Clcn2 Cas9-CKO Strategy

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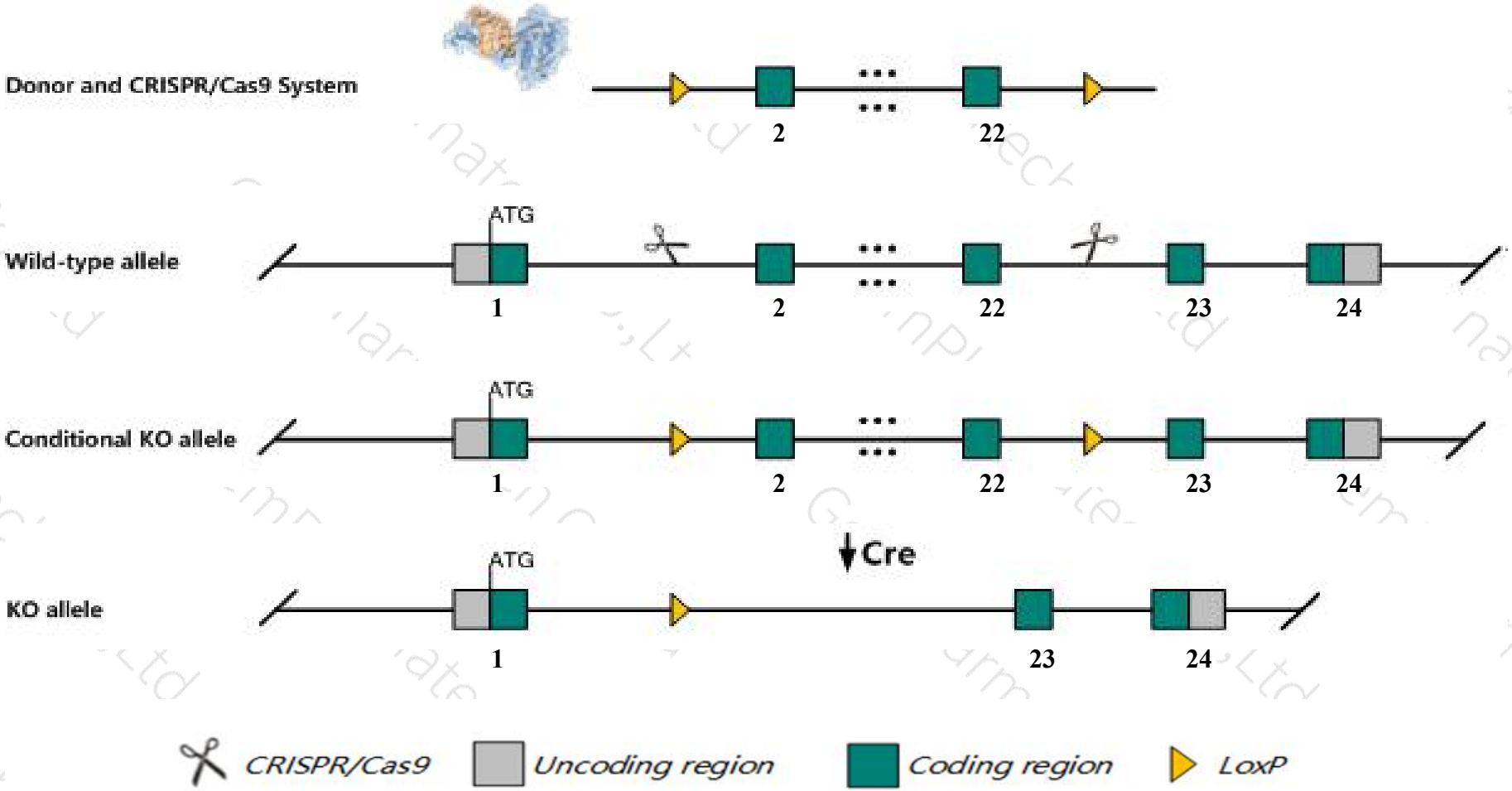
Project Overview

Project Name	***Clcn2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Clcn2* gene. The schematic diagram is as follows:



Technical routes

- The *Clcn2* gene has 11 transcripts. According to the structure of *Clcn2* gene, exon2-exon22 of *Clcn2-201* (ENSMUST00000007207.14) 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 *Clcn2* 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 null allele exhibit abnormal brain morphology, male infertility, and abnormal eye morphology.

➢ The *Clcn2* 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.

➢ The knockout region is about 3.4kb away from the 5-terminal of Polr2h gene, which may affect its 5-terminal regulation after knockout.

➢ 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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Clcn2 chloride channel, voltage-sensitive 2 [*Mus musculus* (house mouse)]

Gene ID: 12724, updated on 17-Aug-2019

Summary

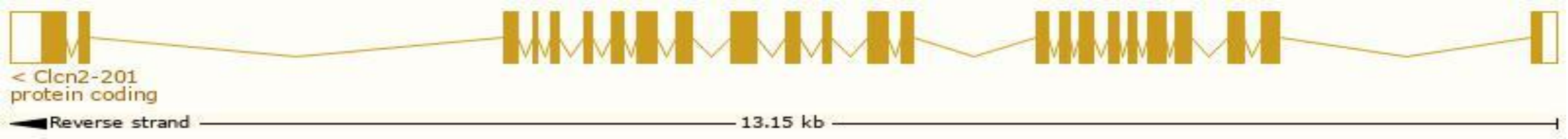
Official Symbol	Clcn2 provided by MGI
Official Full Name	chloride channel, voltage-sensitive 2 provided by MGI
Primary source	MGI:MGI:105061
See related	Ensembl:ENSMUSG00000022843
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Clc2; ClC-2; nmf240; AL118368
Expression	Broad expression in colon adult (RPKM 62.6), adrenal adult (RPKM 45.6) and 23 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

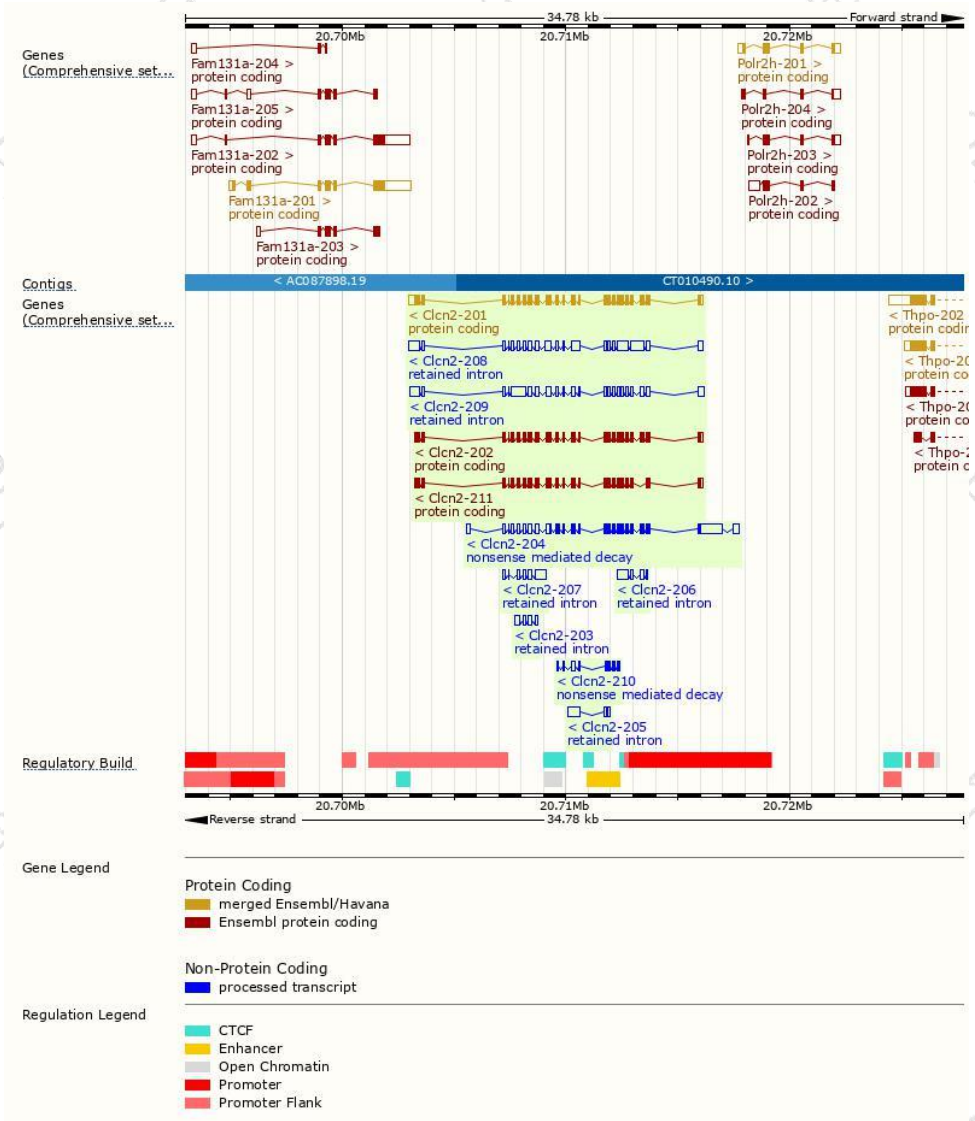
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Clcn2-201	ENSMUST00000007207.14	3128	908aa	Protein coding	CCDS28057	Q9R0A1	TSL:1 GENCODE basic APPRIS P2
Clcn2-202	ENSMUST00000120099.7	2812	891aa	Protein coding	-	A9C437	TSL:5 GENCODE basic APPRIS ALT2
Clcn2-211	ENSMUST00000232309.1	2730	864aa	Protein coding	-	A0A338P673	GENCODE basic APPRIS ALT2
Clcn2-204	ENSMUST00000131522.7	3944	533aa	Nonsense mediated decay	-	D6RFS3	TSL:1
Clcn2-210	ENSMUST00000231381.1	733	155aa	Nonsense mediated decay	-	A0A338P6C2	CDS 5' incomplete
Clcn2-208	ENSMUST00000148131.7	3757	No protein	Retained intron	-	-	TSL:2
Clcn2-209	ENSMUST00000153075.7	3511	No protein	Retained intron	-	-	TSL:2
Clcn2-207	ENSMUST00000144400.7	999	No protein	Retained intron	-	-	TSL:3
Clcn2-206	ENSMUST00000132512.1	794	No protein	Retained intron	-	-	TSL:2
Clcn2-205	ENSMUST00000131833.1	725	No protein	Retained intron	-	-	TSL:3
Clcn2-203	ENSMUST00000123417.1	600	No protein	Retained intron	-	-	TSL:3

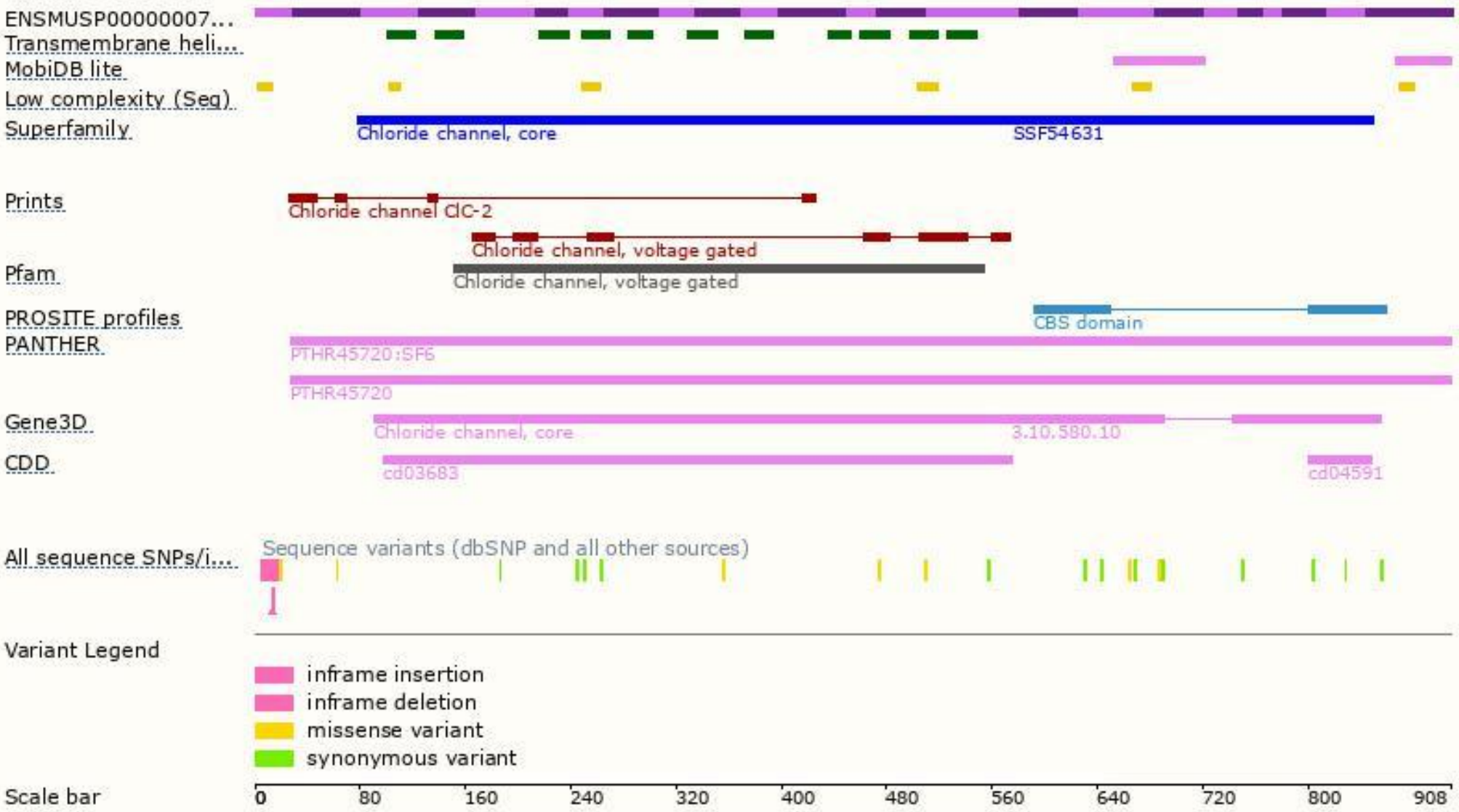
The strategy is based on the design of *Clcn2-201* transcript,The transcription is shown below



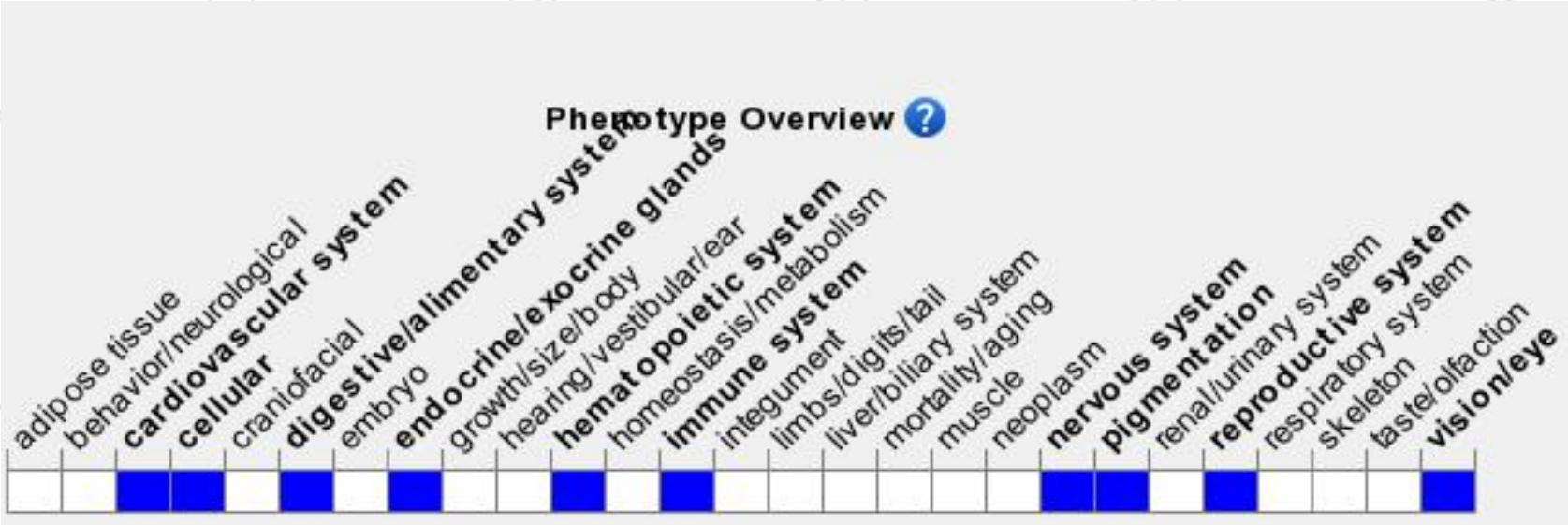
Genomic location distribution



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,Mice homozygous for a null allele exhibit abnormal brain morphology, male infertility, and abnormal eye morphology.

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

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