Sag Cas9-CKO Strategy

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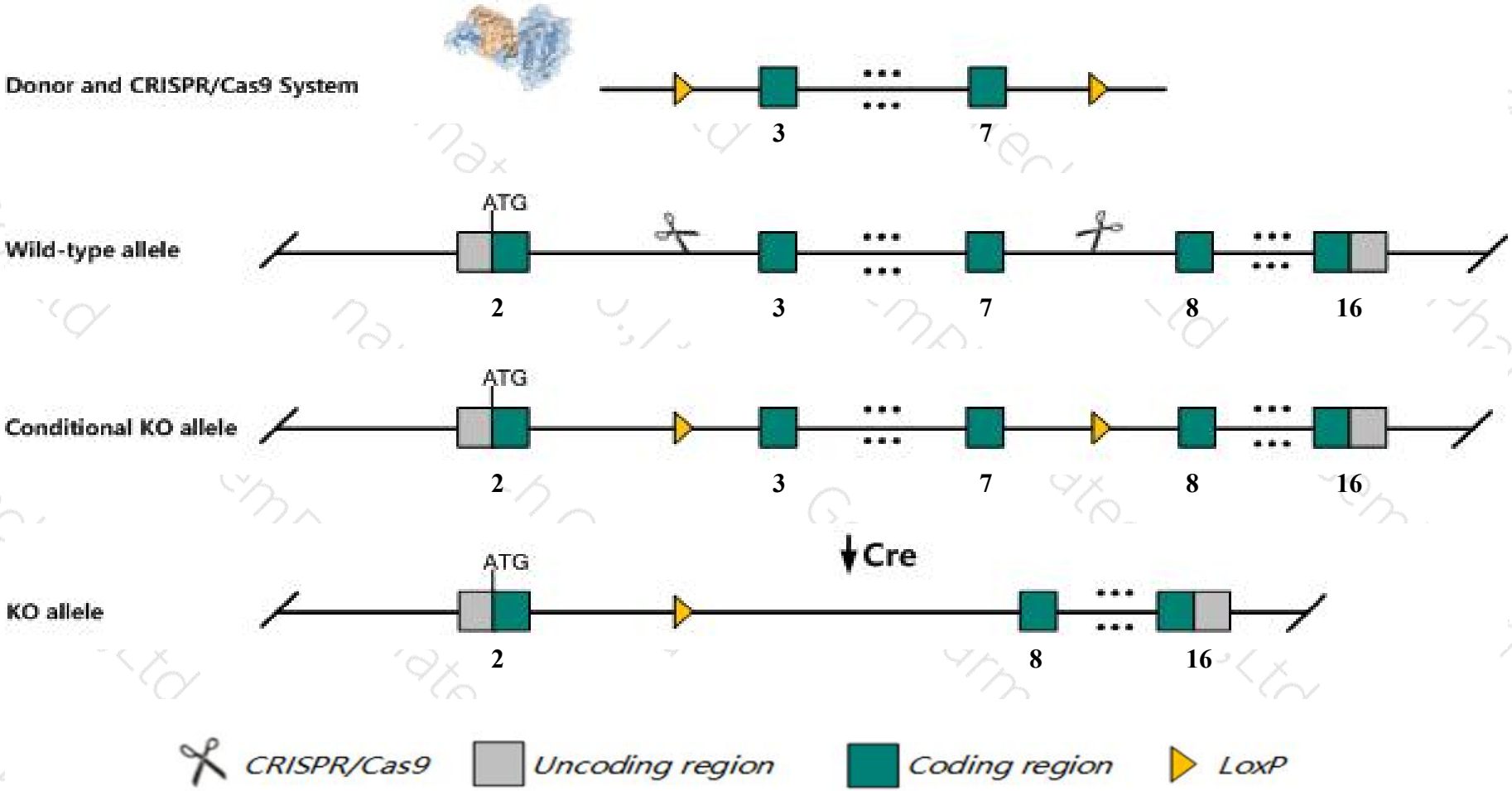
Project Overview

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

Conditional Knockout strategy

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



Technical routes

➢ The *Sag* gene has 9 transcripts. According to the structure of *Sag* gene, exon3-exon7 of *Sag-201*(ENSMUST00000077772.11) transcript is recommended as the knockout region.The region contains 437bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Sag* 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 abnormalities in retinal rod cell outer segment morphology and rod electrophysiology.

➢ The *Sag* gene is located on the Chr1. 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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Sag S-antigen, retina and pineal gland (arrestin) [Mus musculus (house mouse)]

Gene ID: 20215, updated on 13-Mar-2020

Summary

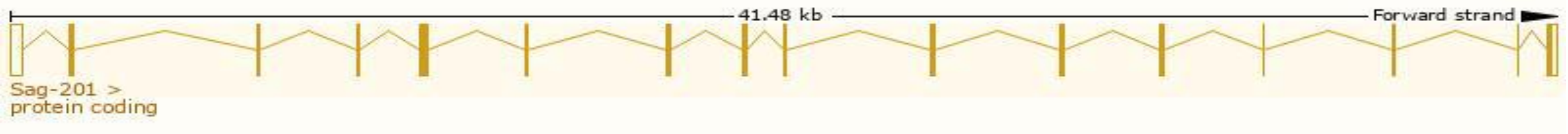
Official Symbol	Sag provided by MGI
Official Full Name	S-antigen, retina and pineal gland (arrestin) provided byMGI
Primary source	MGI:MGI:98227
See related	Ensembl:ENSMUSG00000056055
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	A930001K18Rik, Arr1, Irbp, arrestin
Expression	Biased expression in testis adult (RPKM 1.9), genital fat pad adult (RPKM 0.3) and 7 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

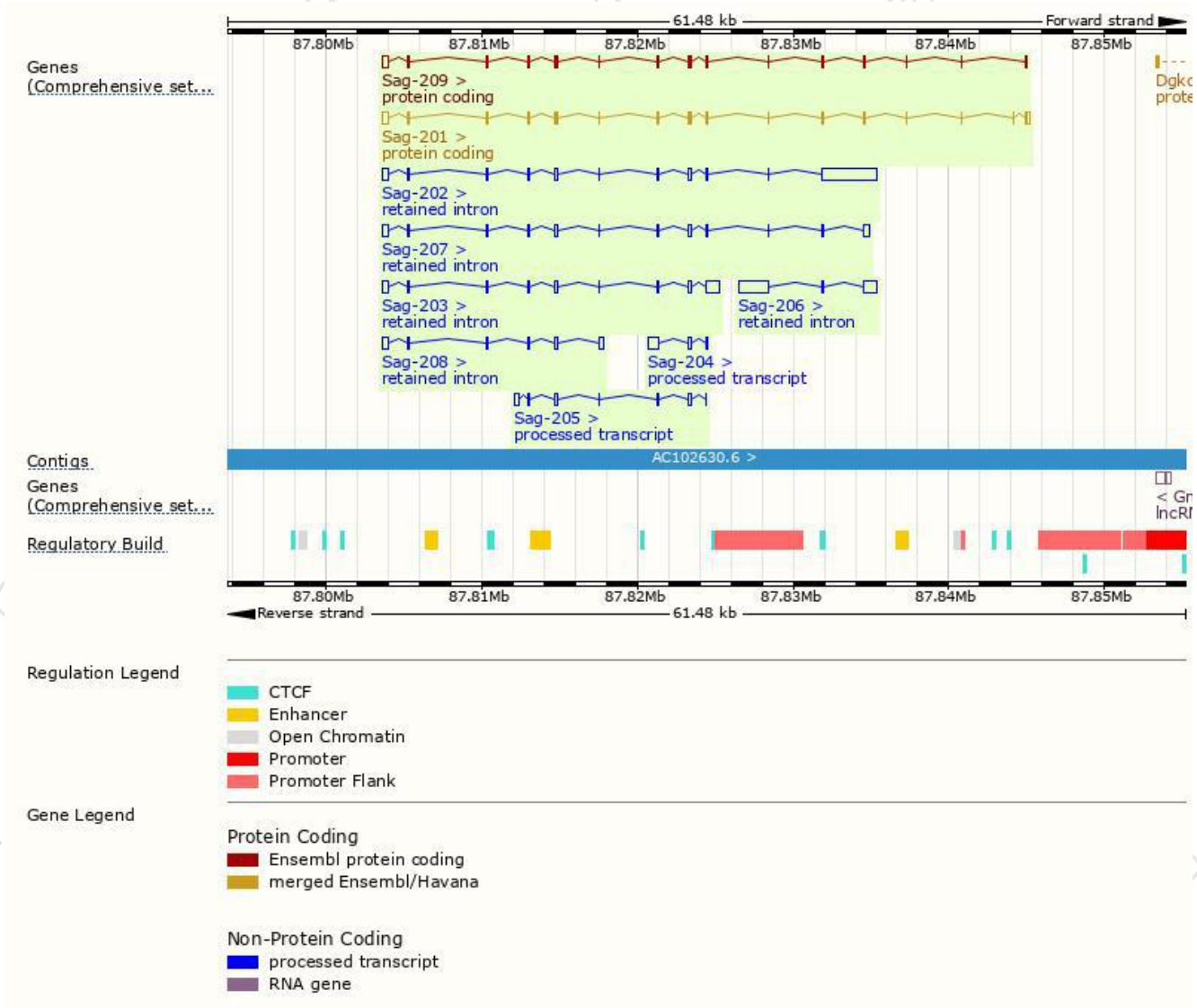
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sag-201	ENSMUST00000077772.11	1719	403aa	Protein coding	CCDS35656	P20443 Q3UPX6	TSL:1 GENCODE basic APPRIS P2
Sag-209	ENSMUST00000177757.1	1584	403aa	Protein coding	-	J3QNF6	TSL:5 GENCODE basic APPRIS ALT2
Sag-204	ENSMUST00000136708.1	857	No protein	Processed transcript	-	-	TSL:2
Sag-205	ENSMUST00000144334.7	774	No protein	Processed transcript	-	-	TSL:5
Sag-202	ENSMUST00000128761.7	4727	No protein	Retained intron	-	-	TSL:1
Sag-206	ENSMUST00000145385.1	2983	No protein	Retained intron	-	-	TSL:1
Sag-203	ENSMUST00000130886.7	1886	No protein	Retained intron	-	-	TSL:1
Sag-207	ENSMUST00000155393.7	1668	No protein	Retained intron	-	-	TSL:1
Sag-208	ENSMUST00000156381.7	1086	No protein	Retained intron	-	-	TSL:2

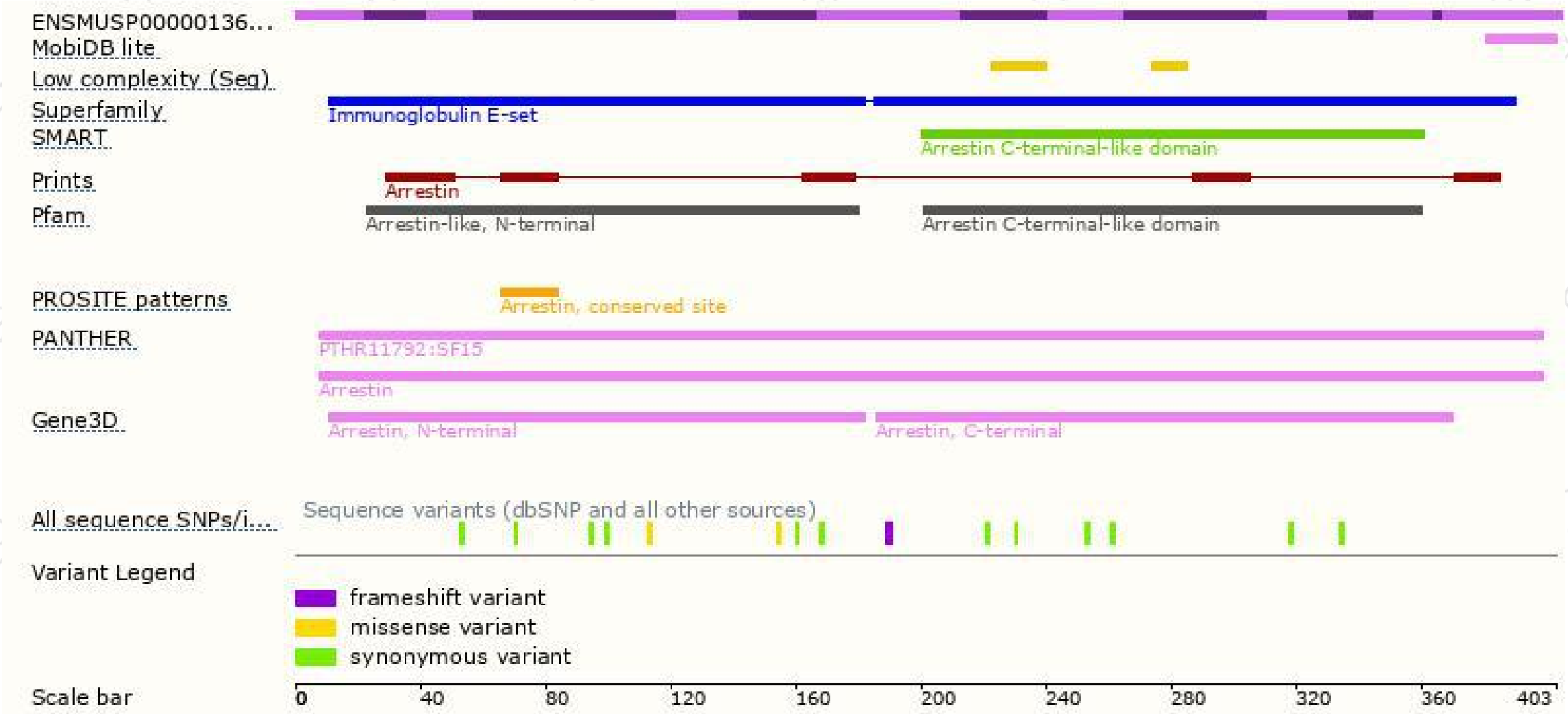
The strategy is based on the design of *Sag-201* transcript,the transcription is shown below:



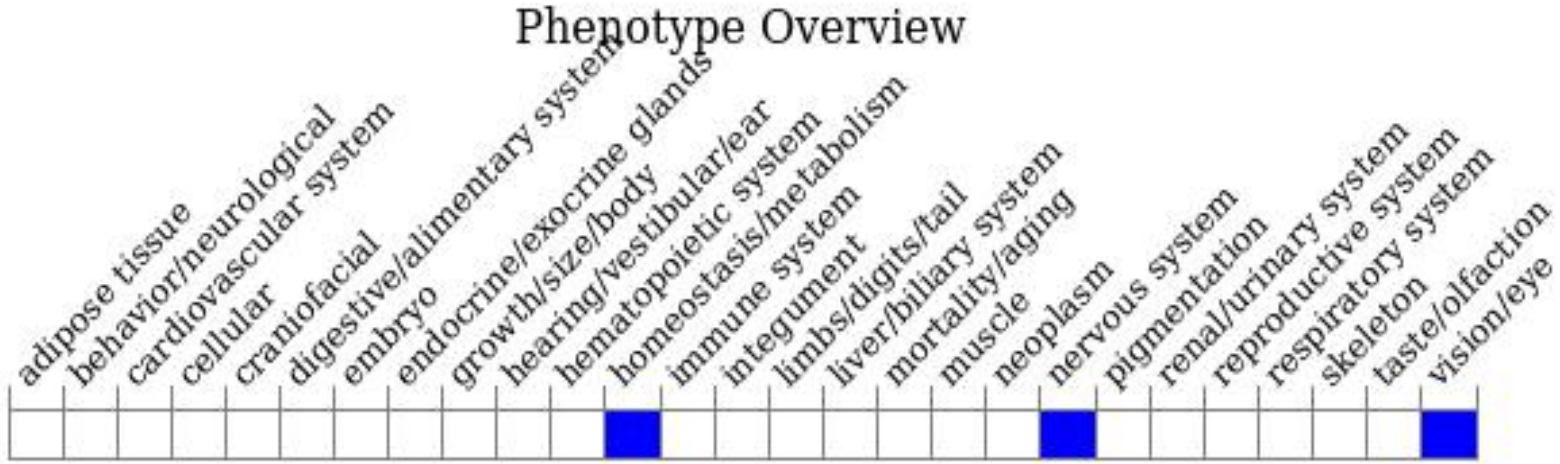
Genomic location distribution



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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