

Clstn3 Cas9-CKO Strategy

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Project Overview

Project Name

Clstn3

Project type

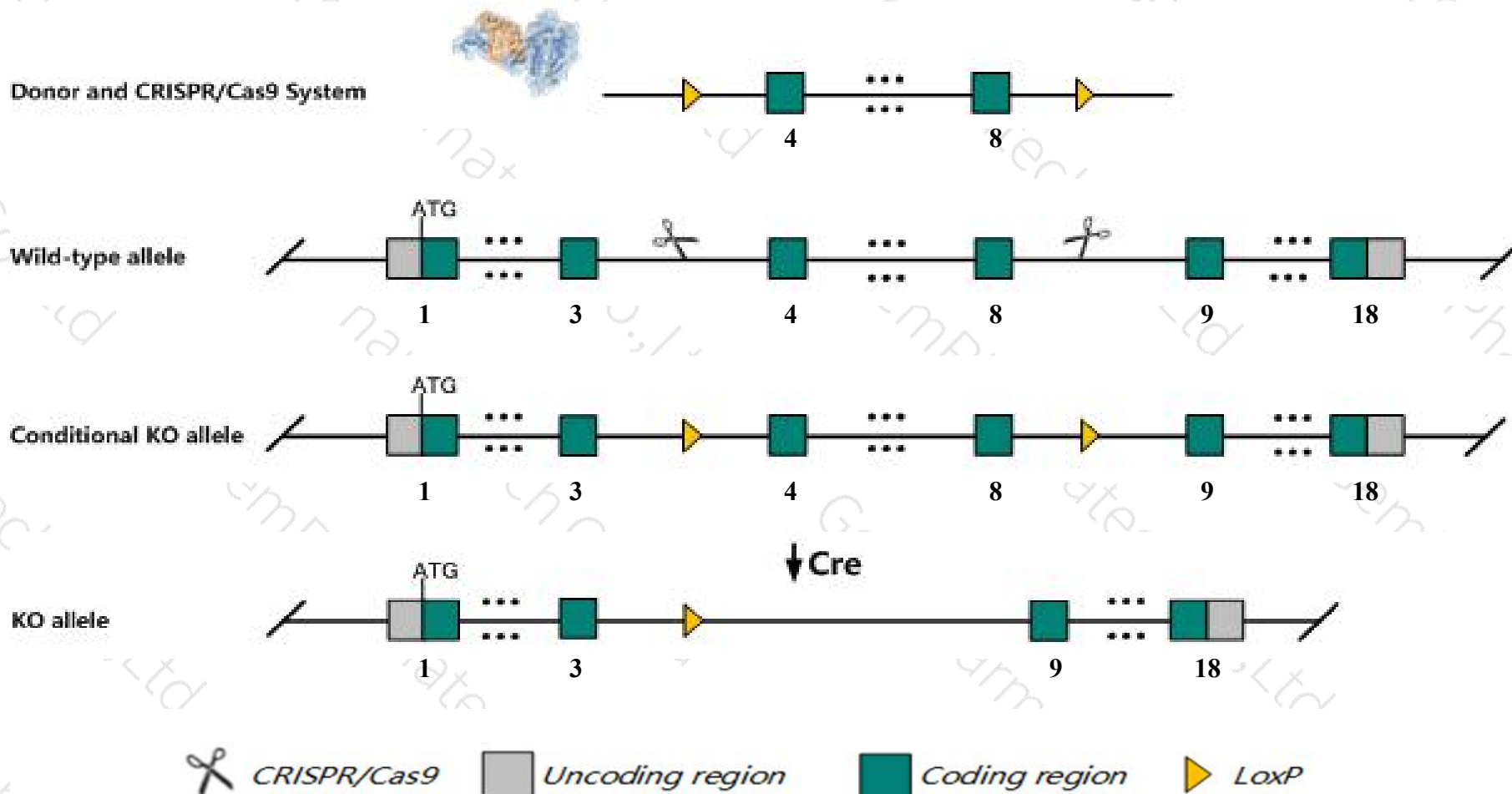
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Clstn3* gene. The schematic diagram is as follows:



Technical routes

- The *Clstn3* gene has 9 transcripts. According to the structure of *Clstn3* gene, exon4-exon8 of *Clstn3-201* (ENSMUST00000008297.4) transcript is recommended as the knockout region. The region contains 940bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Clstn3* 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.

- According to the existing MGI data, mice homozygous for a knock-out allele exhibit reductions in excitatory and inhibitory synapse density and deficits in synaptic transmission.
- Transcript *Clstn3-203*, *Clstn3-206* and *Clstn3-208* may not be affected.
- The *Clstn3* gene is located on the Chr6. 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)

Clstn3 calyntenin 3 [Mus musculus (house mouse)]

Gene ID: 232370, updated on 13-Mar-2020

Summary



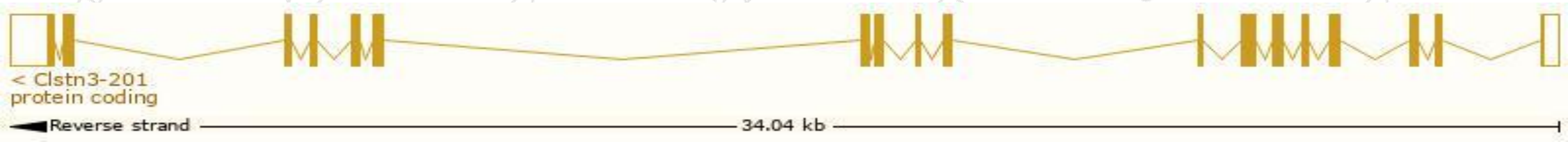
Official Symbol	Clstn3 provided by MGI
Official Full Name	calyntenin 3 provided by MGI
Primary source	MGI:MGI:2178323
See related	Ensembl:ENSMUSG00000008153
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CSTN3, Clstn3b, Cs3, Cst-3, alc-beta
Expression	Biased expression in cerebellum adult (RPKM 97.0), cortex adult (RPKM 62.3) and 8 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

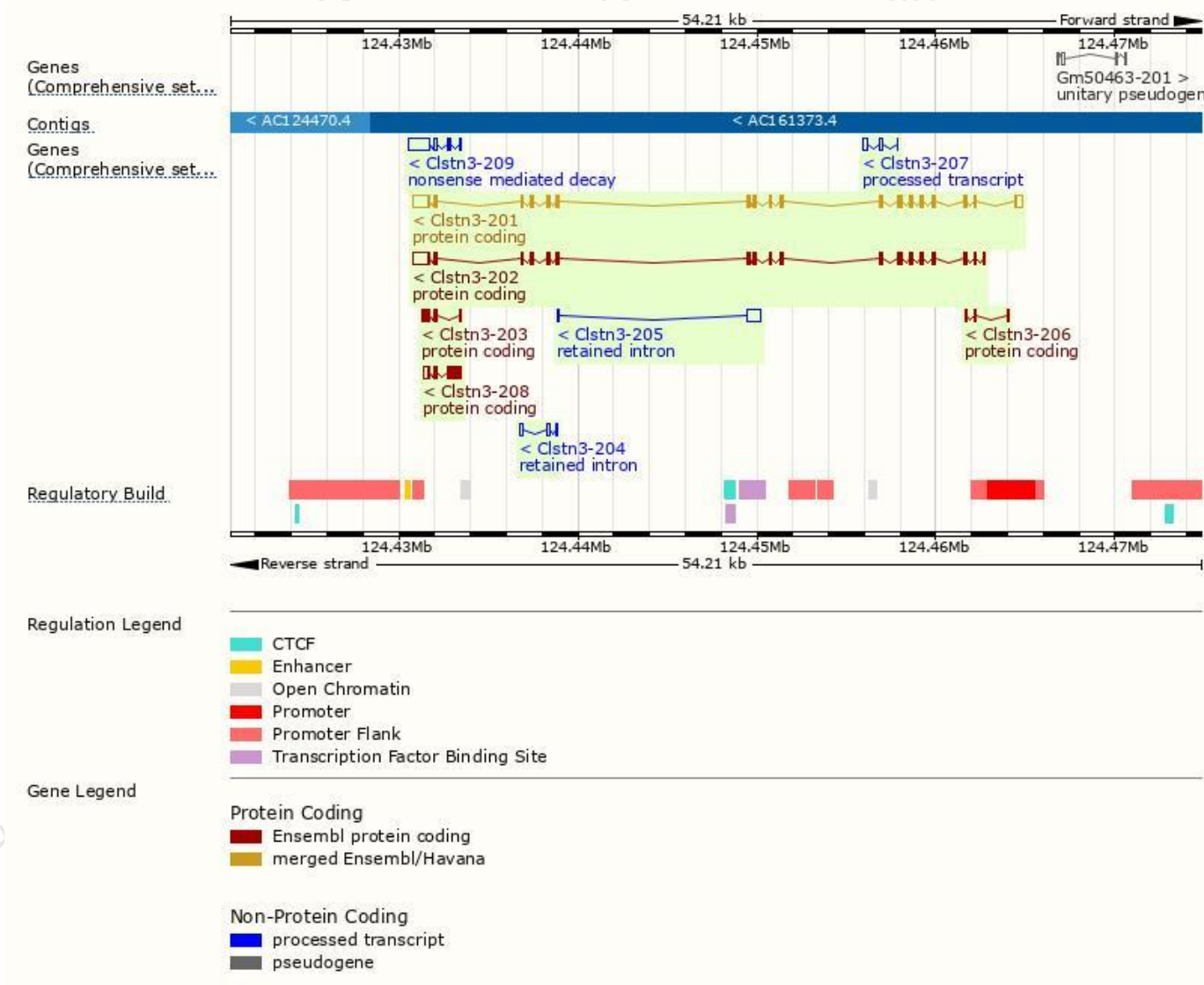
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Clstn3-201	ENSMUST00000008297.4	4014	956aa	Protein coding	CCDS20519	Q99JH7	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 P3
Clstn3-202	ENSMUST00000112523.7	3703	919aa	Protein coding	CCDS85156	D3Z601	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 ALT2
Clstn3-208	ENSMUST00000238807.1	1382	357aa	Protein coding	-	A0A4P8DYG6	GENCODE basic
Clstn3-203	ENSMUST00000124998.1	728	191aa	Protein coding	-	-	TSL:2 GENCODE basic
Clstn3-206	ENSMUST00000150774.2	366	64aa	Protein coding	-	A0A0N4SW90	CDS 3' incomplete TSL:5
Clstn3-209	ENSMUST00000239139.1	1687	78aa	Nonsense mediated decay	-	-	
Clstn3-207	ENSMUST00000156040.1	391	No protein	Processed transcript	-	-	TSL:2
Clstn3-205	ENSMUST00000147947.1	765	No protein	Retained intron	-	-	TSL:2
Clstn3-204	ENSMUST00000143283.1	474	No protein	Retained intron	-	-	TSL:3

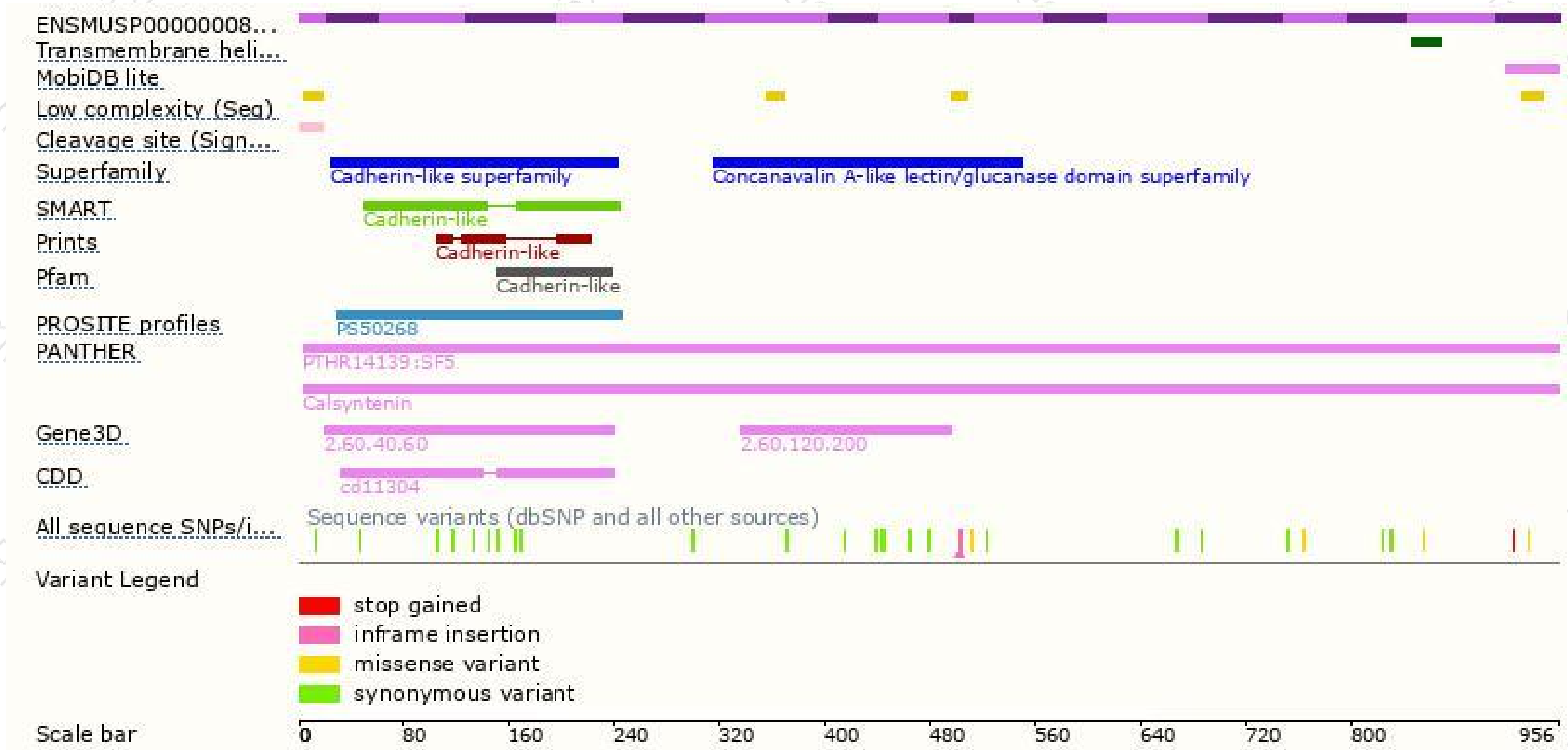
The strategy is based on the design of *Clstn3-201* transcript,the transcription is shown below:



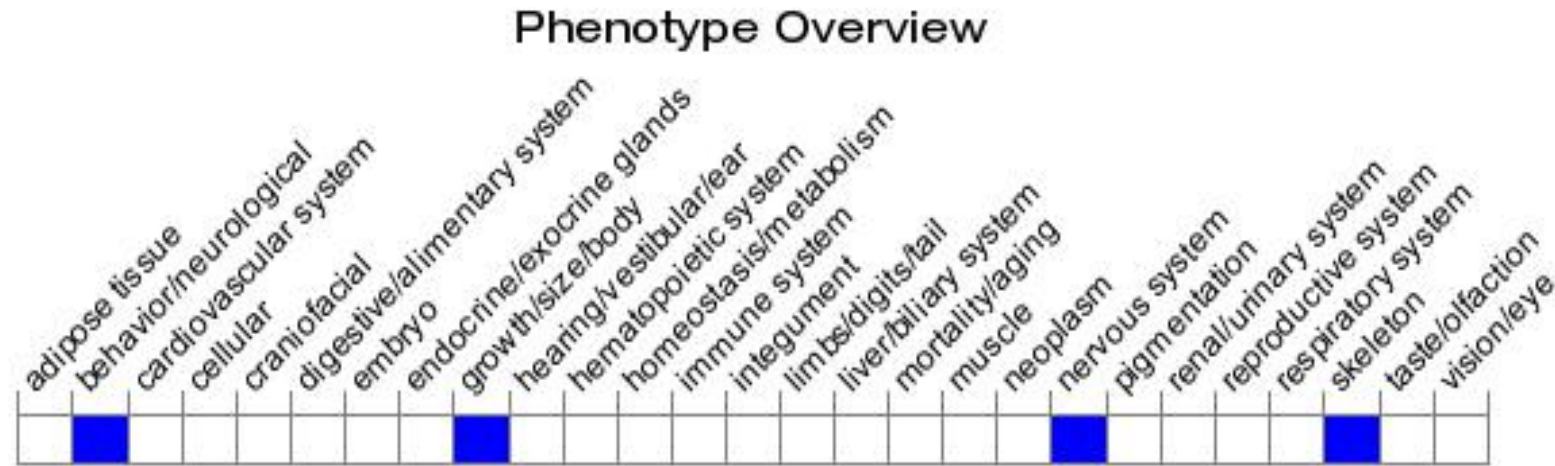
Genomic location distribution



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 knock-out allele exhibit reductions in excitatory and inhibitory synapse density and deficits in synaptic transmission.

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

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