Slc5a3 Cas9-CKO Strategy

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Design Date: 2021-5-26

Project Overview

Project Name	***Slc5a3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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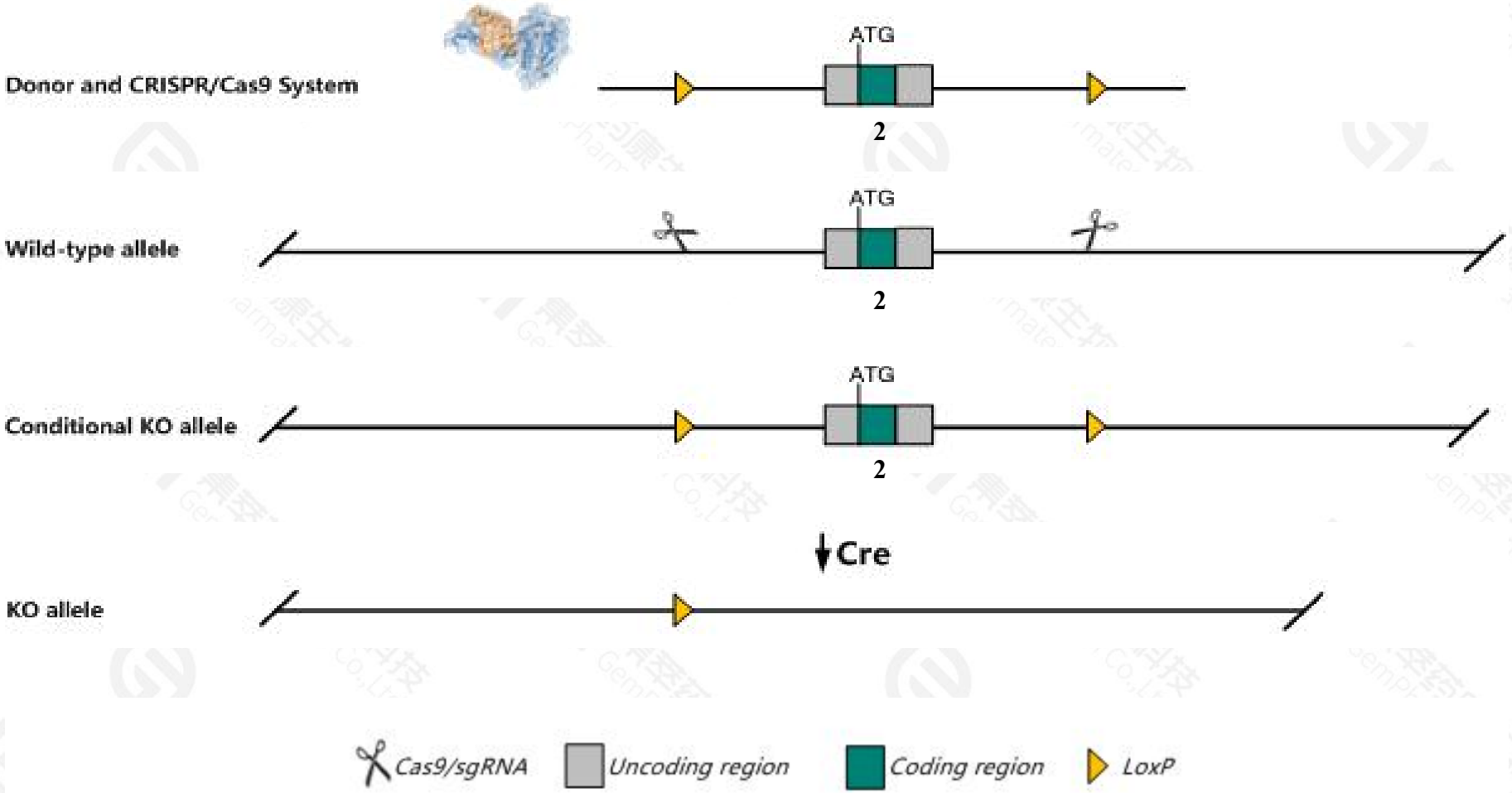
This model will use CRISPR/Cas9 technology to edit the *Slc5a3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Slc5a3* gene has 2 transcripts. According to the structure of *Slc5a3* gene, exon2 of *Slc5a3-201*(ENSMUST00000113975.3) 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 *Slc5a3* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor was constructed.Cas9, sgRNA 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 was 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,homozygous mutation of this gene results in lethality shortly after birth due to respiratory failure and abnormal development of peripheral nerves.

➢ The partial intron of *Gm49711* and *Mrps6* gene will be deleted together after Cre recombination in this strategy.

➢ The *Slc5a3* gene is located on the Chr16. 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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Slc5a3 solute carrier family 5 (inositol transporters), member 3 [Mus musculus (house mouse)]

Gene ID: 53881, updated on 25-Sep-2020

Summary

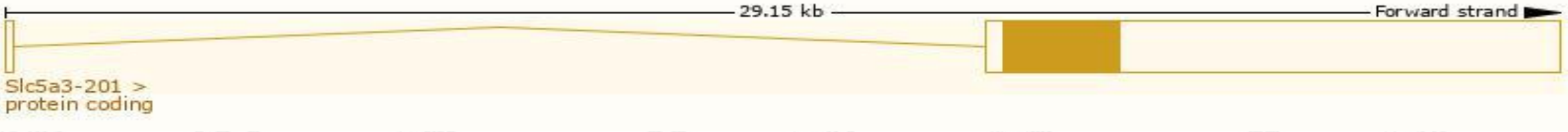
Official Symbol	Slc5a3 provided by MGI
Official Full Name	solute carrier family 5 (inositol transporters), member 3 provided byMGI
Primary source	MGI:MGI:1858226
See related	Ensembl:ENSMUSG00000089774
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AA623876, BF642829, Smi, Smit1
Expression	Broad expression in kidney adult (RPKM 7.8), genital fat pad adult (RPKM 7.5) and 24 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

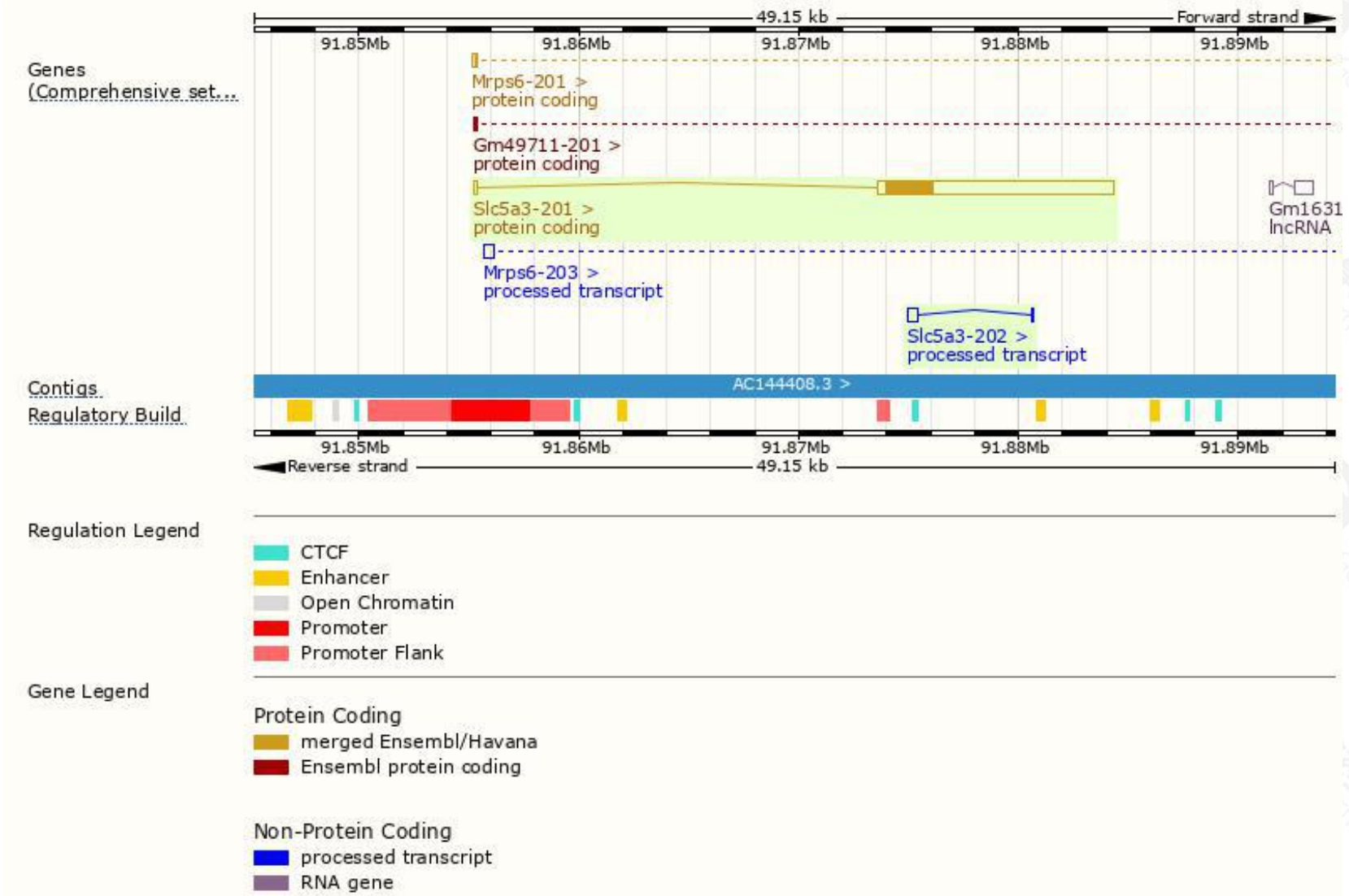
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Slc5a3-201	ENSMUST00000113975.3	10913	718aa	Protein coding	CCDS37403		TSL:1 , GENCODE basic , APPRIS P1 ,
Slc5a3-202	ENSMUST00000131098.2	521	No protein	Processed transcript	-		TSL:3 ,

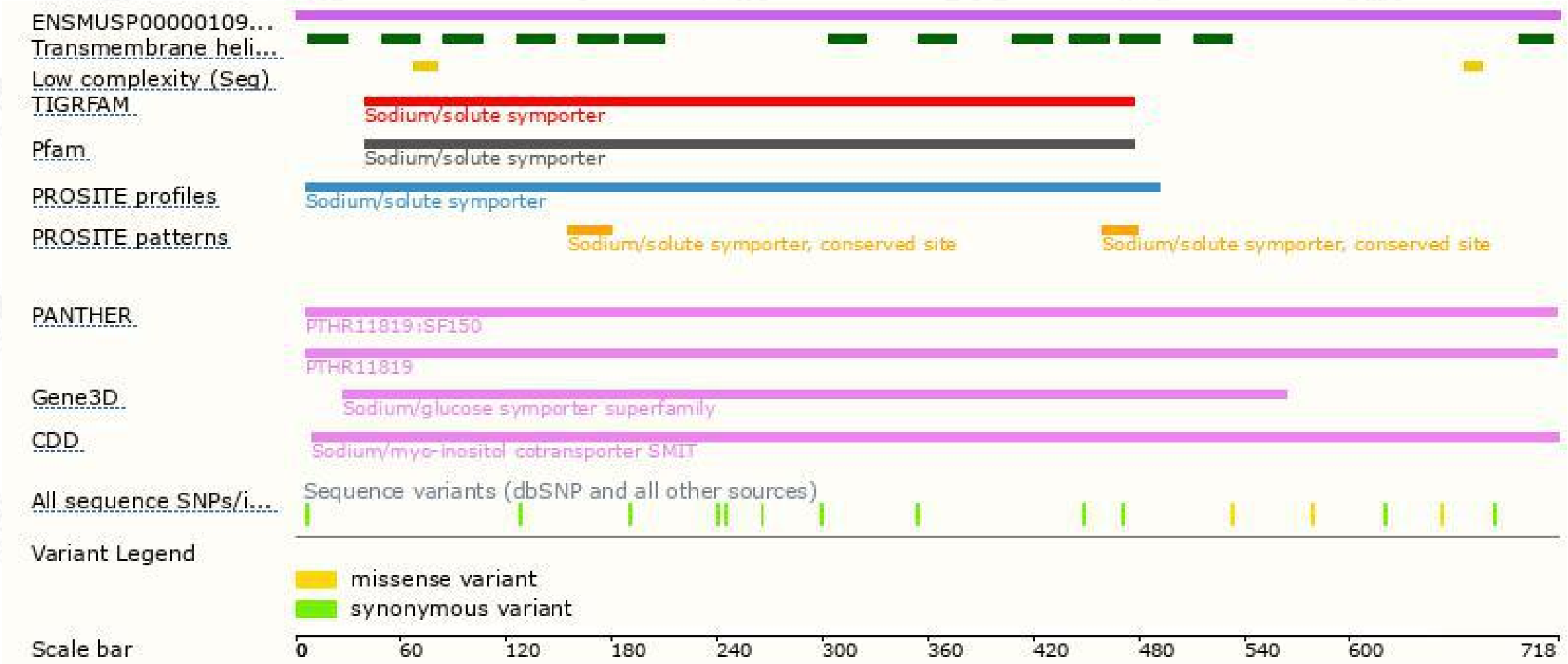
The strategy is based on the design of *Slc5a3-201* transcript,the transcription is shown below:



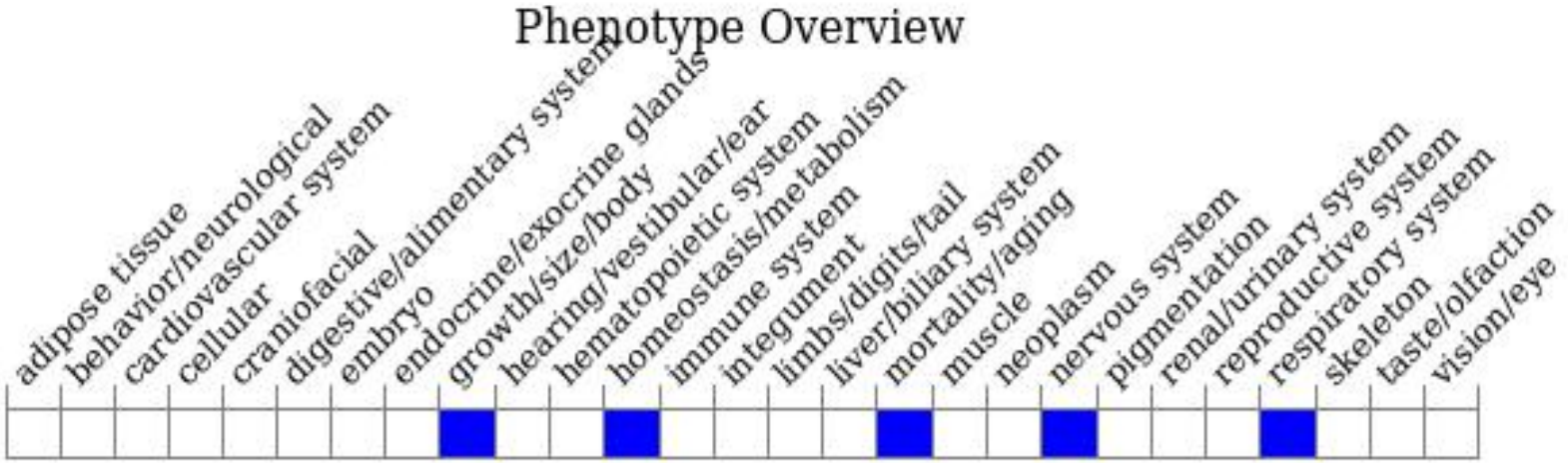
Genomic location distribution



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 mutation of this gene results in lethality shortly after birth due to respiratory failure and abnormal development of peripheral nerves.

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

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