# *Gria4* Cas9-CKO Strategy

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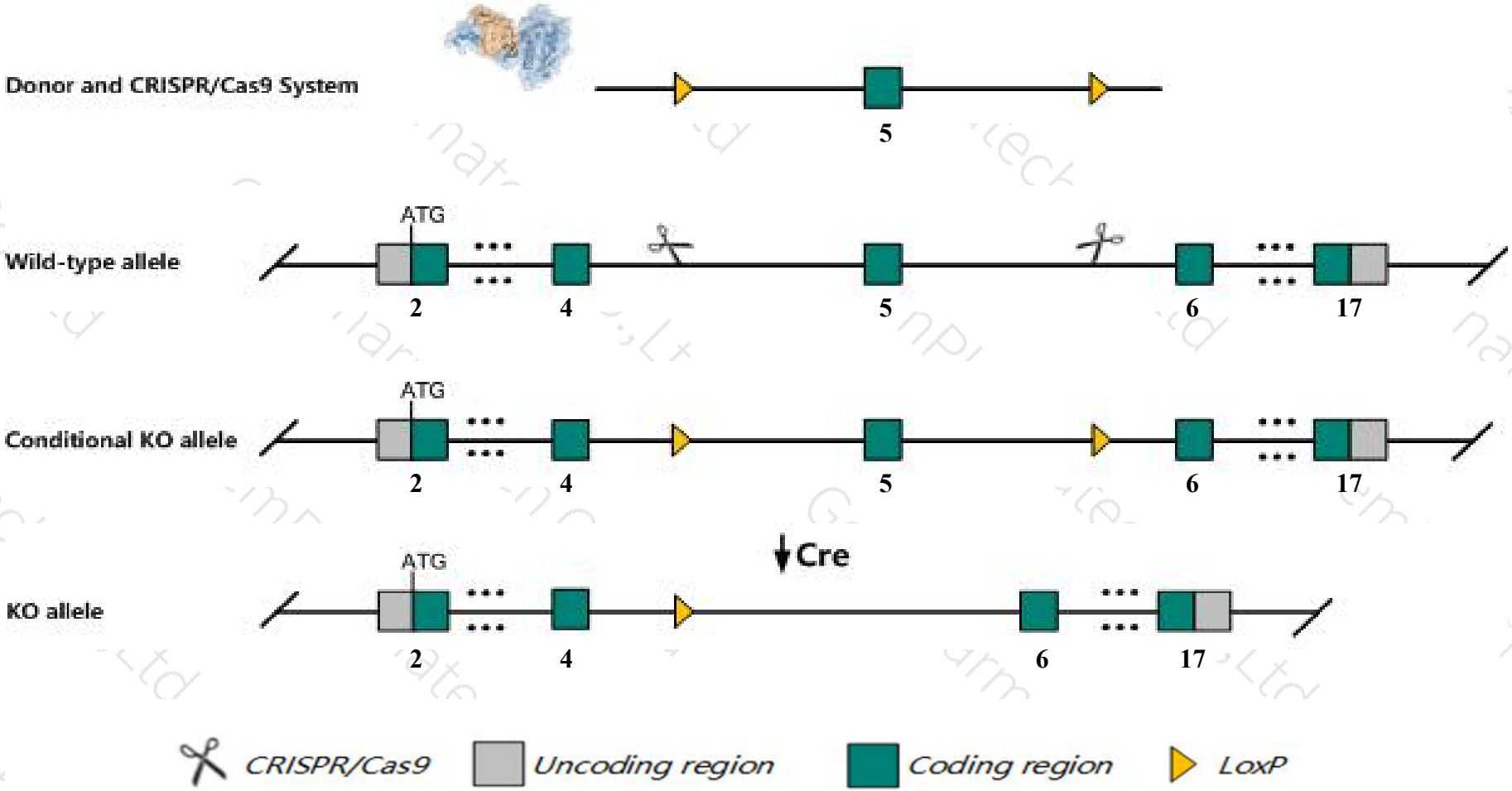
**Reviewer:** Huimin Su
**Design Date:** 2019-11-20

# Project Overview

| | |
|---|---|
| **Project Name** | ***Gria4*** |
| **Project type** | **Cas9-CKO** |
| **Strain background** | **C57BL/6JGpt** |

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Gria4* gene. The schematic diagram is as follows:



# Technical routes

- The *Gria4* gene has 4 transcripts. According to the structure of *Gria4* gene, exon5 of *Gria4-201* (ENSMUST00000027020.12) transcript is recommended as the knockout region.The region contains 185bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Gria4* 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 targeted mutation display hyperactivity, decreased thermal nociception, and abnormal sensitivity to pharmacologically induced seizures.

➢ The *Gria4* gene is located on the Chr9. 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）

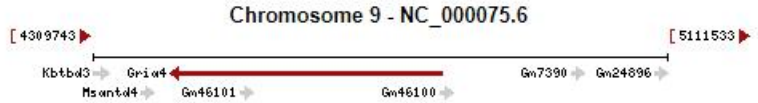
集萃药康 GemPharmatech

**Gria4 glutamate receptor, ionotropic, AMPA4 (alpha 4) [ *Mus musculus* (house mouse) ]**

Gene ID: 14802, updated on 29-Oct-2019

**Summary**

| | |
|---|---|
| **Official Symbol** | Gria4 provided by MGI |
| **Official Full Name** | glutamate receptor, ionotropic, AMPA4 (alpha 4) provided by MGI |
| **Primary source** | MGI:MGI:95811 |
| **See related** | Ensembl:ENSMUSG00000025892 |
| **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** | GluA4; Glur4; spkw1; GluR-D; Glur-4; Gluralpha4 |
| **Summary** | Glutamate receptors are the predominant excitatory neurotransmitter receptors in the mammalian brain and are activated in a variety of normal neurophysiologic processes. These receptors are heteromeric protein complexes composed of multiple subunits, arranged to form ligand-gated ion channels. The classification of glutamate receptors is based on their activation by different pharmacologic agonists. The subunit encoded by this gene belongs to a family of AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate)-sensitive glutamate receptors, and is subject to RNA editing (AGA->GGA; R->G). Alternative splicing of this gene results in transcript variants encoding different isoforms, which may vary in their signal transduction properties. [provided by RefSeq, Jul 2008] |
| **Expression** | Biased expression in cerebellum adult (RPKM 34.2), frontal lobe adult (RPKM 17.3) and 6 other tissues See more |
| **Orthologs** | human all |

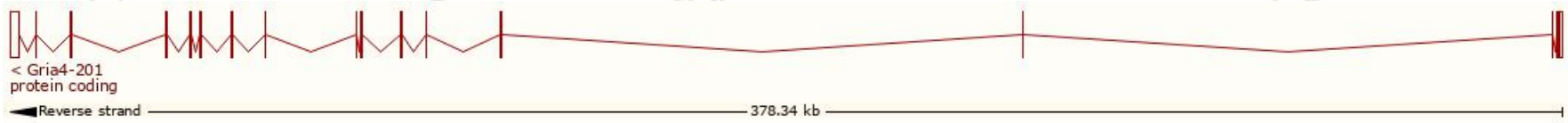


# Transcript information（Ensembl）

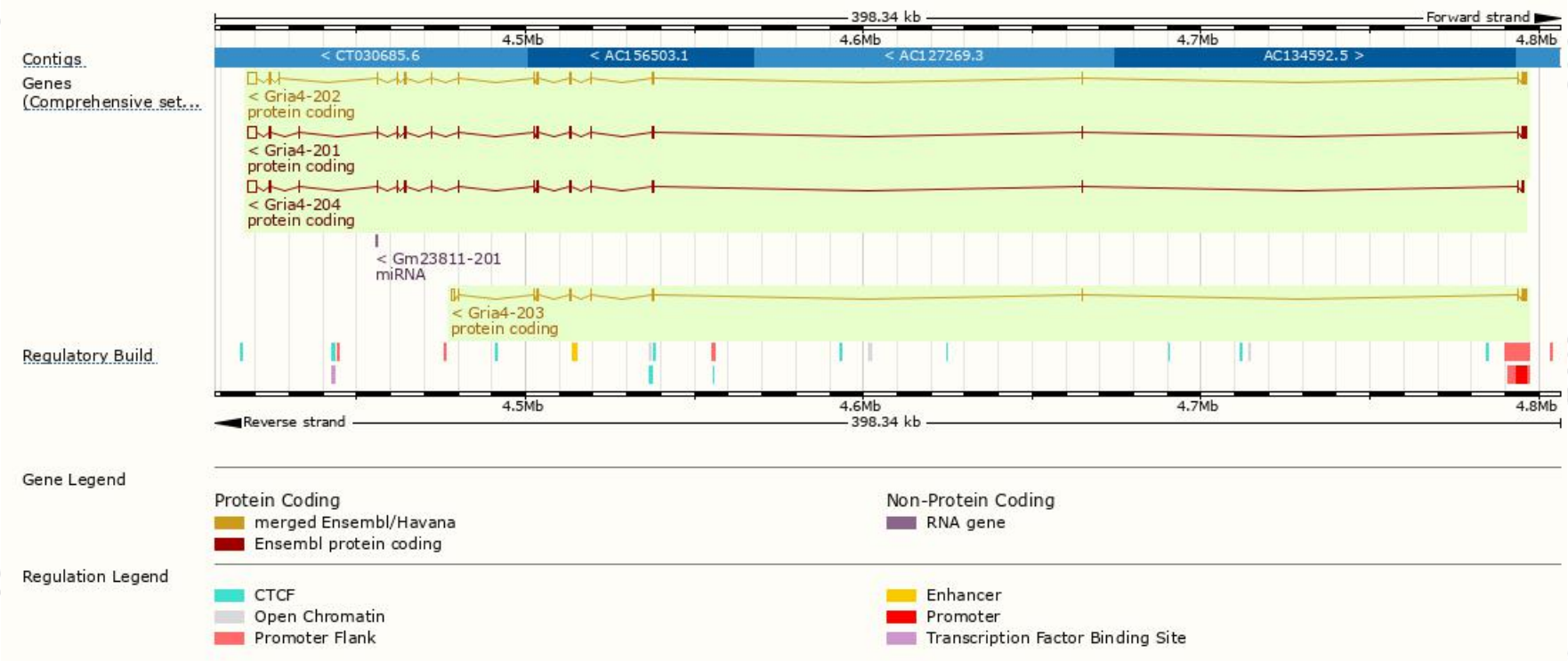
The gene has 4 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|---|---|---|---|---|---|---|---|
| Gria4-201 | ENSMUST00000027020.12 | 5441 | 902aa | Protein coding | CCDS52715 | G5E863 | TSL:5 GENCODE basic APPRIS ALT1 |
| Gria4-202 | ENSMUST00000063508.14 | 5352 | 902aa | Protein coding | CCDS22797 | Q9Z2W8 | TSL:1 GENCODE basic APPRIS P3 |
| Gria4-204 | ENSMUST00000212533.1 | 5207 | 902aa | Protein coding | CCDS52715 | G5E863 | TSL:1 GENCODE basic APPRIS ALT1 |
| Gria4-203 | ENSMUST00000163309.1 | 2338 | 433aa | Protein coding | CCDS52716 | E9PX01 | TSL:1 GENCODE basic |

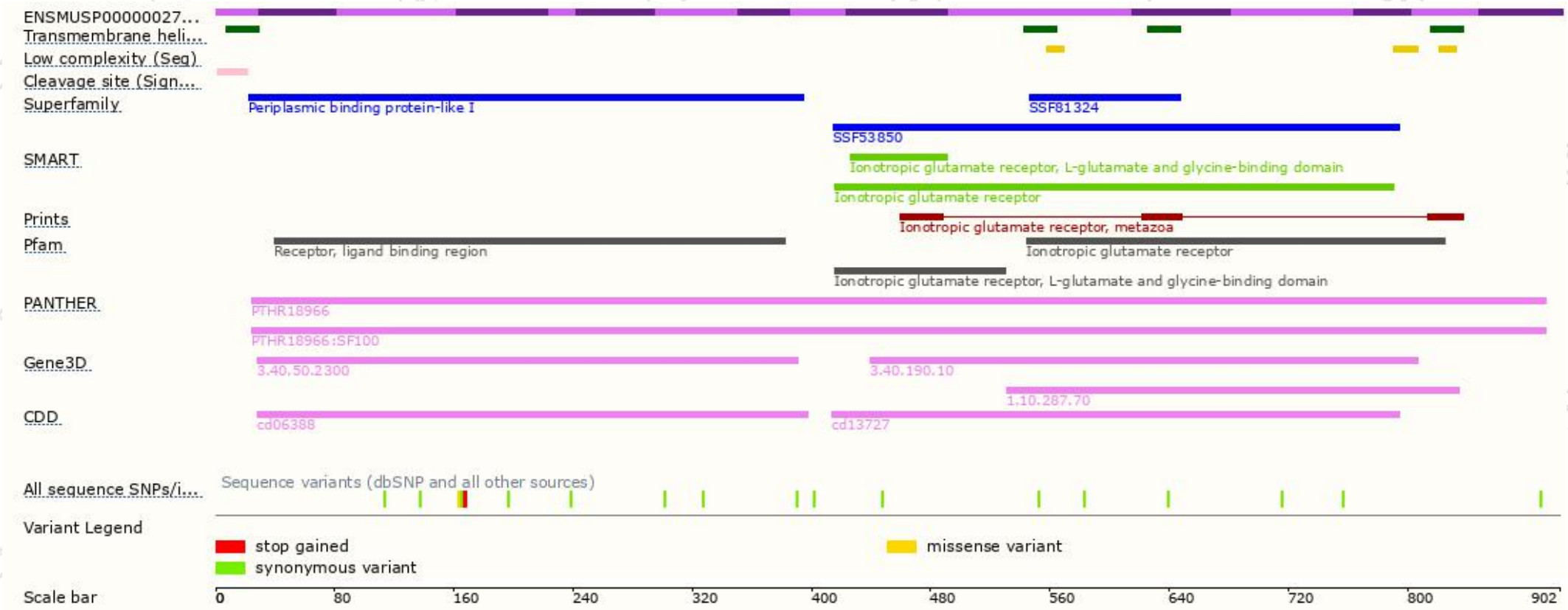
The strategy is based on the design of *Gria4-201* transcript,The transcription is shown below



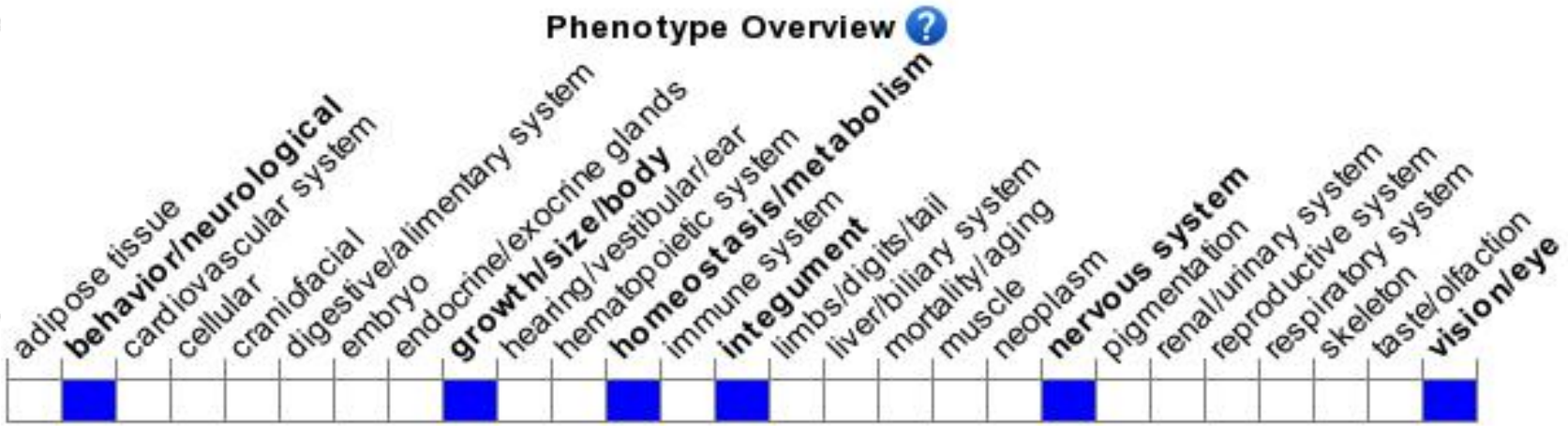
# Genomic location distribution



# 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 targeted mutation display hyperactivity, decreased thermal nociception, and abnormal sensitivity to pharmacologically induced seizures.

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

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