Slc12a2 Cas9-KO Strategy

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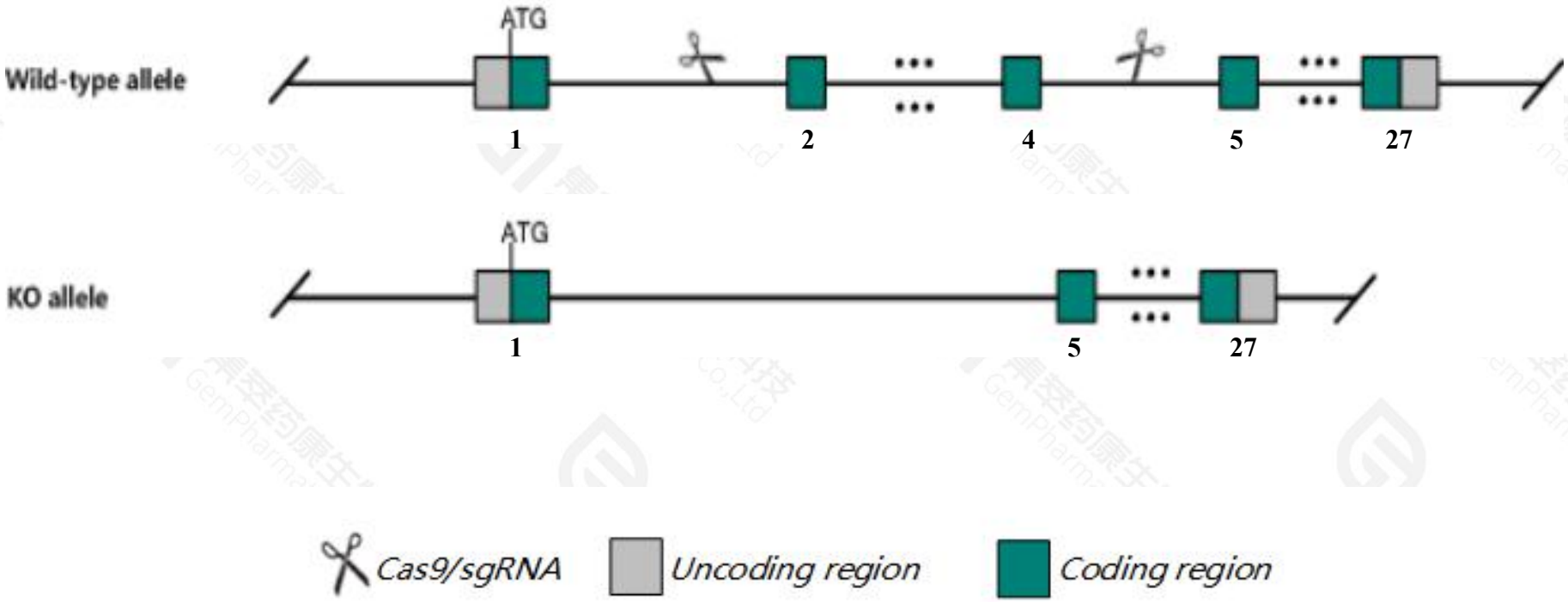
Design Date: 2021-6-10

Project Overview

Project Name	***Slc12a2***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Slc12a2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Slc12a2* gene has 2 transcripts. According to the structure of *Slc12a2* gene, exon2-exon4 of *Slc12a2-201*(ENSMUST00000115366.3) transcript is recommended as the knockout region.The region contains 292bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Slc12a2* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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,homozygous mutants show variably severe deafness, head-shaking, circling, reduced endolymph secretion, male sterility, growth retardation, hypotension, reduced salivation, delayed ductal outgrowth of mammary epithelium and increased periweaning mortality.

➢ The *Slc12a2* gene is located on the Chr18. 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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Slc12a2 solute carrier family 12, member 2 [Mus musculus (house mouse)]

Gene ID: 20496, updated on 15-Mar-2020

Summary

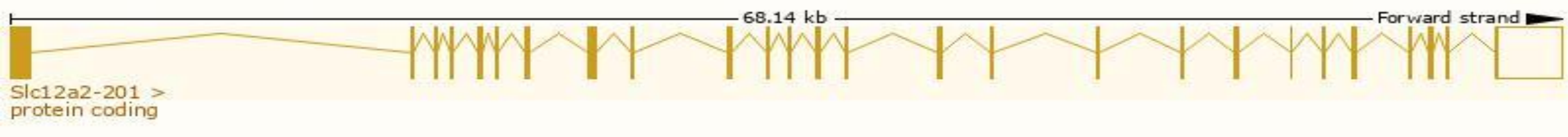
Official Symbol	Slc12a2 provided by MGI
Official Full Name	solute carrier family 12, member 2 provided by MGI
Primary source	MGI:MGI:101924
See related	Ensembl:ENSMUSG00000024597
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	9330166H04Rik, Nkcc1, mBSC2, sy-ns
Expression	Broad expression in mammary gland adult (RPKM 43.3), colon adult (RPKM 19.3) and 20 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

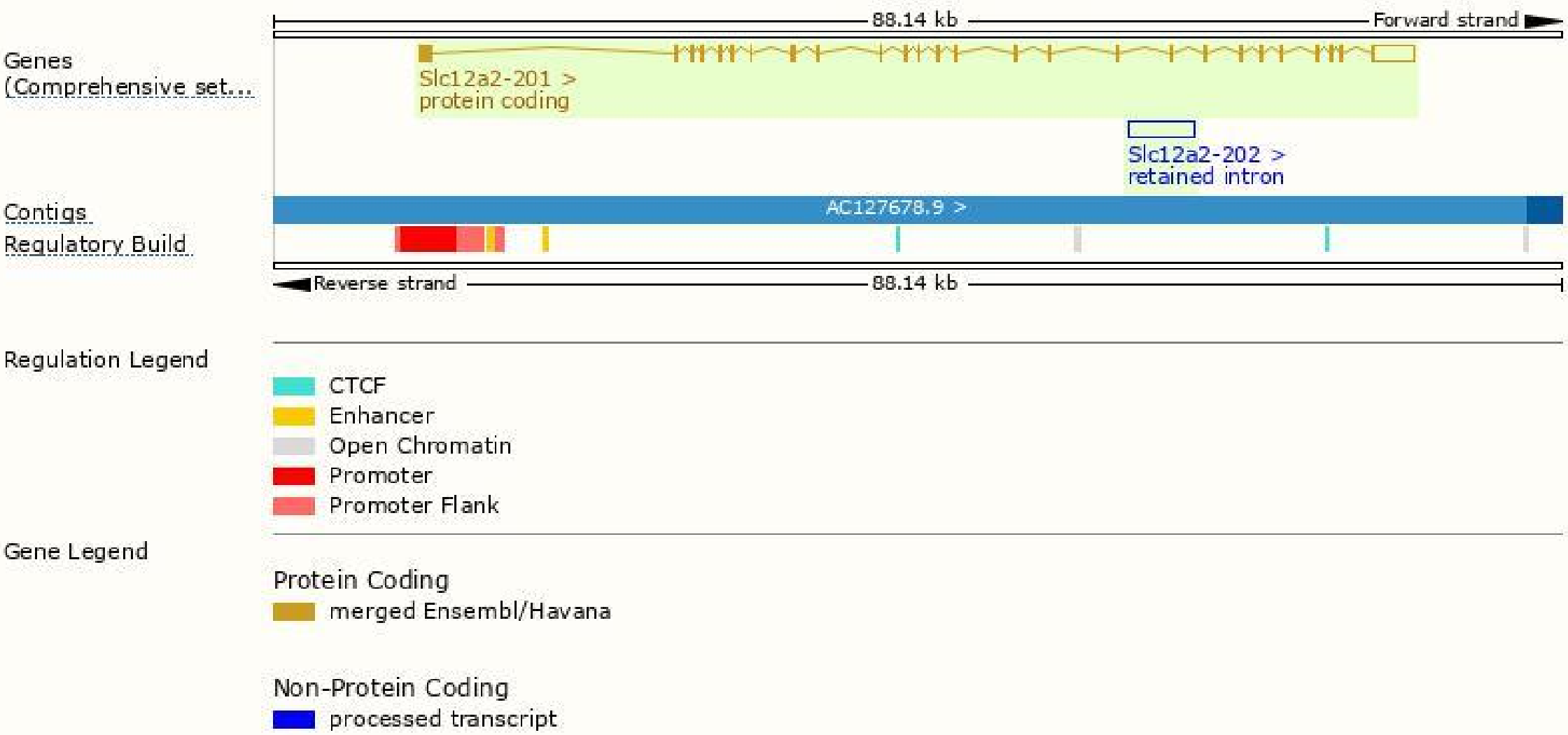
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Slc12a2-201	ENSMUST00000115366.2	6520	1206aa	Protein coding	CCDS37826	E9QM38	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Slc12a2-202	ENSMUST00000236673.1	4480	No protein	Retained intron	-	-	

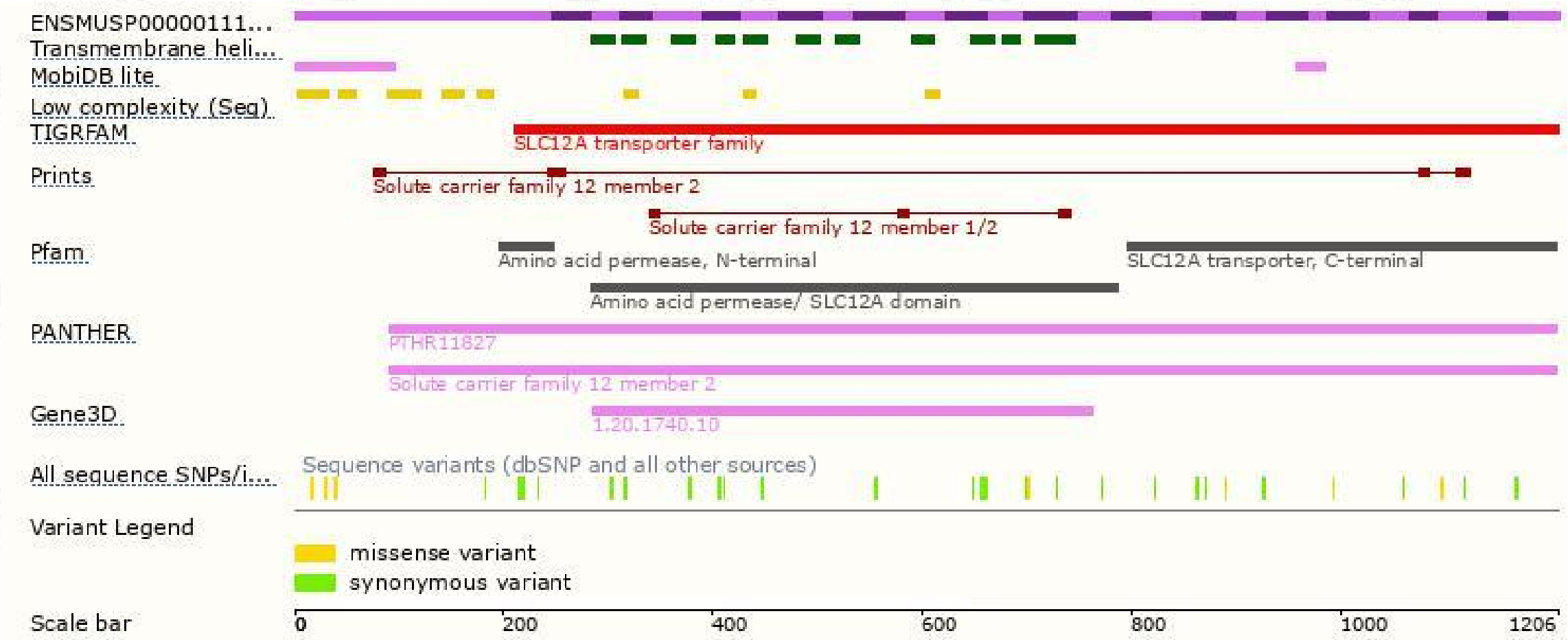
The strategy is based on the design of *Slc12a2-201* transcript,the transcription is shown below:



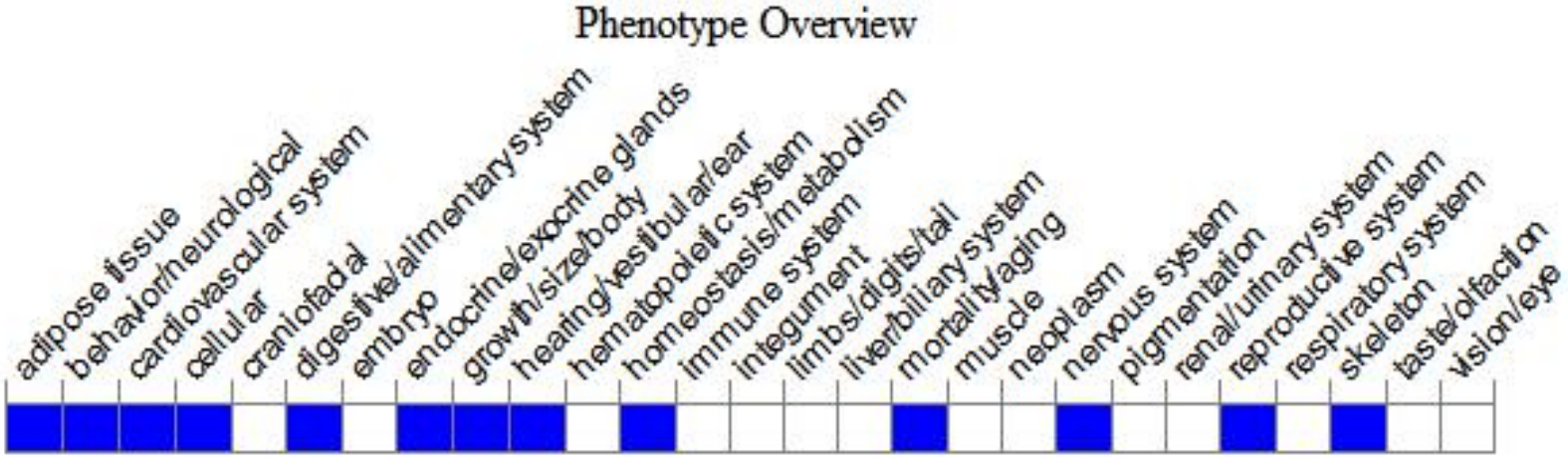
Genomic location distribution



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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