Gria2 Cas9-CKO Strategy

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Project Overview

Project Name	***Gria2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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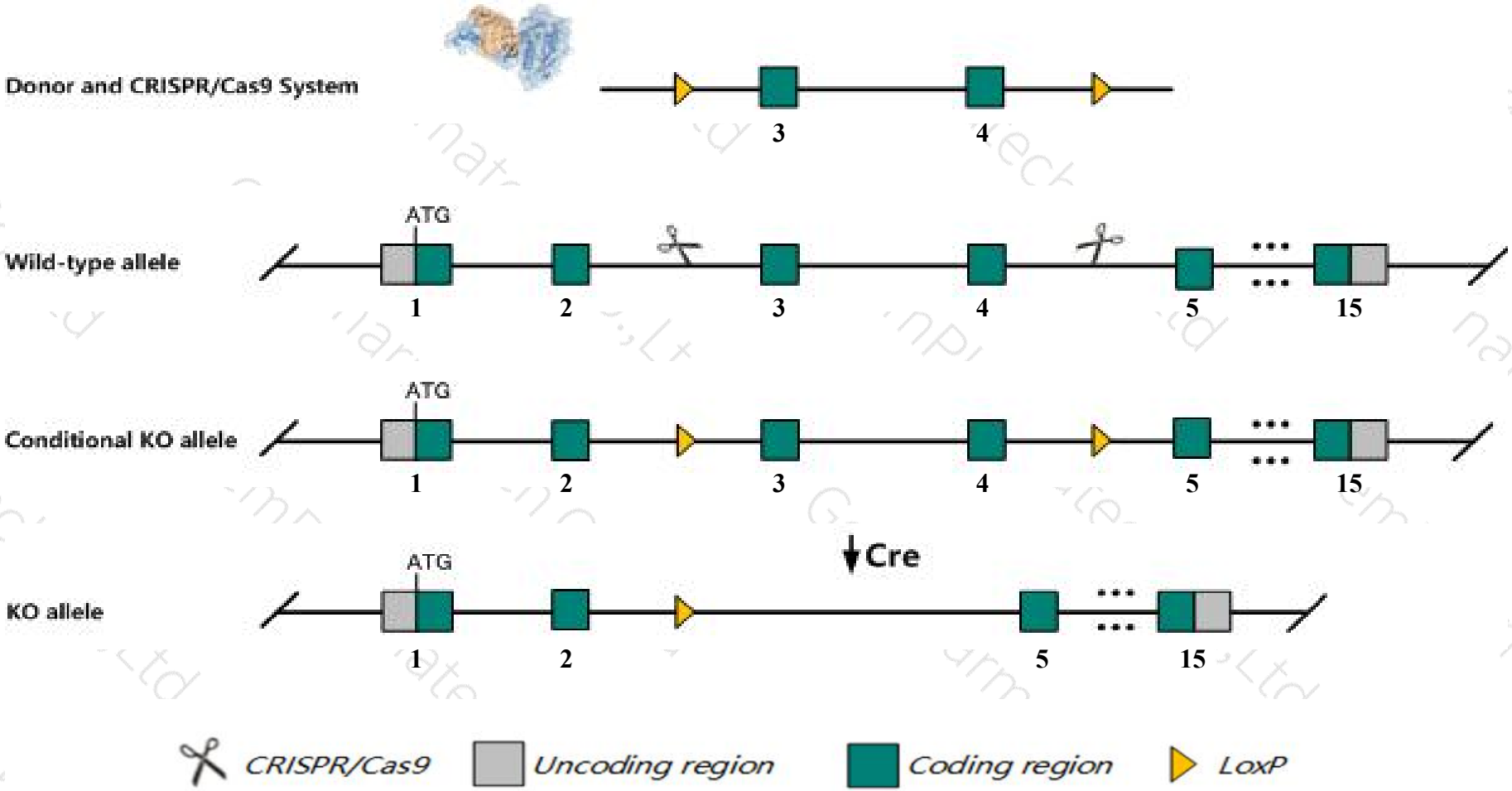
This model will use CRISPR/Cas9 technology to edit the *Gria2* gene. The schematic diagram is as follows:



Technical routes

- The *Gria2* gene has 8 transcripts. According to the structure of *Gria2* gene, exon3-exon4 of *Gria2-201* (ENSMUST00000075316.9) transcript is recommended as the knockout region.The region contains 437bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Gria2* 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,Homozygotes for targeted null mutations exhibit epilepsy, deficient dendritic architecture, altered exploratory behavior, impaired motor and learning performance, and increased mortality.

- The *Gria2* gene is located on the Chr3. 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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Gria2 glutamate receptor, ionotropic, AMPA2 (alpha 2) [Mus musculus (house mouse)]

Gene ID: 14800, updated on 25-Mar-2019

Summary

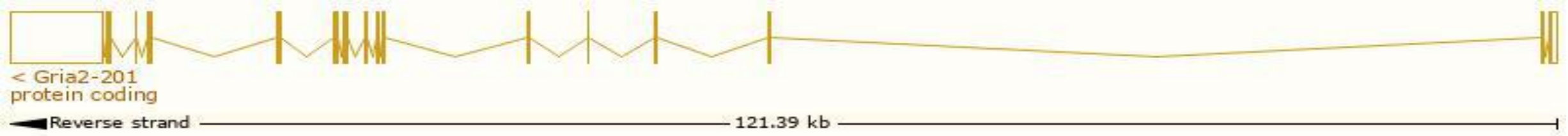
Official Symbol	Gria2 provided by MGI
Official Full Name	glutamate receptor, ionotropic, AMPA2 (alpha 2) provided byMGI
Primary source	MGI:MGI:95809
See related	Ensembl:ENSMUSG00000033981
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	GluA2, GluR-B, Glur-2, Glur2, gluR-K2
Summary	Glutamate receptors are the predominant excitatory neurotransmitter receptors in the mammalian brain and are activated in a variety of normal neurophysiologic processes. This gene product belongs to a family of glutamate receptors that are sensitive to alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), and function as ligand-activated cation channels. These channels are assembled from 4 related subunits, Gria1-4. The subunit encoded by this gene (Gria2) is subject to RNA editing (CAG->CGG; Q->R) within the second transmembrane domain, which is thought to render the channel impermeable to Ca(2+). Alternative splicing, resulting in transcript variants encoding different isoforms, (including the flip and flop isoforms that vary in their signal transduction properties), has been noted for this gene. [provided by RefSeq, Jul 2008]
Expression	Biased expression in CNS E18 (RPKM 50.8), frontal lobe adult (RPKM 41.0) and 5 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

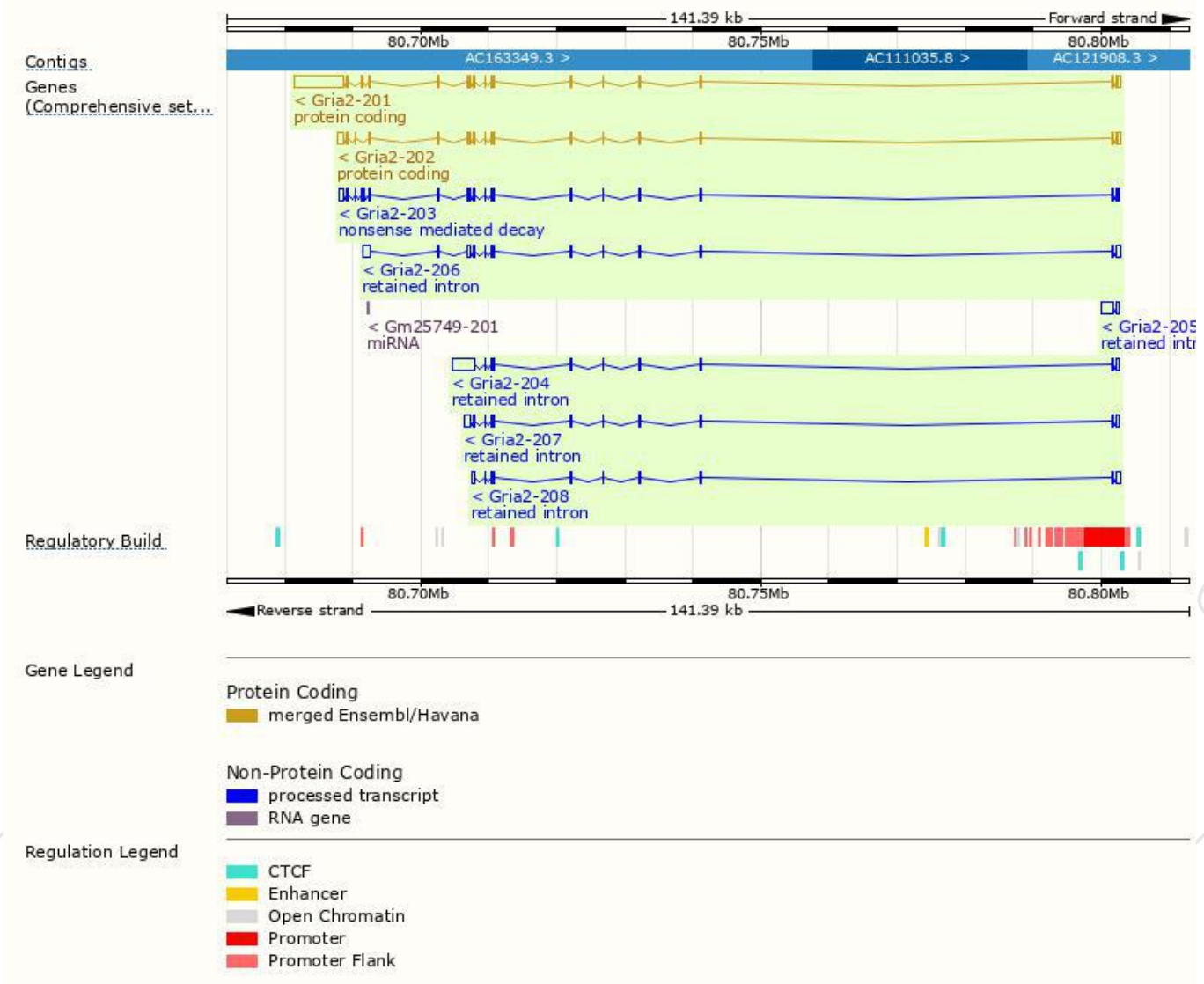
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gria2-201	ENSMUST00000075316.9	10371	883aa	Protein coding	CCDS17423	G5E8H1	TSL:1 GENCODE basic APPRIS P3
Gria2-202	ENSMUST00000107745.7	4026	883aa	Protein coding	CCDS38459	E9QKC0	TSL:5 GENCODE basic APPRIS ALT1
Gria2-203	ENSMUST00000192463.1	3681	777aa	Nonsense mediated decay	-	A0A0A6YW90	TSL:1
Gria2-204	ENSMUST00000193645.5	4799	No protein	Retained intron	-	-	TSL:1
Gria2-206	ENSMUST00000194383.5	3462	No protein	Retained intron	-	-	TSL:1
Gria2-207	ENSMUST00000194523.5	2719	No protein	Retained intron	-	-	TSL:1
Gria2-205	ENSMUST00000194164.1	2147	No protein	Retained intron	-	-	TSL:1
Gria2-208	ENSMUST00000195062.1	1975	No protein	Retained intron	-	-	TSL:1

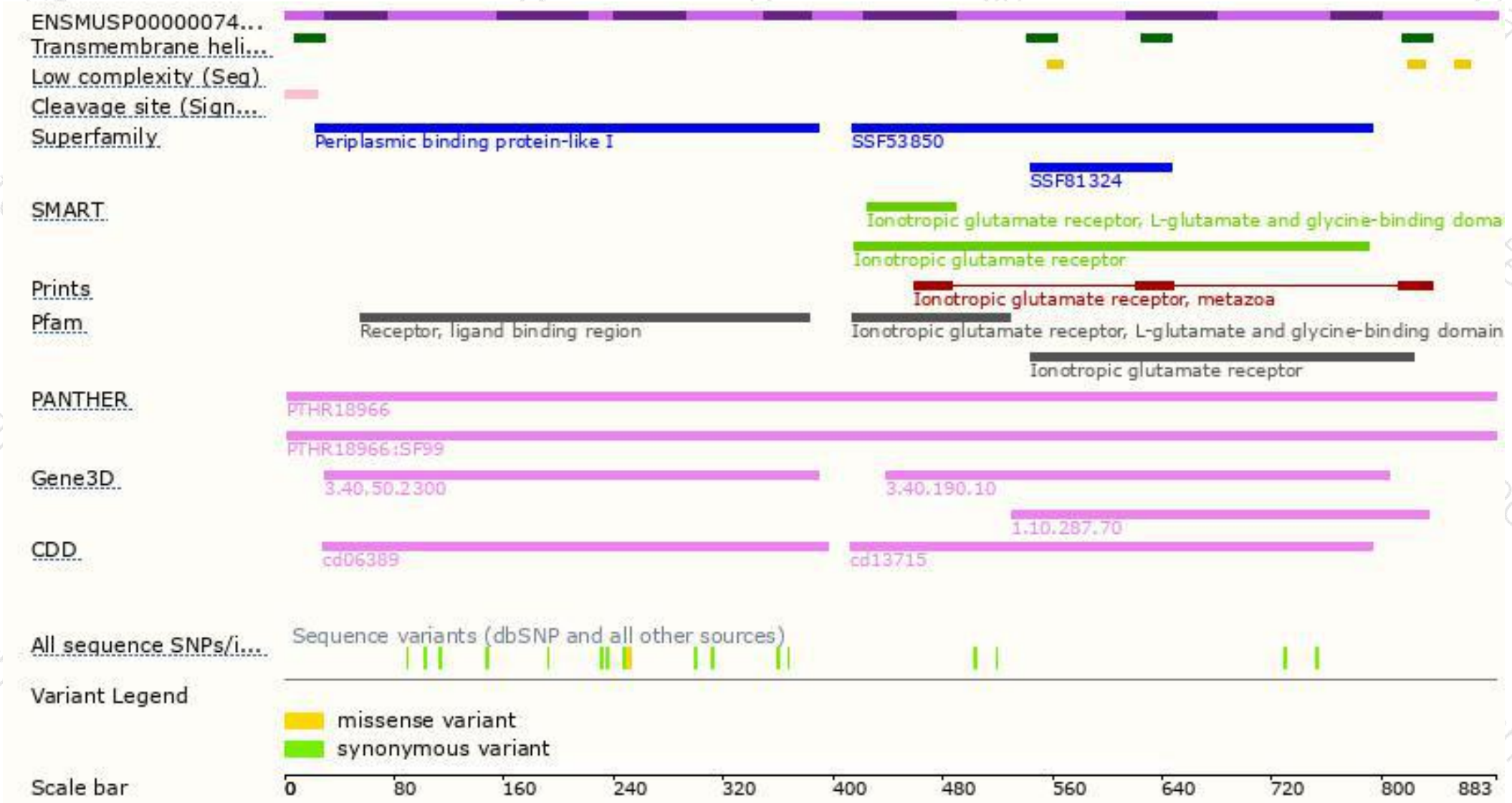
The strategy is based on the design of *Gria2-201* transcript,The transcription is shown below



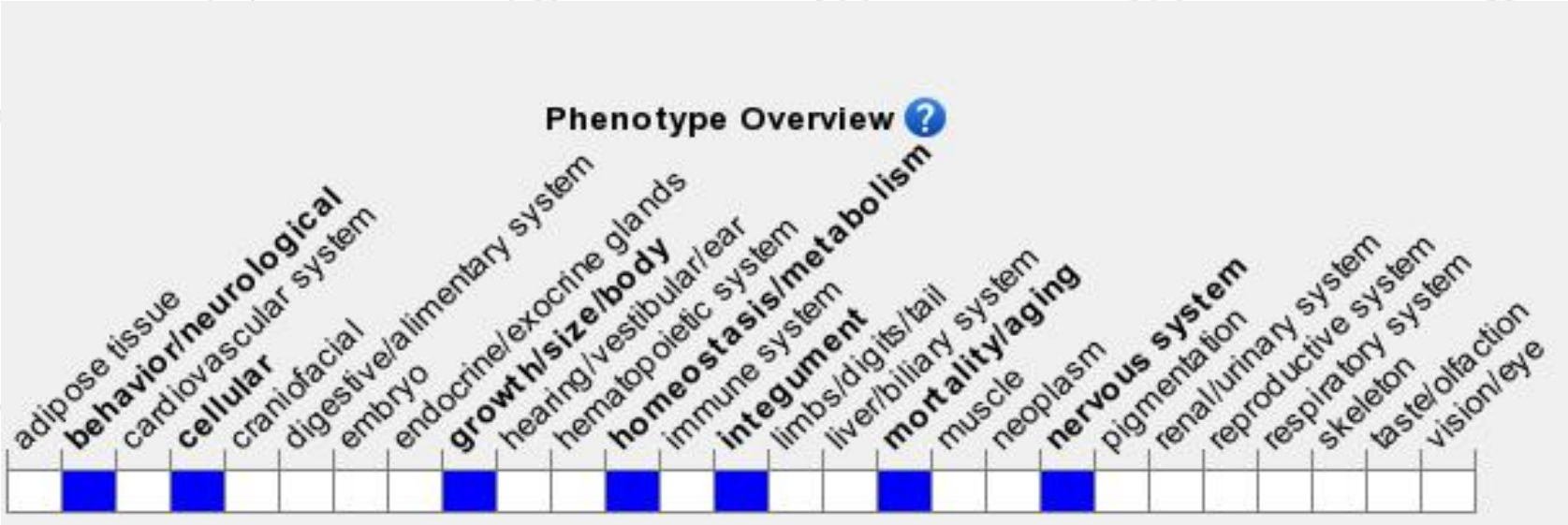
Genomic location distribution



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,Homozygotes for targeted null mutations exhibit epilepsy, deficient dendritic architecture, altered exploratory behavior, impaired motor and learning performance, and increased mortality.

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

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