

Glra2 Cas9-KO Strategy Romphamaxon College

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Project Overview



Project Name Glra2

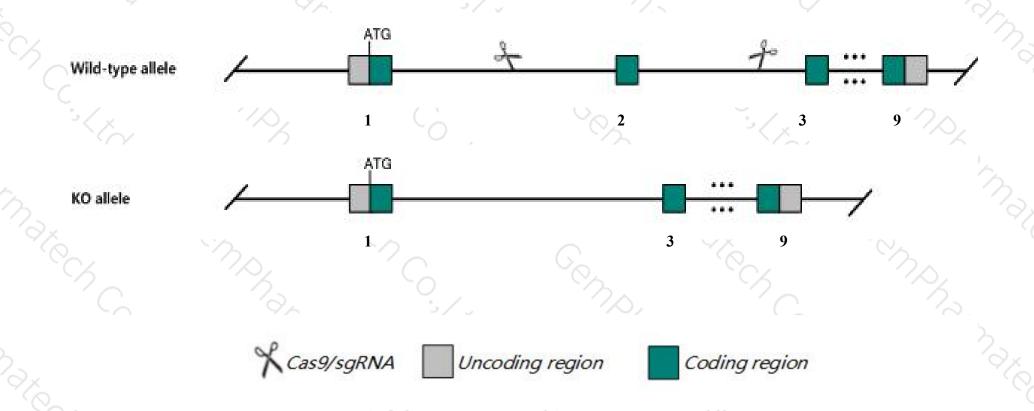
Project type Cas9-KO

Strain background C57BL/6J

Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Glra2* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Glra2* gene has 1 transcript. According to the structure of *Glra2* gene, exon2 of *Glra2-201* (
 ENSMUST00000058787.8) transcript is recommended as the knockout region. The region contains 134bp coding sequence.

 Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Glra2* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.

Notice



- ➤ According to the existing MGI data, Mice homozygous for a null allele lack cortical neuron responses to glycine and taurine but are otherwise normal. Mice homozygous for another targeted allele exhibit impaired interneuron migration into the cortical wall.
- > The *Glra2* 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)



Glra2 glycine receptor, alpha 2 subunit [Mus musculus (house mouse)]

Gene ID: 237213, updated on 31-Jan-2019

Summary

☆ ?

Official Symbol Glra2 provided by MGI

Official Full Name glycine receptor, alpha 2 subunit provided by MGI

Primary source MGI:MGI:95748

See related Ensembl:ENSMUSG00000018589

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

Expression Biased expression in CNS E18 (RPKM 14.3), whole brain E14.5 (RPKM 8.6) and 4 other tissues See more

Orthologs <u>human</u> all

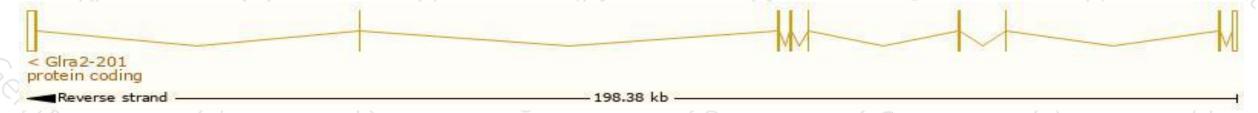
Transcript information (Ensembl)



The gene has 1 transcript, and the transcript is shown below:

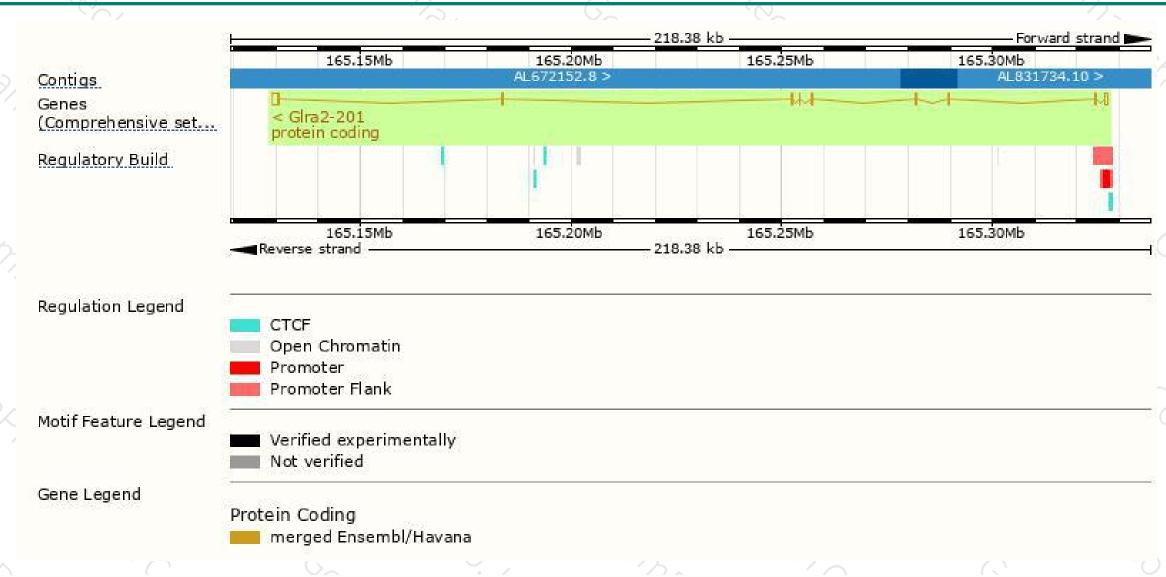
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Glra2-201	ENSMUST00000058787.8	3150	452aa	Protein coding	CCDS41206	Q3UTL8 Q7TNC8	TSL:1 GENCODE basic APPRIS P1	

The strategy is based on the design of *Glra2-201* transcript, The transcription is shown below



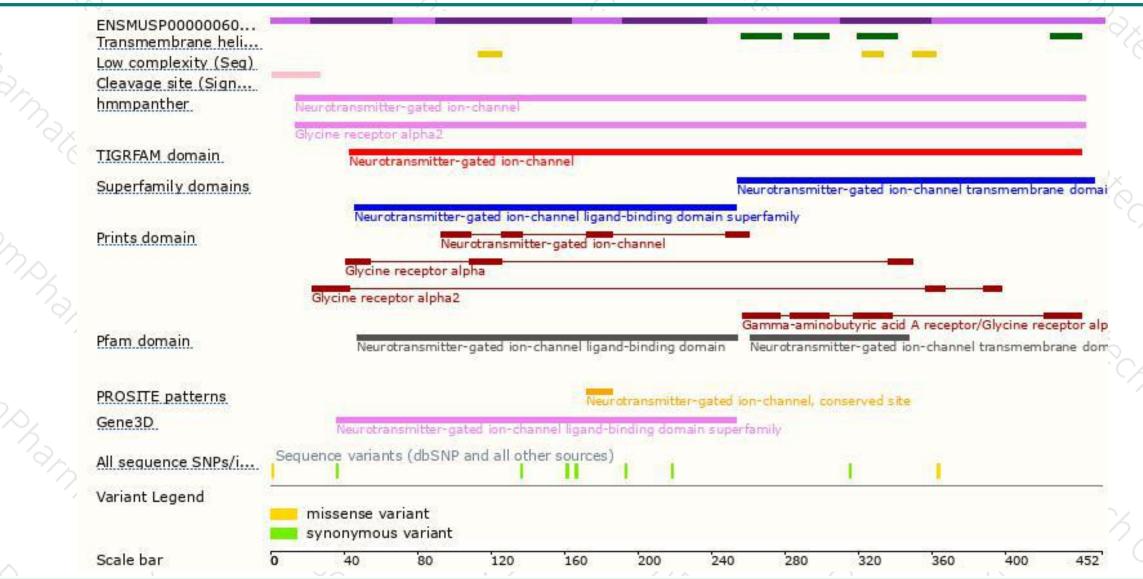
Genomic location distribution





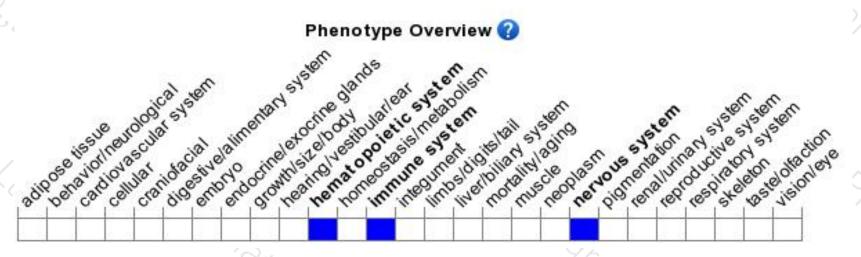
Protein domain





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 lack cortical neuron responses to glycine and taurine but are otherwise normal. Mice homozygous for another targeted allele exhibit impaired interneuron migration into the cortical wall.



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

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