Slc6a9 Cas9-CKO Strategy

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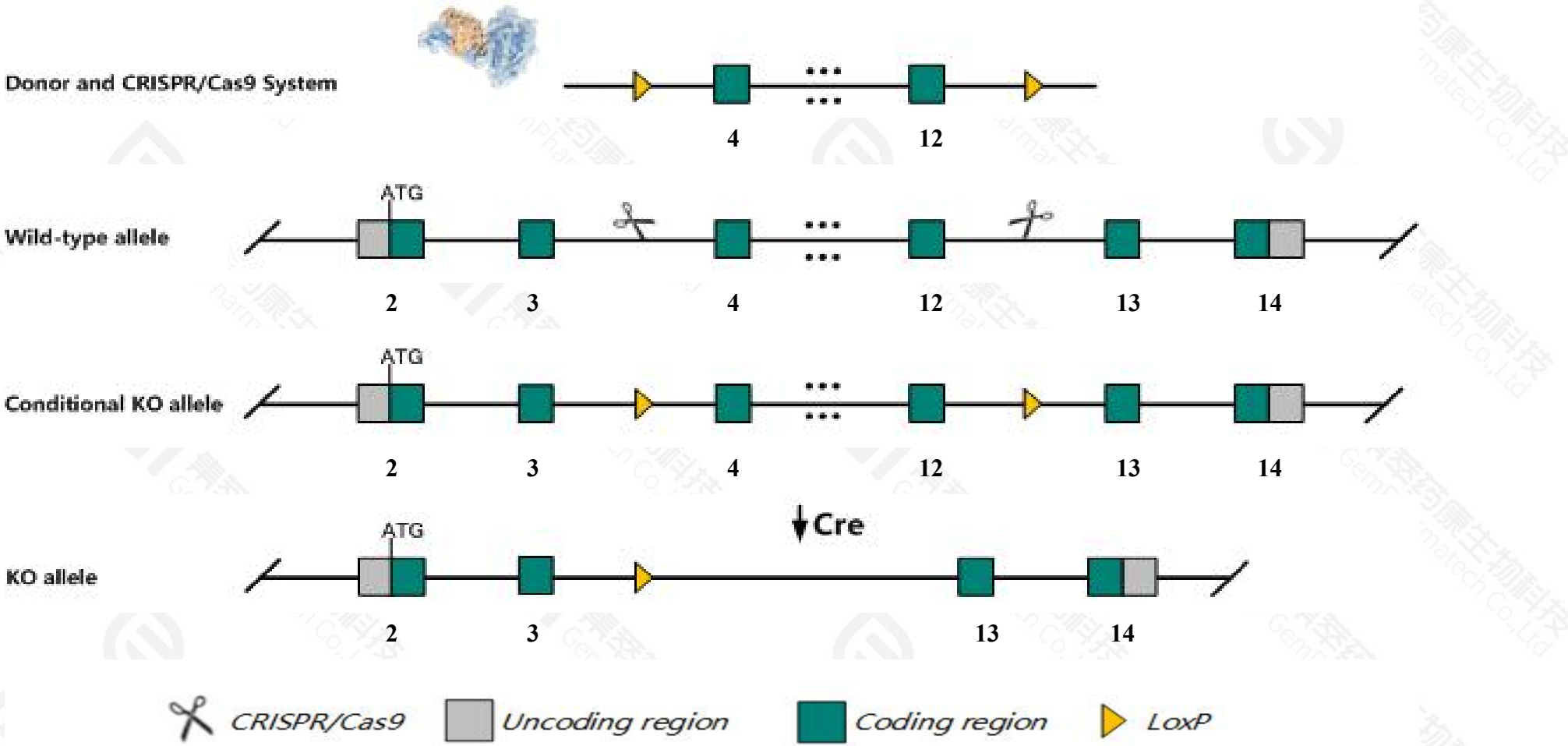
Design Date: 2022-1-7

Project Overview

Project Name	***Slc6a9***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Slc6a9* gene. The schematic diagram is as follows:



Technical routes

➢ The *Slc6a9* gene has 12 transcripts. According to the structure of *Slc6a9* gene, exon4-exon12 of *Slc6a9-201*(ENSMUST00000030269.14) transcript is recommended as the knockout region.The region contains 1349bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Slc6a9* 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

➢ The partial intron of *Gm17114* gene will be deleted together after Cre recombination in this strategy.

➢ Transcript *Slc6a9-208* may not be affected.

➢ According to the existing MGI data,homozygous null mice die shortly after birth exhibiting breathing and movement deficiencies.

➢ The *Slc6a9* gene is located on the Chr4. 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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Slc6a9 solute carrier family 6 (neurotransmitter transporter, glycine), member 9 [Mus musculus (house mouse)]

Gene ID: 14664, updated on 2-Mar-2021

Summary

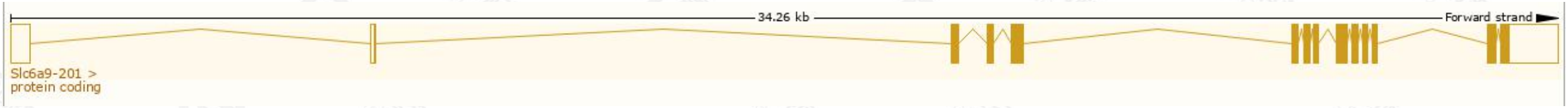
Official Symbol	Slc6a9 provided by MGI
Official Full Name	solute carrier family 6 (neurotransmitter transporter, glycine), member 9 provided by MGI
Primary source	MGI:MGI:95760
See related	Ensembl:ENSMUSG00000028542
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Glyt, Glyt-, Glyt-1, Glyt1
Expression	Ubiquitous expression in liver E14.5 (RPKM 33.4), cerebellum adult (RPKM 30.9) and 27 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

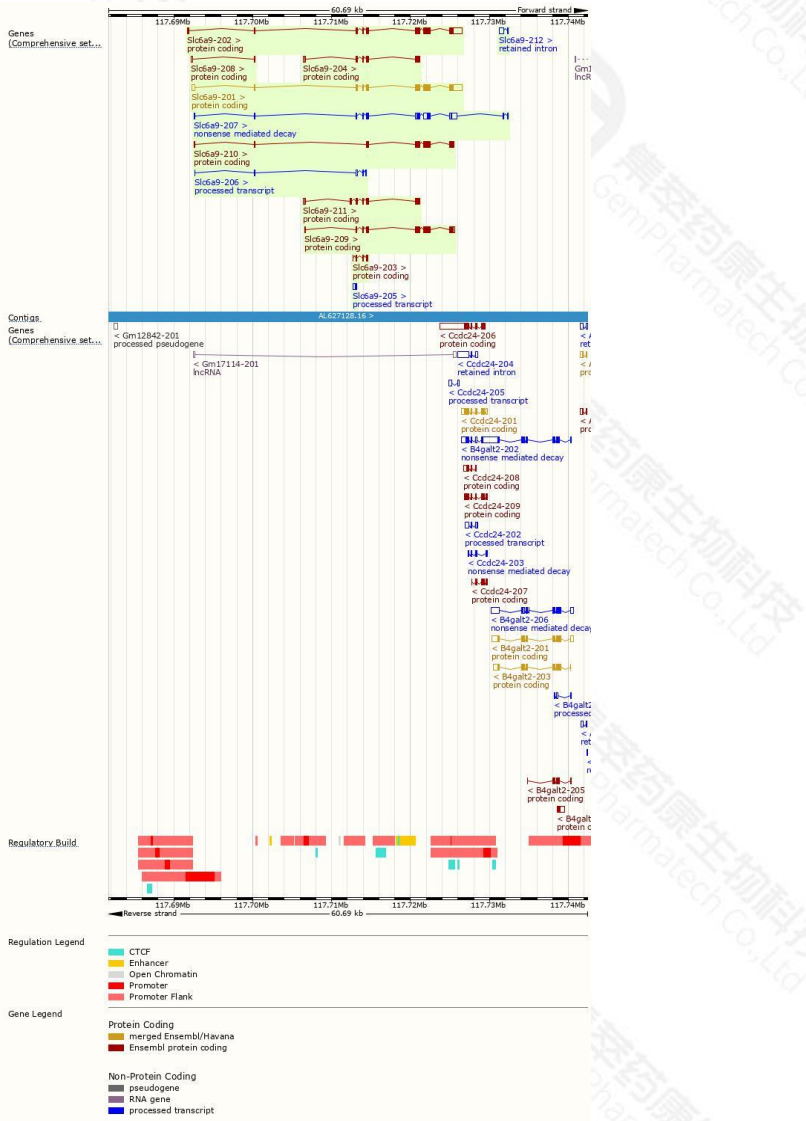
The gene has 12 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Slc6a9-201	ENSMUST00000030269.14	3479	633aa	Protein coding	CCDS18538		TSL:1 , GENCODE basic , APPRIS P1 ,
Slc6a9-202	ENSMUST00000063857.11	3146	633aa	Protein coding	CCDS18538		TSL:1 , GENCODE basic , APPRIS P1 ,
Slc6a9-209	ENSMUST00000163288.2	2107	637aa	Protein coding	-		TSL:5 , GENCODE basic ,
Slc6a9-210	ENSMUST00000169885.8	1876	521aa	Protein coding	-		TSL:5 , GENCODE basic ,
Slc6a9-211	ENSMUST00000169990.8	1344	373aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Slc6a9-204	ENSMUST00000132043.9	1194	321aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Slc6a9-203	ENSMUST00000123994.3	456	116aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Slc6a9-208	ENSMUST00000151316.2	244	1aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Slc6a9-207	ENSMUST00000149168.2	2834	126aa	Nonsense mediated decay	-		TSL:1 ,
Slc6a9-206	ENSMUST00000136476.2	488	No protein	Processed transcript	-		TSL:5 ,
Slc6a9-205	ENSMUST00000135769.2	370	No protein	Processed transcript	-		TSL:2 ,
Slc6a9-212	ENSMUST00000170733.2	678	No protein	Retained intron	-		TSL:3 ,

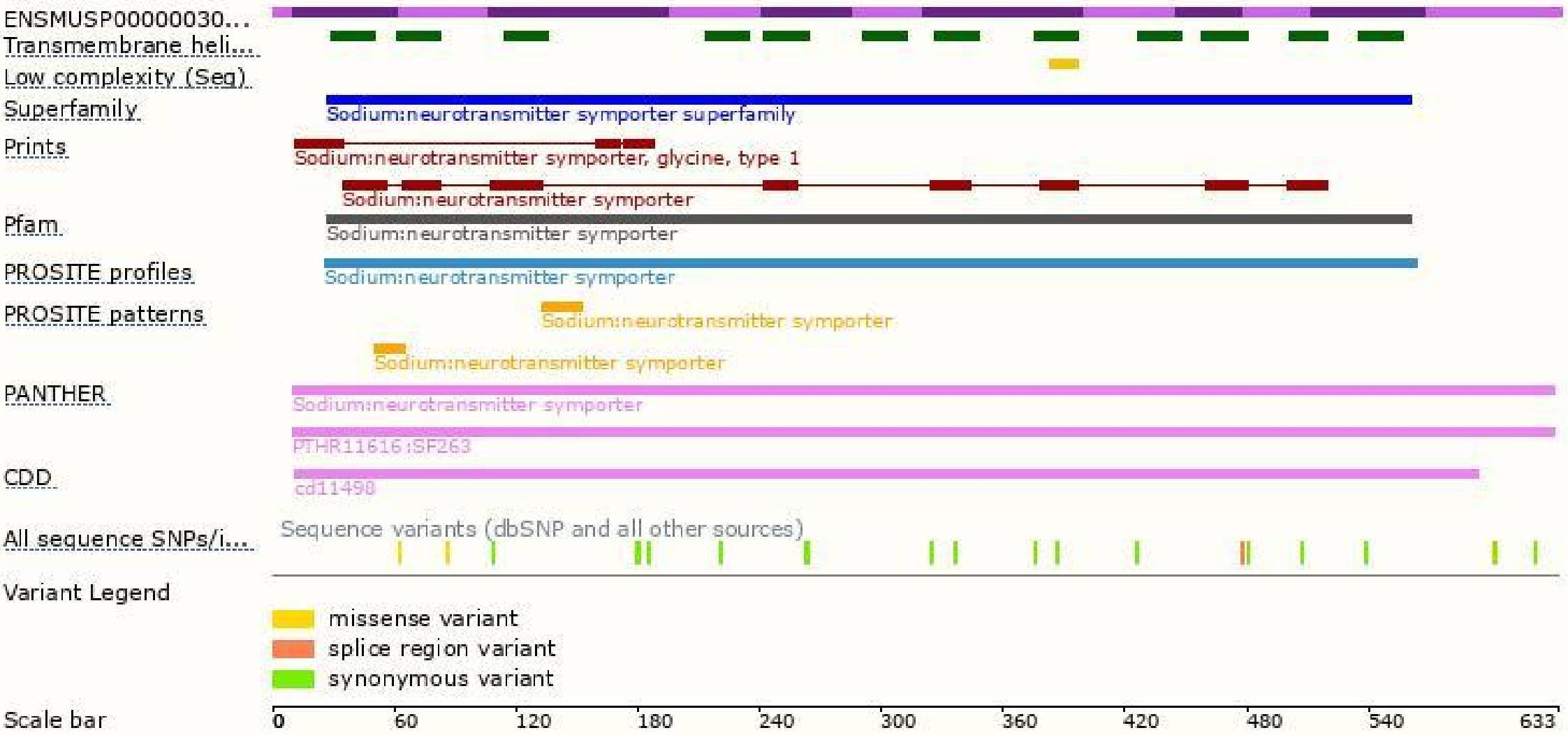
The strategy is based on the design of *Slc6a9-201* transcript,the transcription is shown below:



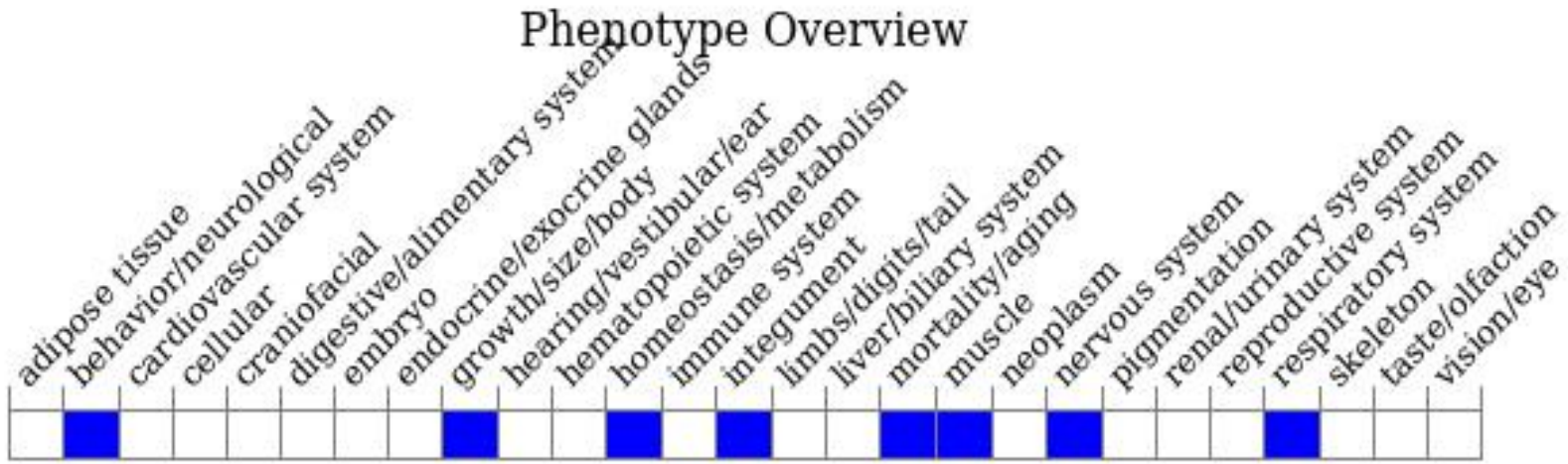
Genomic location distribution



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/).

According to the existing MGI data,homozygous null mice die shortly after birth exhibiting breathing and movement deficiencies.

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If you have any questions, you are welcome to inquire.
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