Nrxn2 Cas9-CKO Strategy

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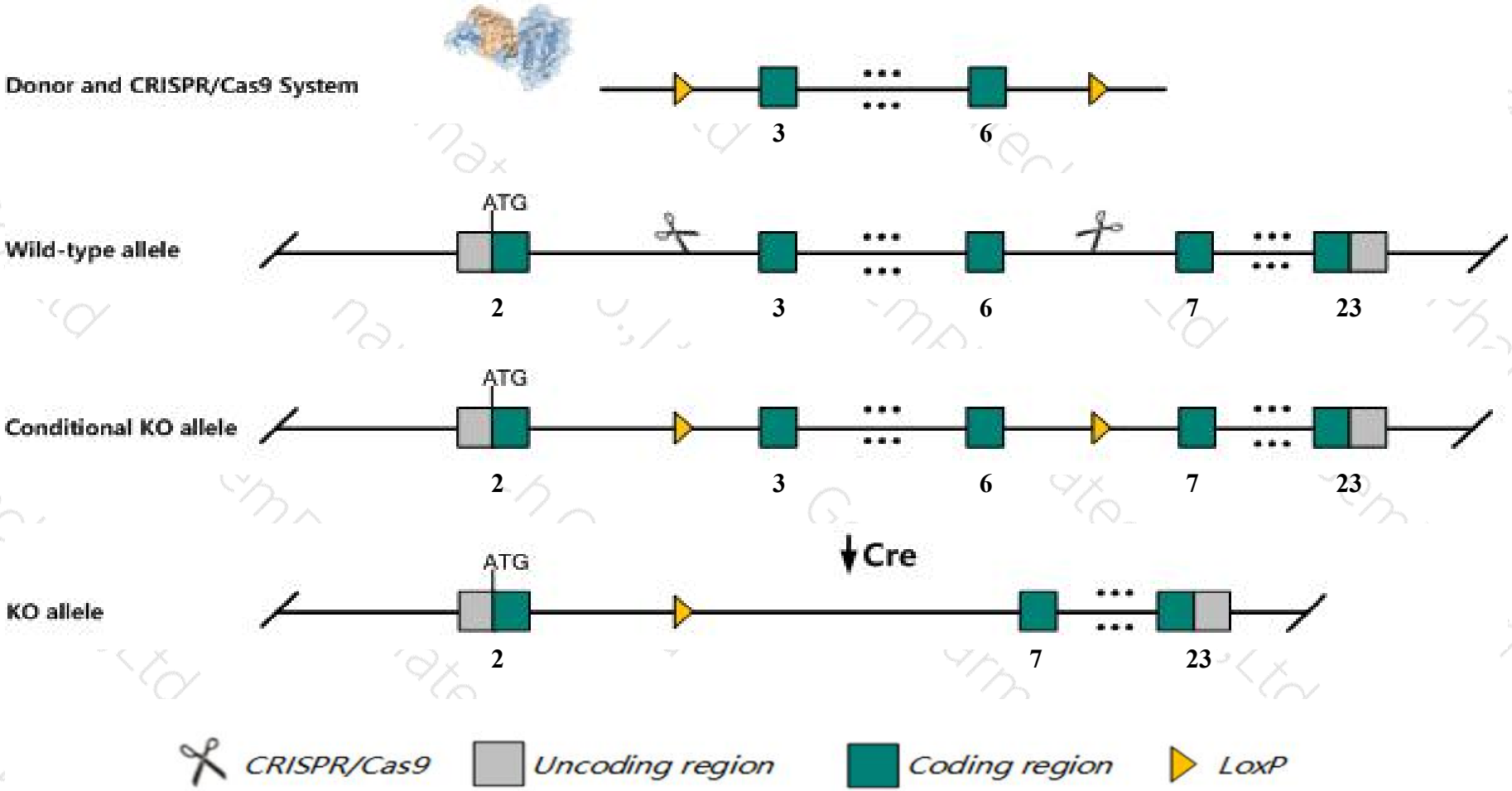
Project Overview

Project Name	***Nrxn2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nrxn2* gene. The schematic diagram is as follows:



Technical routes

- The *Nrxn2* gene has 18 transcripts. According to the structure of *Nrxn2* gene, exon3-exon6 of *Nrxn2-205* (ENSMUST00000113462.7) transcript is recommended as the knockout region.The region contains 422bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Nrxn2* 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 knock-out allele are generally non-viable; surviving homozygotes show a 30-40% decrease in body weight and their inhibitory postsynaptic currents (ipscs) are decreased in cortical slice cultures.
- The *Nrxn2* gene is located on the Chr19. 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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Nrxn2 neurexin II [Mus musculus (house mouse)]

Gene ID: 18190, updated on 13-Mar-2020

Summary

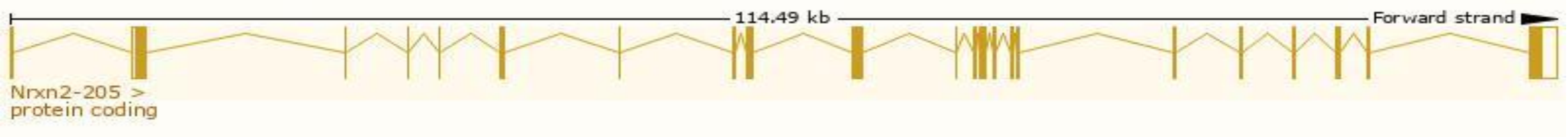
Official Symbol	Nrxn2 provided by MGI
Official Full Name	neurexin II provided byMGI
Primary source	MGI:MGI:1096362
See related	Ensembl:ENSMUSG00000033768
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	6430591O13Rik, mKIAA0921
Expression	Biased expression in cerebellum adult (RPKM 41.2), frontal lobe adult (RPKM 37.4) and 7 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

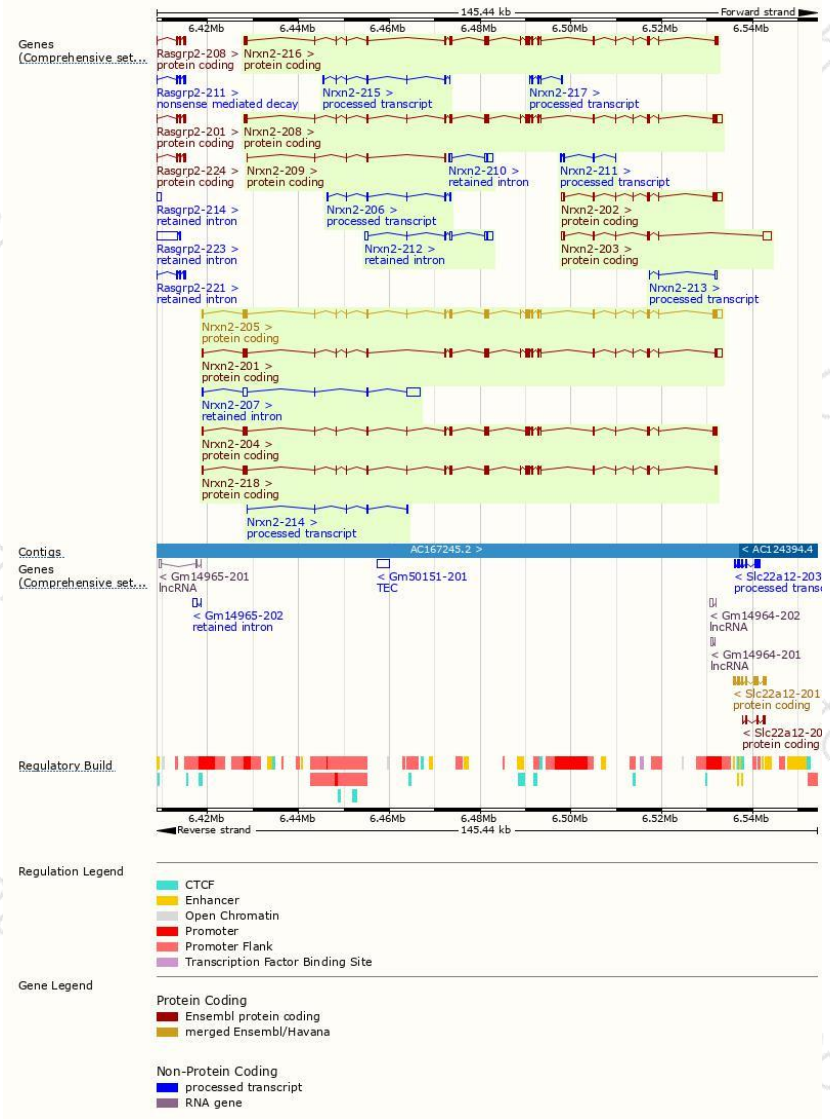
The gene has 18 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nrxn2-205	ENSMUST00000113462.7	6667	1703aa	Protein coding	CCDS57132	E9PUM9	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 P4
Nrxn2-201	ENSMUST00000077182.12	6059	1503aa	Protein coding	CCDS57133	E9Q5N7	TSL:5 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
Nrxn2-216	ENSMUST00000235714.1	5001	1503aa	Protein coding	CCDS57133	E9Q5N7	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
Nrxn2-208	ENSMUST00000137166.7	6314	1710aa	Protein coding	-	E9Q7X7	TSL:5 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
Nrxn2-204	ENSMUST00000113461.7	5505	1640aa	Protein coding	-	E9PUN0	TSL:5 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
Nrxn2-218	ENSMUST00000236635.1	5118	1511aa	Protein coding	-	A0A494B8Y4	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
Nrxn2-202	ENSMUST00000113458.7	3503	660aa	Protein coding	-	E9PUN2	TSL:5 GENCODE basic
Nrxn2-203	ENSMUST00000113459.1	3285	353aa	Protein coding	-	E9PUN1	TSL:2 GENCODE basic
Nrxn2-209	ENSMUST00000137821.7	648	216aa	Protein coding	-	F6Y027	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Nrxn2-206	ENSMUST00000124815.7	818	No protein	Processed transcript	-	-	TSL:3
Nrxn2-215	ENSMUST00000157014.7	739	No protein	Processed transcript	-	-	TSL:3
Nrxn2-217	ENSMUST00000236610.1	725	No protein	Processed transcript	-	-	
Nrxn2-214	ENSMUST00000155158.2	604	No protein	Processed transcript	-	-	TSL:5
Nrxn2-213	ENSMUST00000154580.1	576	No protein	Processed transcript	-	-	TSL:3
Nrxn2-211	ENSMUST00000148391.1	547	No protein	Processed transcript	-	-	TSL:5
Nrxn2-207	ENSMUST00000128272.7	4456	No protein	Retained intron	-	-	TSL:1
Nrxn2-212	ENSMUST00000150792.7	2748	No protein	Retained intron	-	-	TSL:5
Nrxn2-210	ENSMUST00000140435.1	1933	No protein	Retained intron	-	-	TSL:1

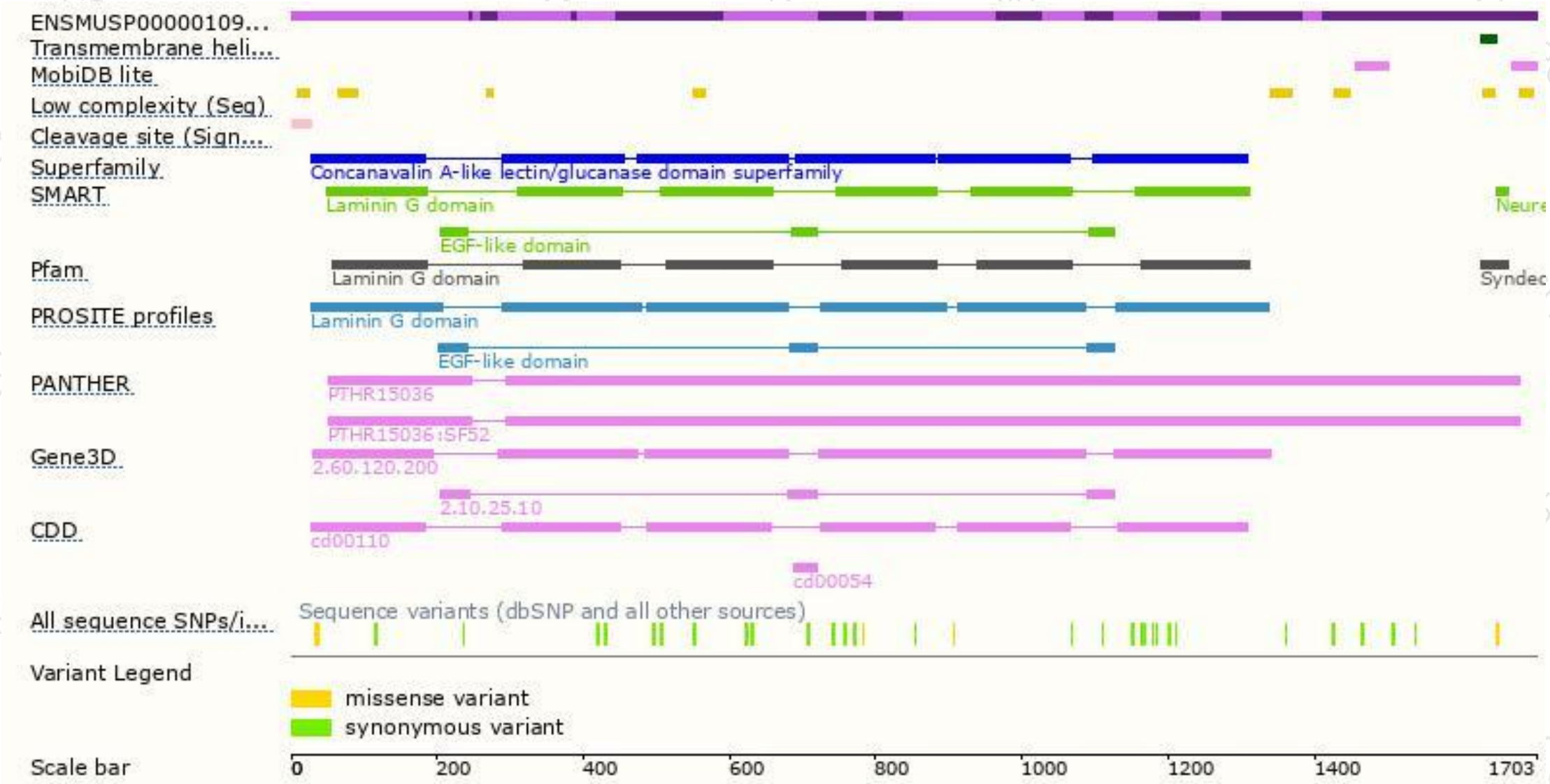
The strategy is based on the design of *Nrxn2-205* transcript,the transcription is shown below:



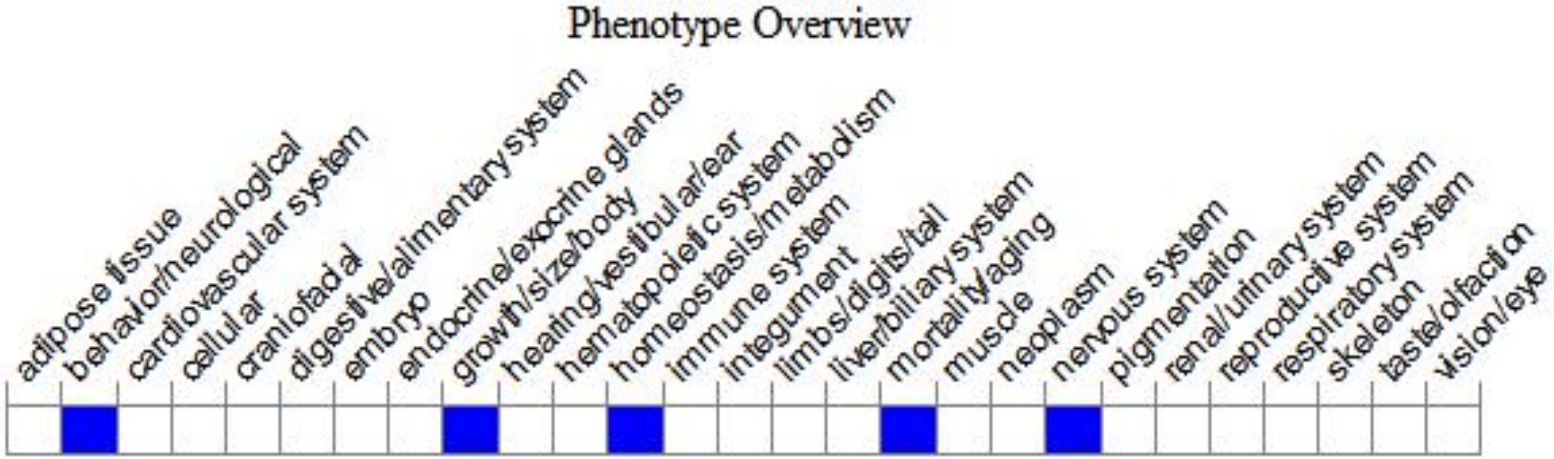
Genomic location distribution



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 are generally non-viable; surviving homozygotes show a 30-40% decrease in body weight and their inhibitory postsynaptic currents (IPSCs) are decreased in cort slice cultures.

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

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