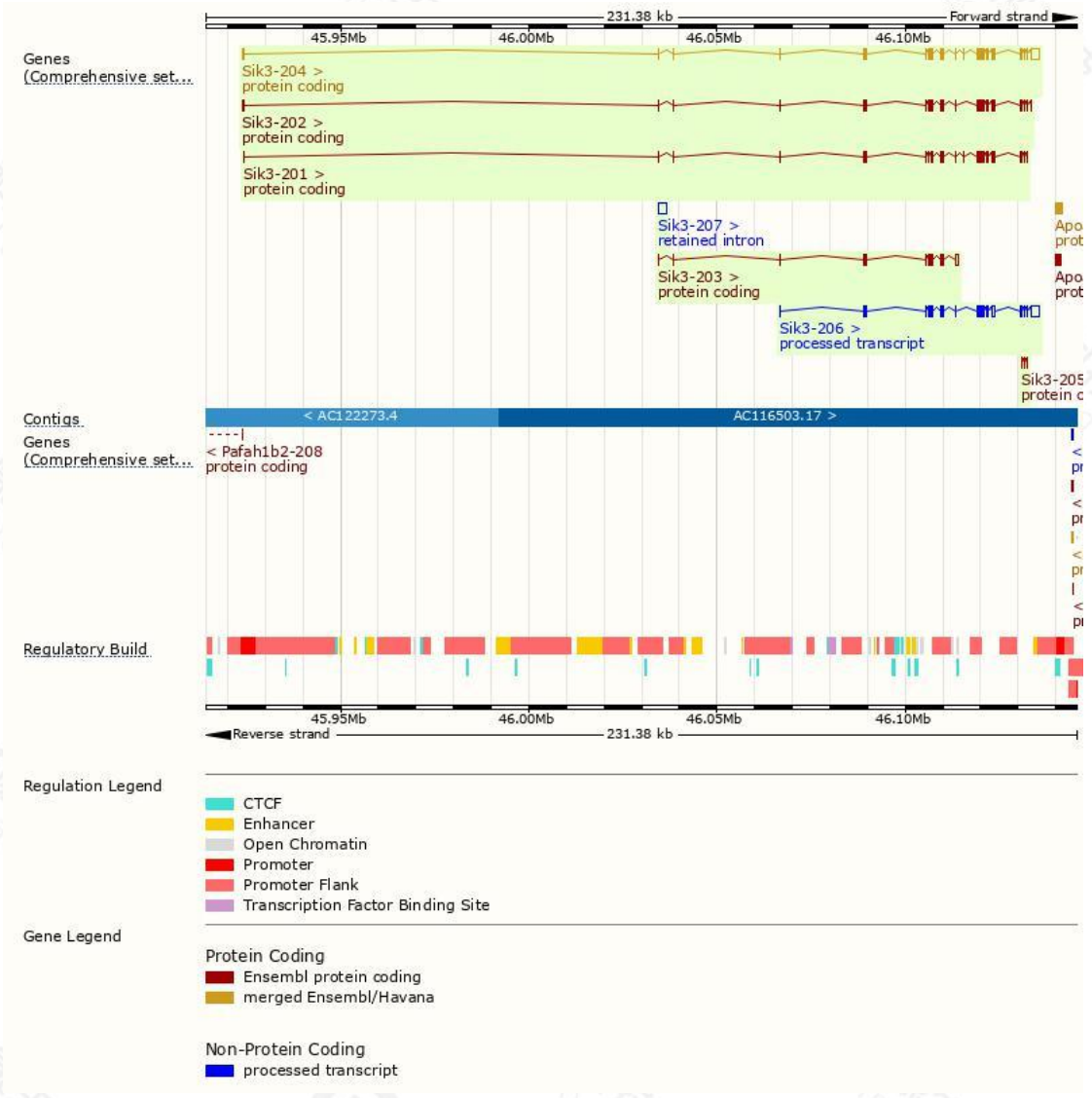
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sik3-204	ENSMUST00000126865.8	6287	1369aa	Protein coding	CCDS23140		TSL:1 , GENCODE basic , APPRIS P1 ,
Sik3-202	ENSMUST00000120463.9	4180	1319aa	Protein coding	-		CDS 5' incomplete , TSL:5 ,
Sik3-201	ENSMUST00000120247.8	3657	1214aa	Protein coding	-		CDS 5' incomplete , TSL:5 ,
Sik3-203	ENSMUST00000122865.2	2330	506aa	Protein coding	-		CDS 5' incomplete , TSL:1 ,
Sik3-205	ENSMUST00000138338.2	227	71aa	Protein coding	-		CDS 5' incomplete , TSL:5 ,
Sik3-206	ENSMUST00000153152.2	5465	No protein	Processed transcript	-		TSL:5 ,
Sik3-207	ENSMUST00000215954.2	2333	No protein	Retained intron	-		TSL:NA ,

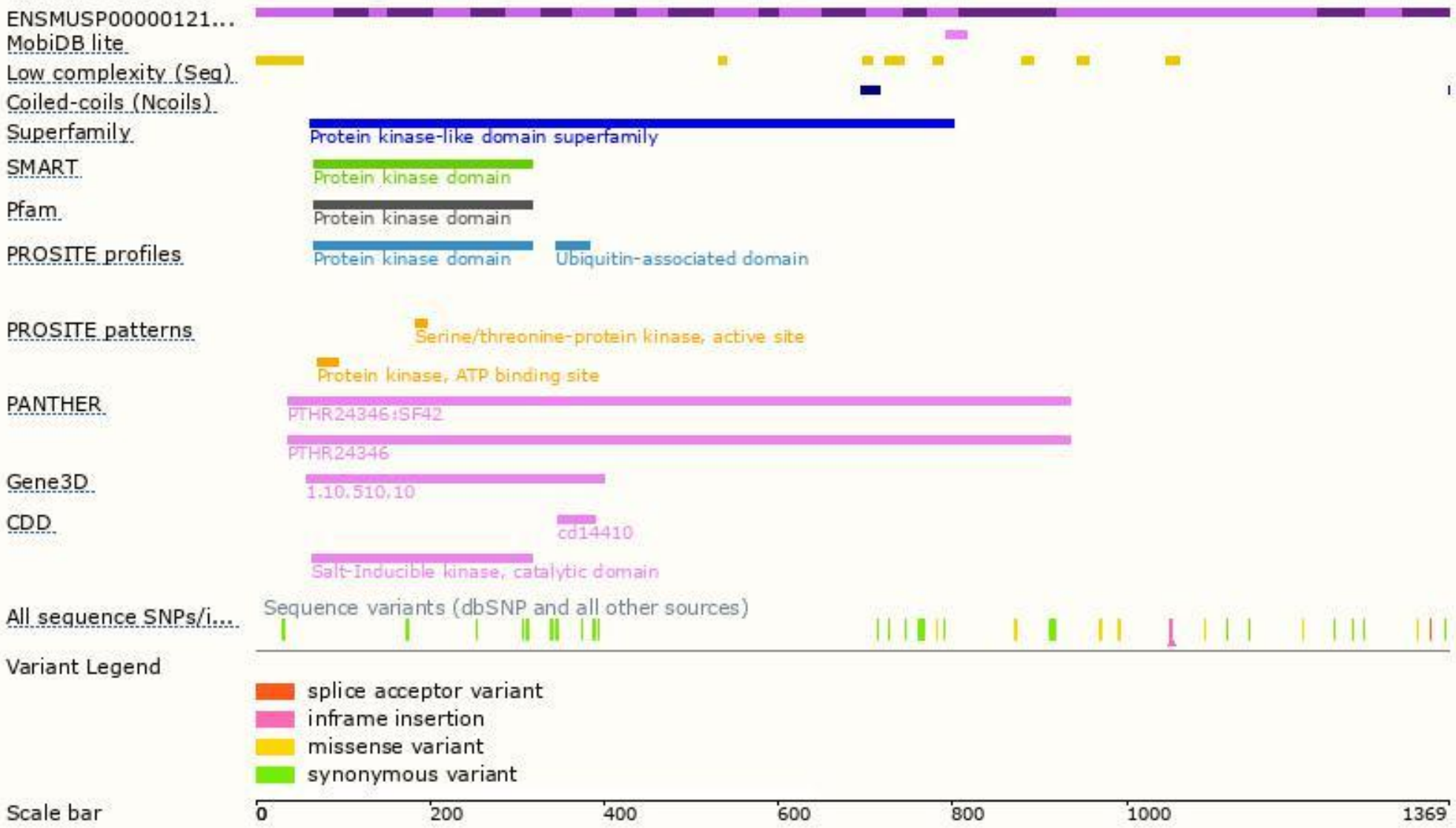
The strategy is based on the design of *Sik3-204* transcript,the transcription is shown below:



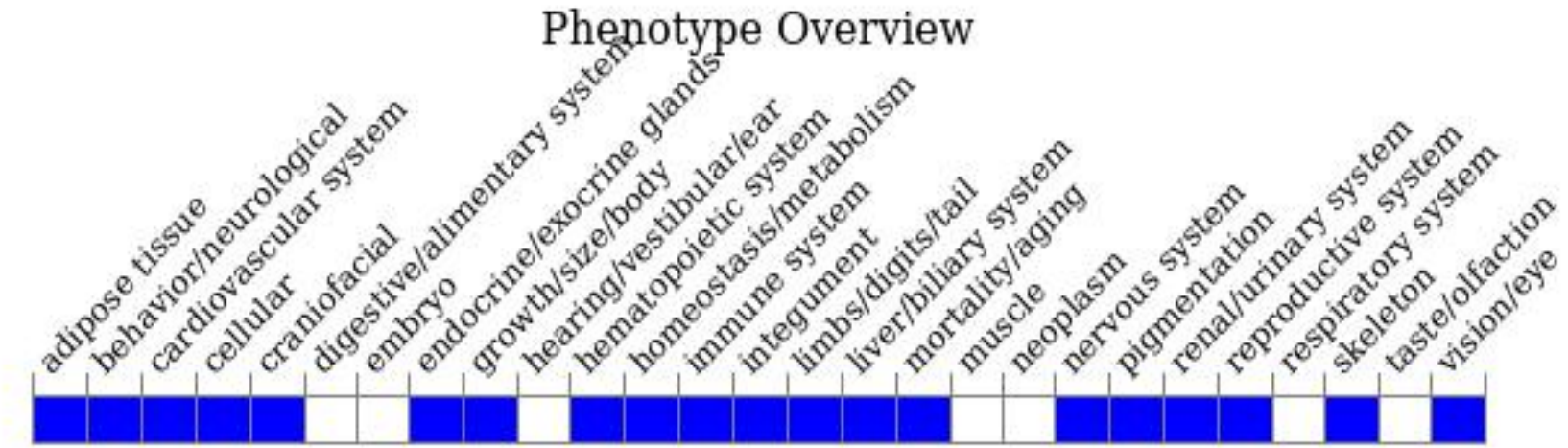
Genomic location distribution



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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