Slc6a8 Cas9-CKO Strategy

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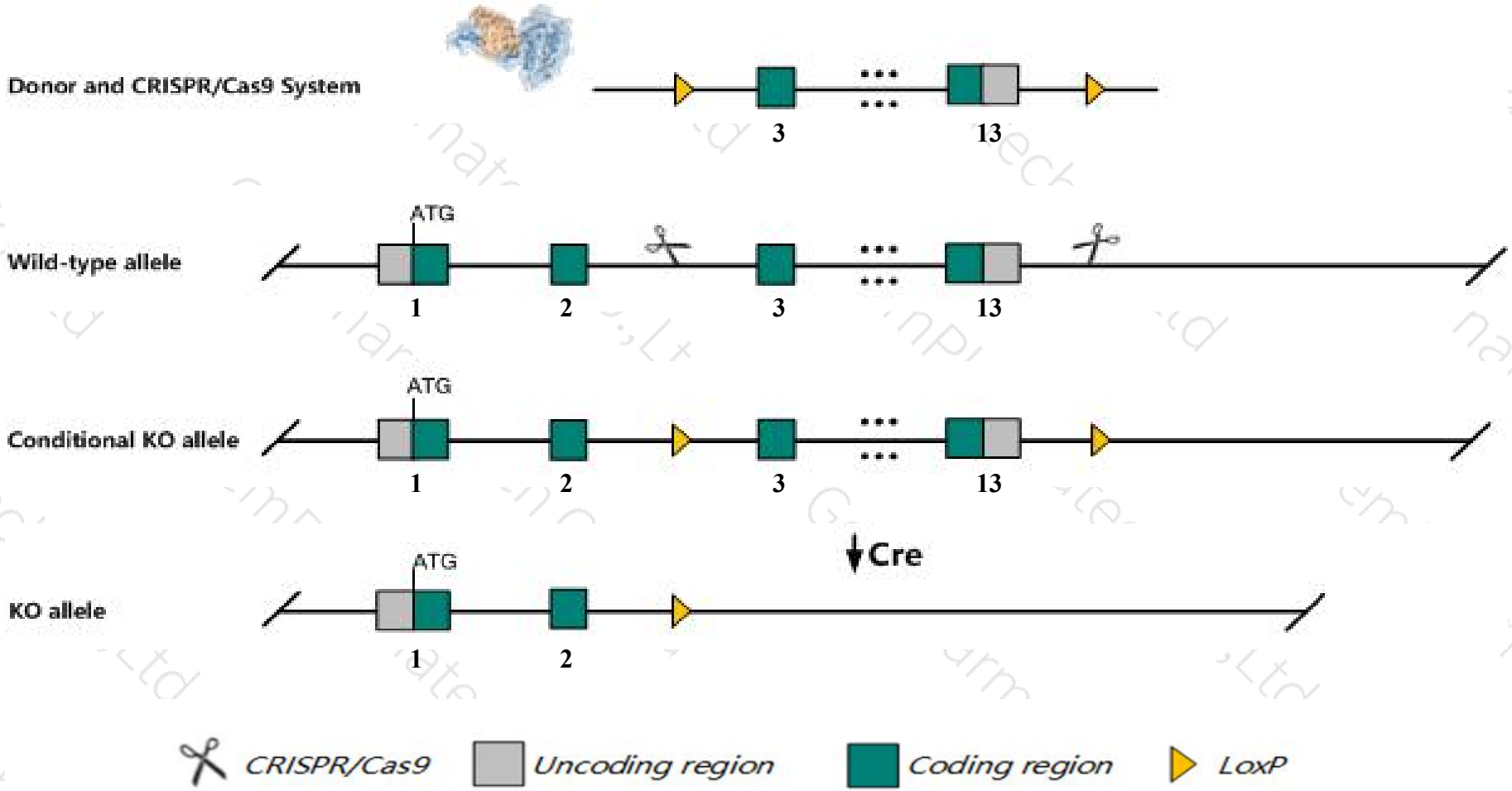
Project Overview

Project Name	***Slc6a8***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Slc6a8* gene. The schematic diagram is as follows:



Technical routes

➢ The *Slc6a8* gene has 13 transcripts. According to the structure of *Slc6a8* gene, exon3-exon13 of *Slc6a8-201* (ENSMUST00000033752.13) transcript is recommended as the knockout region.The region contains most 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 *Slc6a8* 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,Male mice hemizygous for a targeted allele exhibit decreased body weight, decreased creatine concentrations, impaired short term object recognition, impaired contextual conditioning, altered locomotor activity, and increased serotonine levels in the brain.
- The N-terminal of *Slc6a8* gene will remain several amino acids ,it may remain the partial function of *Slc6a8* gene.
- The *Slc6a8* gene is located on the ChrX. 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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Slc6a8 solute carrier family 6 (neurotransmitter transporter, creatine), member 8 [*Mus musculus* (house mouse)]

Gene ID: 102857, updated on 10-Oct-2019

Summary

Official Symbol	Slc6a8 provided by MGI
Official Full Name	solute carrier family 6 (neurotransmitter transporter, creatine), member 8 provided by MGI
Primary source	MGI:MGI:2147834
See related	Ensembl:ENSMUSG00000019558
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	CRT; CT1; CRTR; CTR5; Creat; AA589632
Expression	Broad expression in duodenum adult (RPKM 226.8), small intestine adult (RPKM 214.6) and 18 other tissues See more
Orthologs	human all

Genomic context

Location: X A7.3; X 37.38 cM

See Slc6a8 in Genome Data Viewer

Exon count: 13

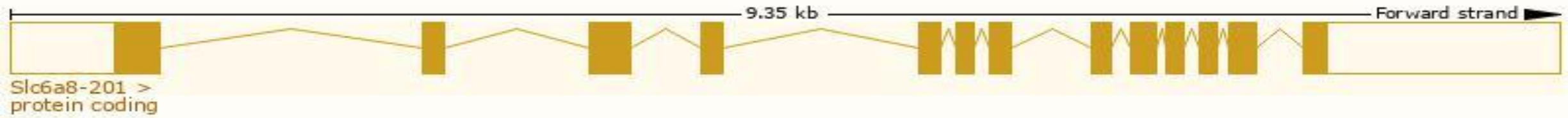
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	X	NC_000086.7 (73673133..73682502)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	X	NC_000086.6 (70918472..70927841)

Transcript information（Ensembl）

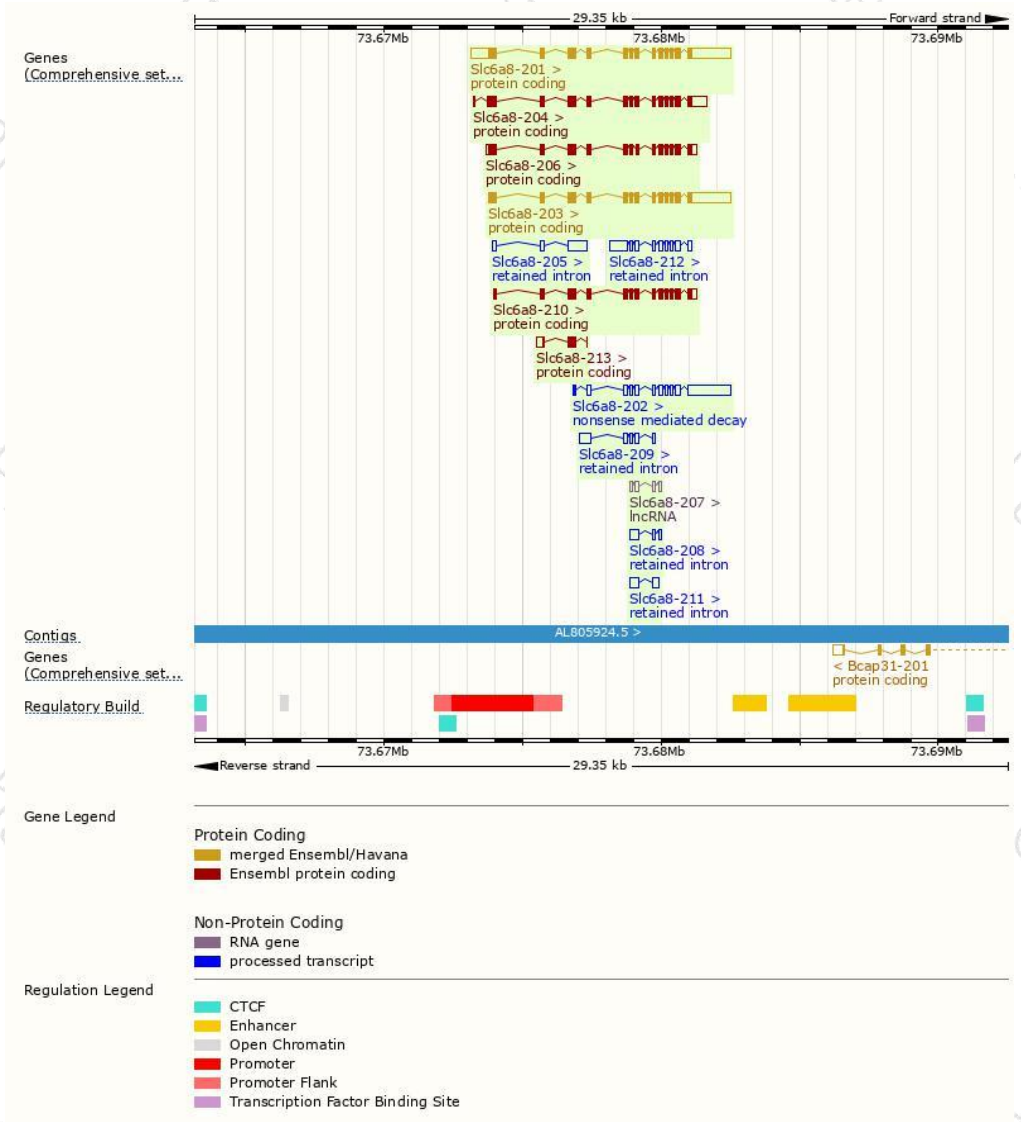
The gene has 13 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Slc6a8-201	ENSMUST00000033752.13	3964	640aa	Protein coding	CCDS30208	Q8VBW1	TSL:1 GENCODE basic
Slc6a8-203	ENSMUST00000114465.8	3314	635aa	Protein coding	CCDS53098	A2ALM6 Q8VBW1	TSL:1 GENCODE basic APPRIS P1
Slc6a8-204	ENSMUST00000114467.8	2509	635aa	Protein coding	CCDS53098	A2ALM6 Q8VBW1	TSL:1 GENCODE basic APPRIS P1
Slc6a8-206	ENSMUST00000164449.7	2151	625aa	Protein coding	-	E9Q151	TSL:5 GENCODE basic
Slc6a8-210	ENSMUST00000168831.7	1919	576aa	Protein coding	-	F6UKB4	CDS 5' incomplete TSL:1
Slc6a8-213	ENSMUST00000171398.1	537	107aa	Protein coding	-	E9Q6T2	CDS 3' incomplete TSL:3
Slc6a8-202	ENSMUST00000114464.8	2729	19aa	Nonsense mediated decay	-	F7C668	CDS 5' incomplete TSL:1
Slc6a8-212	ENSMUST00000170614.7	1613	No protein	Retained intron	-	-	TSL:2
Slc6a8-205	ENSMUST00000146796.2	940	No protein	Retained intron	-	-	TSL:2
Slc6a8-209	ENSMUST00000168764.7	843	No protein	Retained intron	-	-	TSL:3
Slc6a8-211	ENSMUST00000170574.7	527	No protein	Retained intron	-	-	TSL:3
Slc6a8-208	ENSMUST00000167828.1	493	No protein	Retained intron	-	-	TSL:1
Slc6a8-207	ENSMUST00000166200.7	404	No protein	lncRNA	-	-	TSL:3

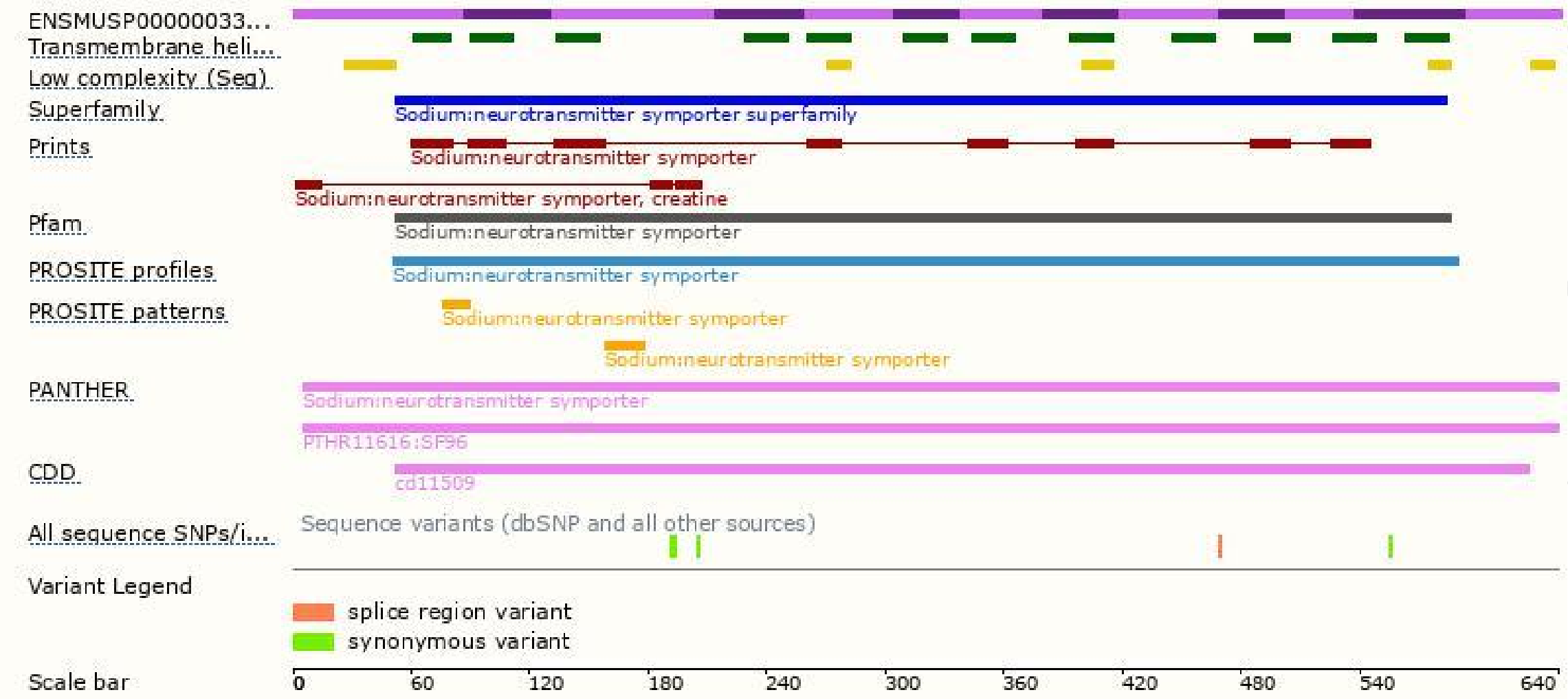
The strategy is based on the design of *Slc6a8-201* transcript,The transcription is shown below



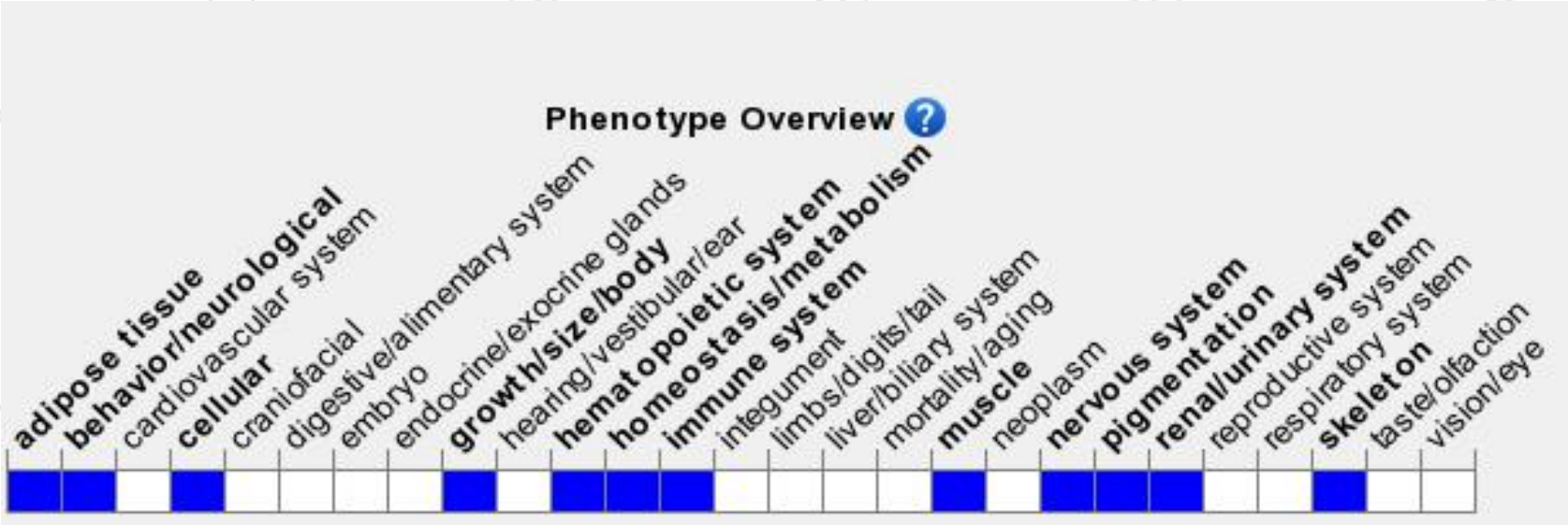
Genomic location distribution



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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