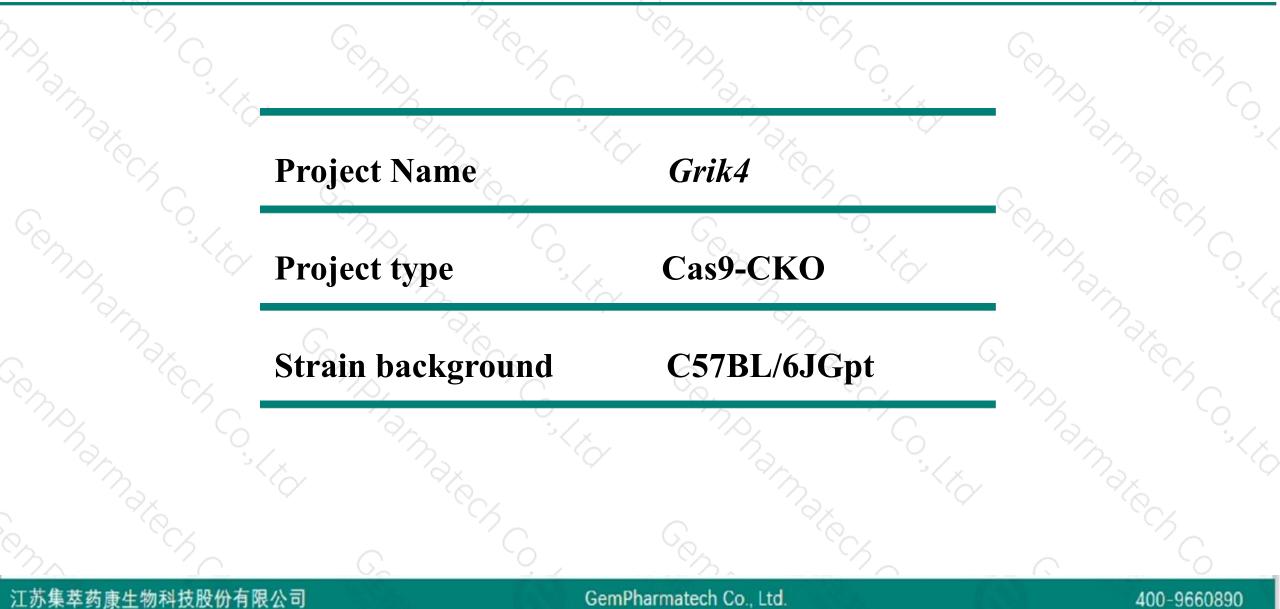


Grik4 Cas9-CKO Strategy

Designer:Xueting Zhang Reviewer:Yanhua Shen Date:2020-1-14

Project Overview



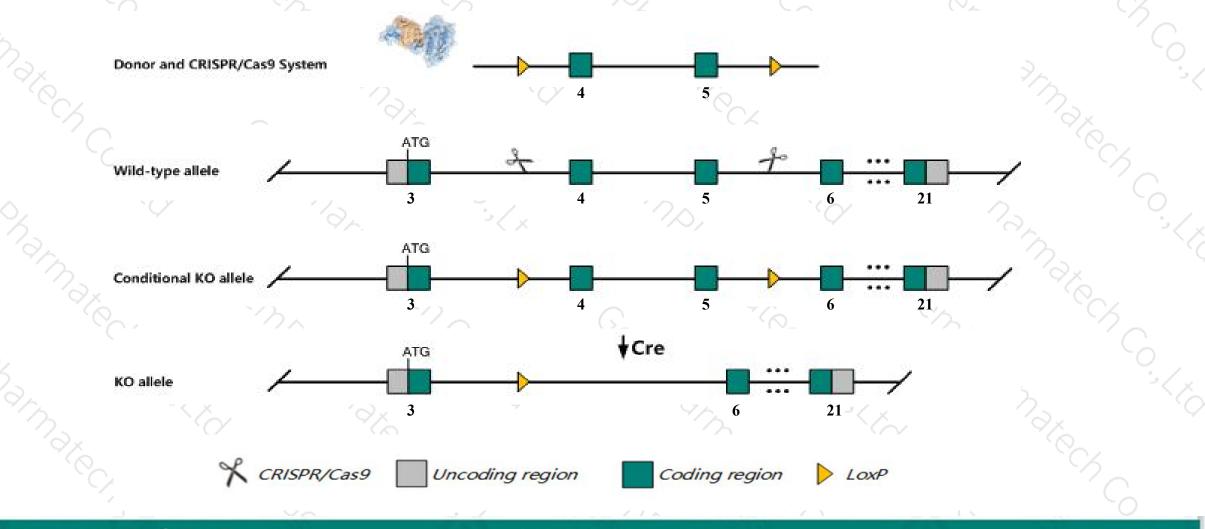


Conditional Knockout strategy



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This model will use CRISPR/Cas9 technology to edit the *Grik4* gene. The schematic diagram is as follows:



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The Grik4 gene has 2 transcripts. According to the structure of Grik4 gene, exon4-exon5 of Grik4-202 (ENSMUST00000114865.7) transcript is recommended as the knockout region. The region contains 263bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Grik4* 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.

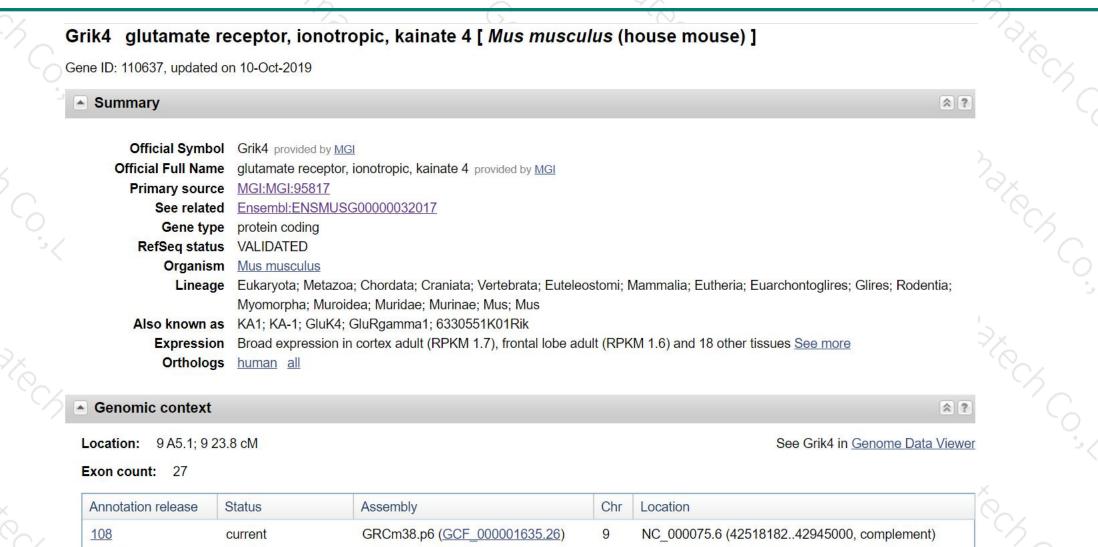
Notice



- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit reduced GYKI-resistant excitatory postsynaptic current.
- > The Grik4 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)





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Build 37.2

previous assembly

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MGSCv37 (GCF 000001635.18)

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NC 000075.5 (42328519..42752454, complement)

Transcript information (Ensembl)



The gene has 2 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Grik4-202	ENSMUST00000114865.7	5887	<u>956aa</u>	Protein coding	CCDS23089	Q8BMF5	TSL:1 GENCODE basic APPRIS P1
Grik4-201	ENSMUST0000034515.6	3413	<u>956aa</u>	Protein coding	CCDS23089	Q8BMF5	TSL:5 GENCODE basic APPRIS P1

The strategy is based on the design of *Grik4-202* transcript, The transcription is shown below

< Grik4-202 protein coding

Reverse strand -

426.36 kb -----

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Genomic location distribution



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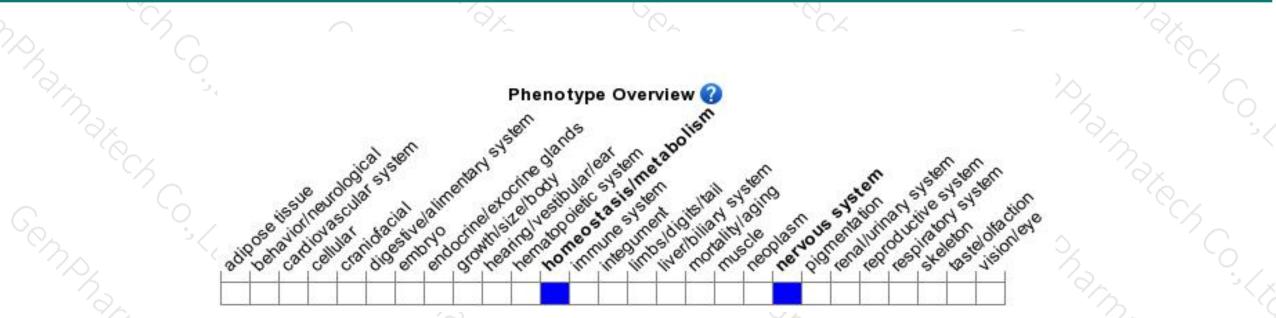
Protein domain



nonarta.	ENSMUSP00000110 Transmembrane heli MobiDB lite Low complexity (Seg) Coiled-coils (Ncoils) Cleavage site (Sign		3×8 78 78 70
(9)	Superfamily	Periplasmic binding protein-like I SSF53850	
Č,	SMART	Ion otropic glutamate receptor, L-glutamate and glycine-binding domain	
Genphan Mar	Prints Pfam	Ionotropic glutamate receptor Ionotropic glutamate receptor, metazoa Receptor, ligand binding region Ionotropic glutamate receptor, L-glutamate and glycine-binding domain Ionotropic glutamate receptor	CA Co
narm.	PROSITE profiles PANTHER	PS51257 PTHR18966	
	Gene3D	PTHR18966:SF171 3,40.50.2300 1.10.287.70	× K
	CDD	3.40.190.10 cd13724	~ 0
9172	All sequence SNPs/i	Sequence variants (dbSNP and all other sources)	
	Variant Legend	missense variant synonymous variant	7
<u>`</u>	Scale bar	0 80 160 240 320 400 480 560 640 720 800 956	0
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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 exhibit reduced GYKI-resistant excitatory postsynaptic current.



If you have any questions, you are welcome to inquire. Tel: 400-9660890



