Grik2(GluR6)-P2A-iCre Cas9-KI Strategy

Designer:	**Yanhua Shen**
Reviewer:	**Xueting Zhang**
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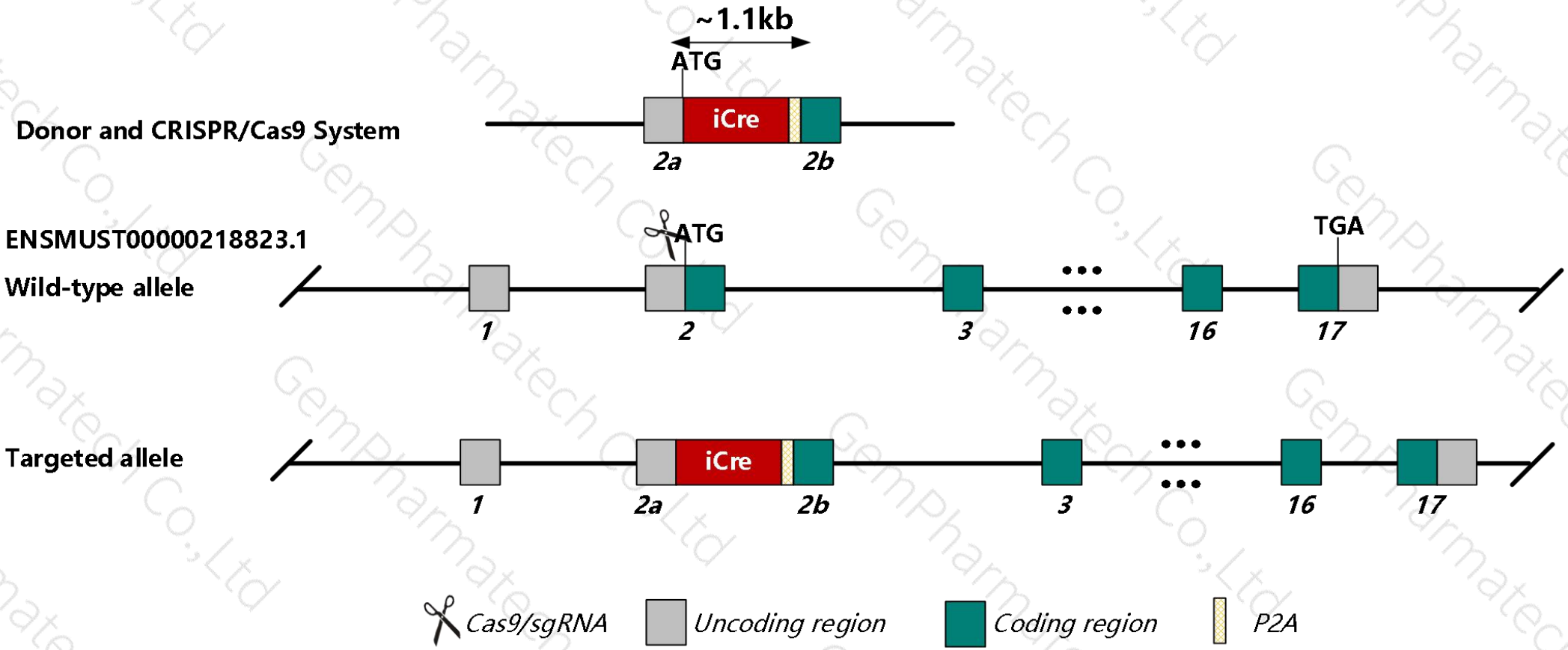
Project Overview

Project Name	***Grik2(GluR6)-P2A-iCre***
Project type	**Cas9-KI**
Strain background	**C57BL/6J**

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Grik2* gene. The schematic diagram is as follows:



Technical routes

- The *Grik2* gene has 11 transcripts.According to the structure of *Grik2* gene, *Grik2-207*(ENSMUST00000218823.1) is selected for presentation of the recommended strategy.

- *Grik2-207* gene has 17 exons, with the ATG start codon in exon2 and TGA stop codon in exon17.

- We make *Grik2-P2A-iCre* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near start coding(ATG) of *Grik2* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in *P2A-iCre* after start coding(ATG) of *Grik2* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the existing MGI data, homozygotes for a targeted null mutation exhibit hippocampal neurons with reduced sensitivity to kainate and reduced susceptibility to the seizure-inducing effects of kainate administration.
- Insertion of iCre may affect the regulation of the 5' end of the *Grik2* gene.
- There will be 1 to 2 amino acid synonymous mutation in exon2 of *Grik2* gene in this strategy.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- The *Grik2* gene is located on the Chr10. If the knockin 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)

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Grik2 glutamate receptor, ionotropic, kainate 2 (beta 2) [*Mus musculus* (house mouse)]

Gene ID: 14806, updated on 10-Aug-2019

Summary

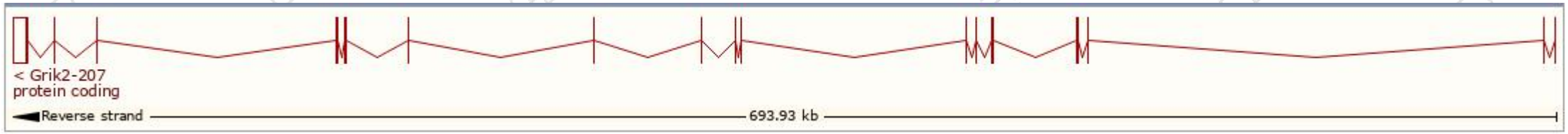
Official Symbol	Grik2 provided by MGI
Official Full Name	glutamate receptor, ionotropic, kainate 2 (beta 2) provided by MGI
Primary source	MGI:MGI:95815
See related	Ensembl:ENSMUSG00000056073
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	GluK2; Glur6; Glur-6; AW124492; Glurbeta2; C130030K03Rik
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 the kainate family of glutamate receptors, which are composed of four subunits and function as ligand-activated ion channels. The subunit encoded by this gene is subject to RNA editing at multiple sites within the first and second transmembrane domains, which is thought to alter the structure and function of the receptor complex. Alternatively spliced transcript variants encoding different isoforms have also been found for this gene. [provided by RefSeq, Jul 2008]
Expression	Biased expression in CNS E18 (RPKM 9.9), whole brain E14.5 (RPKM 6.6) and 5 other tissues See more
Orthologs	human all

Transcript information （Ensembl）

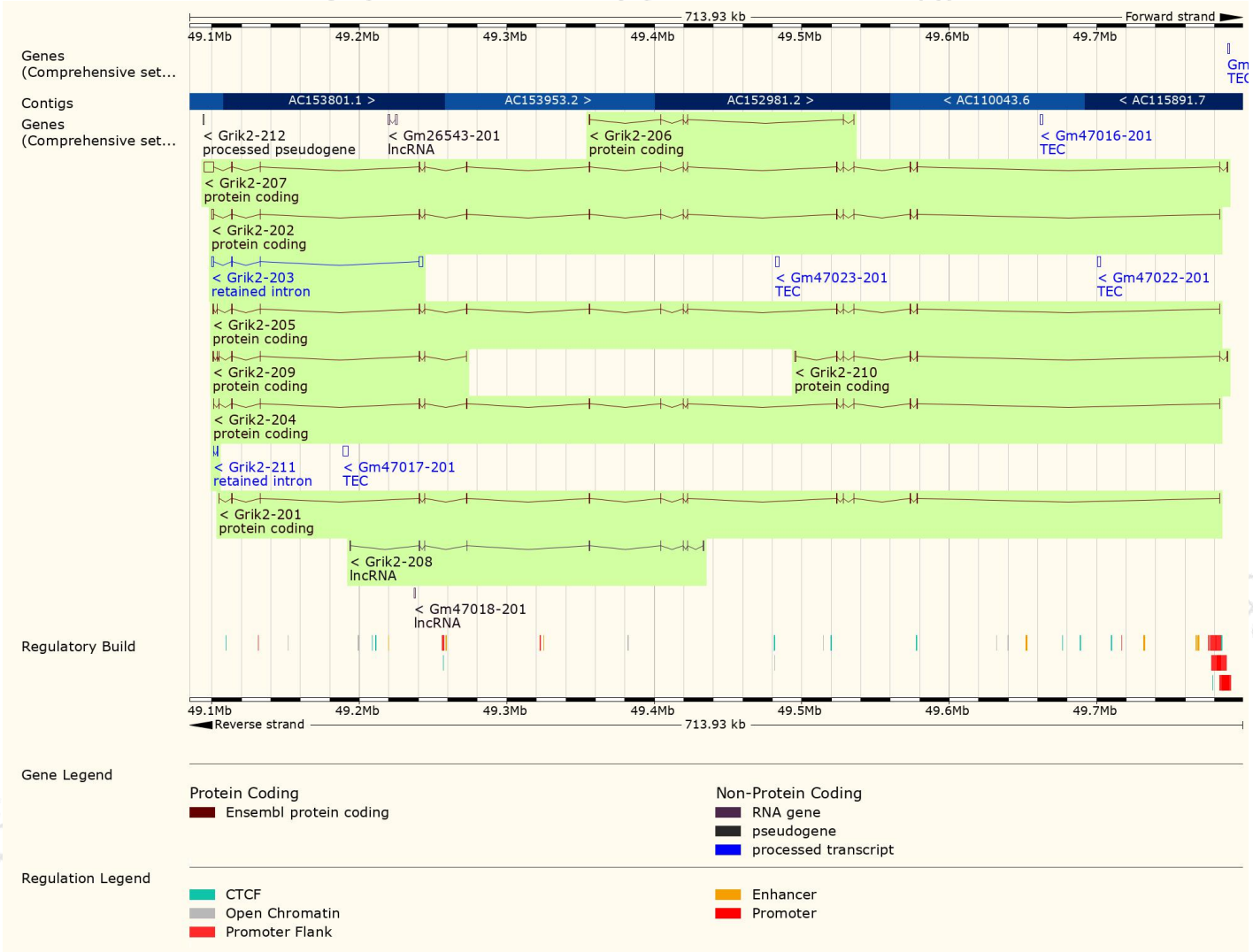
The gene has 11 transcripts, and the transcript is shown below：

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Grik2-207	ENSMUST00000218823.1	9427	908aa	Protein coding	CCDS48554	P39087	TSL:1 GENCODE basic APPRIS P1
Grik2-202	ENSMUST00000105484.9	3992	908aa	Protein coding	CCDS48554	P39087	TSL:1 GENCODE basic APPRIS P1
Grik2-204	ENSMUST00000218441.1	3083	869aa	Protein coding	CCDS23830	P39087	TSL:1 GENCODE basic
Grik2-201	ENSMUST00000079751.8	2610	869aa	Protein coding	CCDS23830	P39087	TSL:2 GENCODE basic
Grik2-205	ENSMUST00000218598.1	3245	893aa	Protein coding	-	A0A1W2P6S5	TSL:5 GENCODE basic
Grik2-210	ENSMUST00000220263.1	2085	338aa	Protein coding	-	Q6PAQ0	TSL:1 GENCODE basic
Grik2-209	ENSMUST00000219509.1	1542	309aa	Protein coding	-	A0A1W2P868	CDS 5' incomplete TSL:5
Grik2-206	ENSMUST00000218669.1	539	179aa	Protein coding	-	A0A1W2P8D9	CDS 5' and 3' incomplete TSL:5
Grik2-203	ENSMUST00000217673.1	4365	No protein	Retained intron	-	-	TSL:1
Grik2-211	ENSMUST00000220330.1	567	No protein	Retained intron	-	-	TSL:3
Grik2-208	ENSMUST00000219051.1	1374	No protein	lncRNA	-	-	TSL:5

The strategy is based on the design of *Grik2-207* transcript,The transcription is shown below

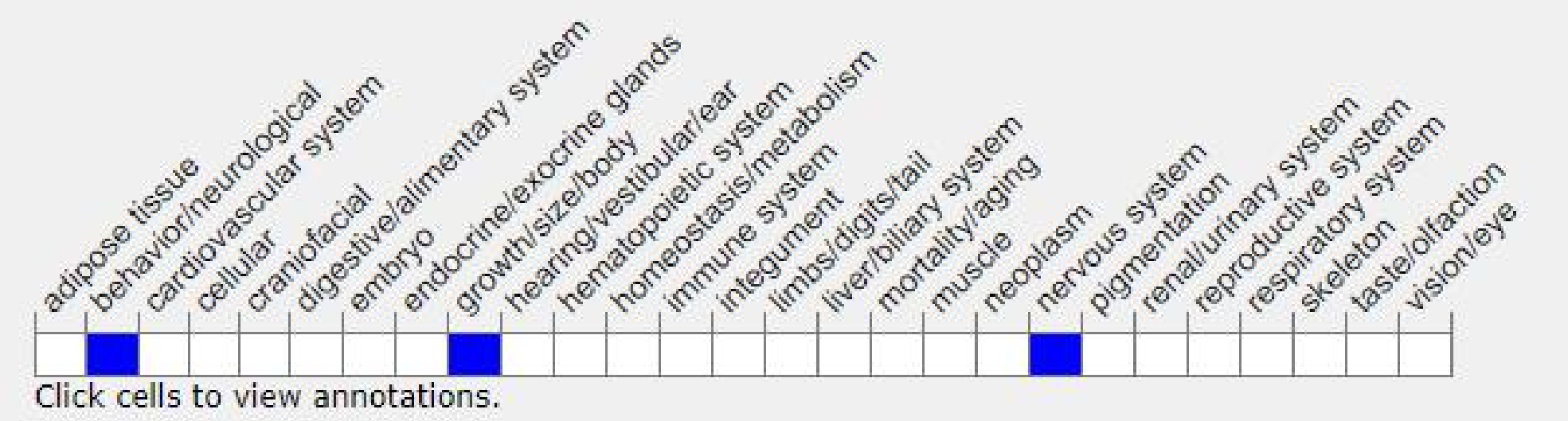


Genomic location distribution



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/) .

Homozygotes for a targeted null mutation exhibit hippocampal neurons with reduced sensitivity to kainate and reduced susceptibility to the seizure-inducing effects of kainate administration.

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

Tel: 025-5864 1534

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