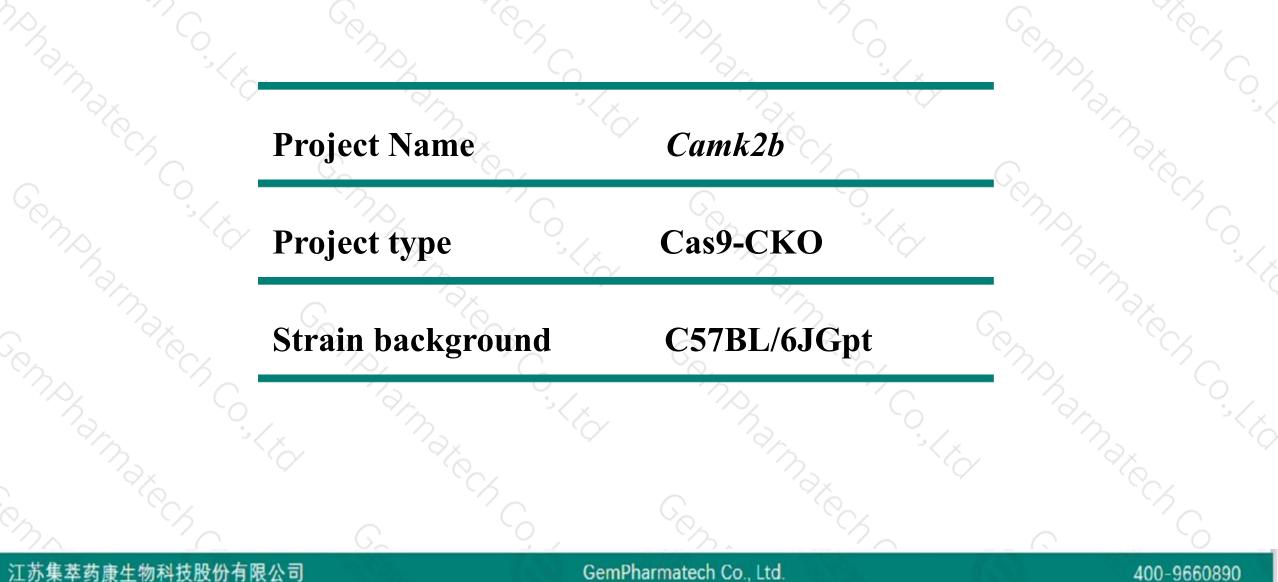


Camk2b Cas9-CKO Strategy

Designer: Huan Fan Design Date: 2019-8-23

Project Overview





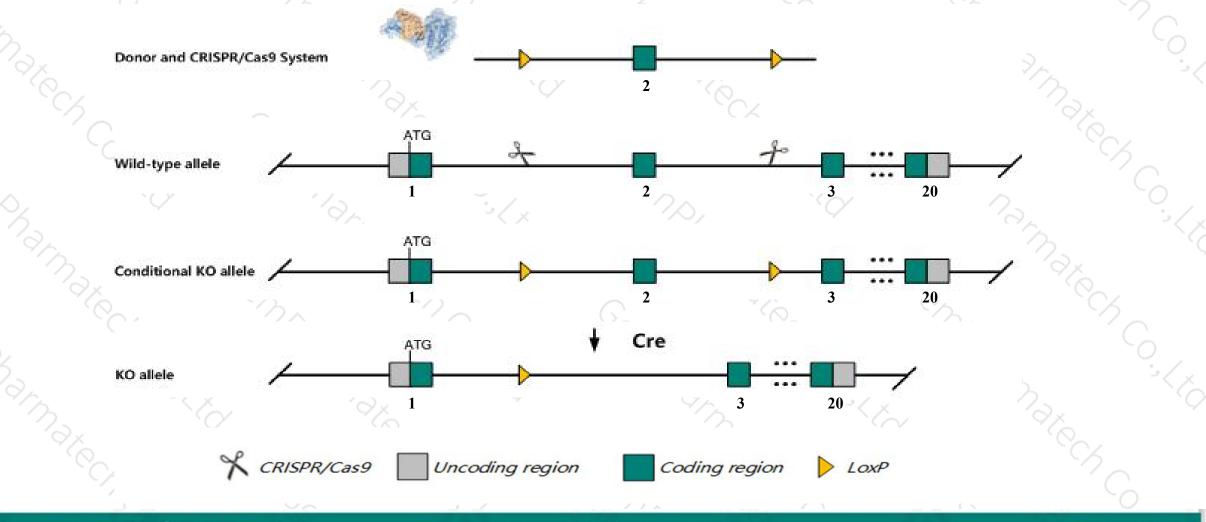
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Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Camk2b* gene. The schematic diagram is as follows:



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The Camk2b gene has 15 transcripts. According to the structure of Camk2b gene, exon2 of Camk2b-209 (ENSMUST00000109813.8) transcript is recommended as the knockout region. The region contains 95bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Camk2b* 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.



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- According to the existing MGI data, Mice homozygous for a null allele exhibit reversal of plasticity direction at parallel fiber-Purkinje cell synapses. Mice homozygous for a different null allele show motor impairments, including ataxia, altered body mass composition, a reduction in anxiety-related behavior, and cognitive deficits.
- The Camk2b gene is located on the Chr11. 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.

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Gene information (NCBI)



\$?

Camk2b calcium/calmodulin-dependent protein kinase II, beta [Mus musculus (house mouse)]

Gene ID: 12323, updated on 7-Apr-2019

Summary

Official Symbol	Camk2b provided by MGI
Official Full Name	calcium/calmodulin-dependent protein kinase II, beta provided by MGI
Primary source	MGI:MGI:88257
See related	Ensembl:ENSMUSG00000057897
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
Also known as	CaMKII
Expression	Biased expression in frontal lobe adult (RPKM 115.3), cortex adult (RPKM 97.8) and 5 other tissues See more
Orthologs	human all



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Transcript information (Ensembl)



The gene has 15 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Camk2b-209	ENSMUST00000109813.8	4023	<u>542aa</u>	Protein coding	CCDS24411	P28652	TSL:1 GENCODE basic APPRIS P2
Camk2b-204	ENSMUST00000090443.9	4004	<u>545aa</u>	Protein coding	CCDS48748	Q68EG2	TSL:1 GENCODE basic
Camk2b-210	ENSMUST00000109815.8	3780	<u>542aa</u>	Protein coding	CCDS24411	P28652	TSL:5 GENCODE basic APPRIS P2
Camk2b-208	ENSMUST00000109812.8	1875	<u>529aa</u>	Protein coding	CCDS48749	Q5SVI2	TSL:1 GENCODE basic
Camk2b-202	ENSMUST00000019133.10	2223	<u>666aa</u>	Protein coding	(15)	Q5SVJ0	TSL:5 GENCODE basic
Camk2b-205	ENSMUST0000093355.11	1973	<u>589aa</u>	Protein coding		Q5SVI1	TSL:5 GENCODE basic
Camk2b-201	ENSMUST0000002817.11	1872	<u>503aa</u>	Protein coding	(12)	Q5SVJ1	TSL:5 GENCODE basic APPRIS ALT
Camk2b-206	ENSMUST00000101585.9	1650	<u>518aa</u>	Protein coding	1.	<u>Q5SVI3</u>	TSL:5 GENCODE basic
Camk2b-203	ENSMUST0000066431.13	1603	<u>479aa</u>	Protein coding	(15)	<u>Q5SVI9</u>	TSL:5 GENCODE basic APPRIS ALT
Camk2b-207	ENSMUST00000101586.2	1590	<u>518aa</u>	Protein coding	691	<u>Q5SVI0</u>	TSL:5 GENCODE basic APPRIS ALT
Camk2b-212	ENSMUST00000129098.1	4125	No protein	Processed transcript	(12)	84	TSL:1
Camk2b-215	ENSMUST00000155755.1	3328	No protein	Processed transcript	N25	12	TSL:5
Camk2b-214	ENSMUST00000154197.7	851	No protein	Processed transcript	1753	65	TSL:5
Camk2b-211	ENSMUST00000123391.7	456	No protein	Processed transcript	694	87	TSL:3
Camk2b-213	ENSMUST00000130427.7	412	No protein	Processed transcript	(11)	84	TSL:5

The strategy is based on the design of Camk2b-209 transcript, The transcription is shown below

< Camk2b-209 protein coding

Reverse strand

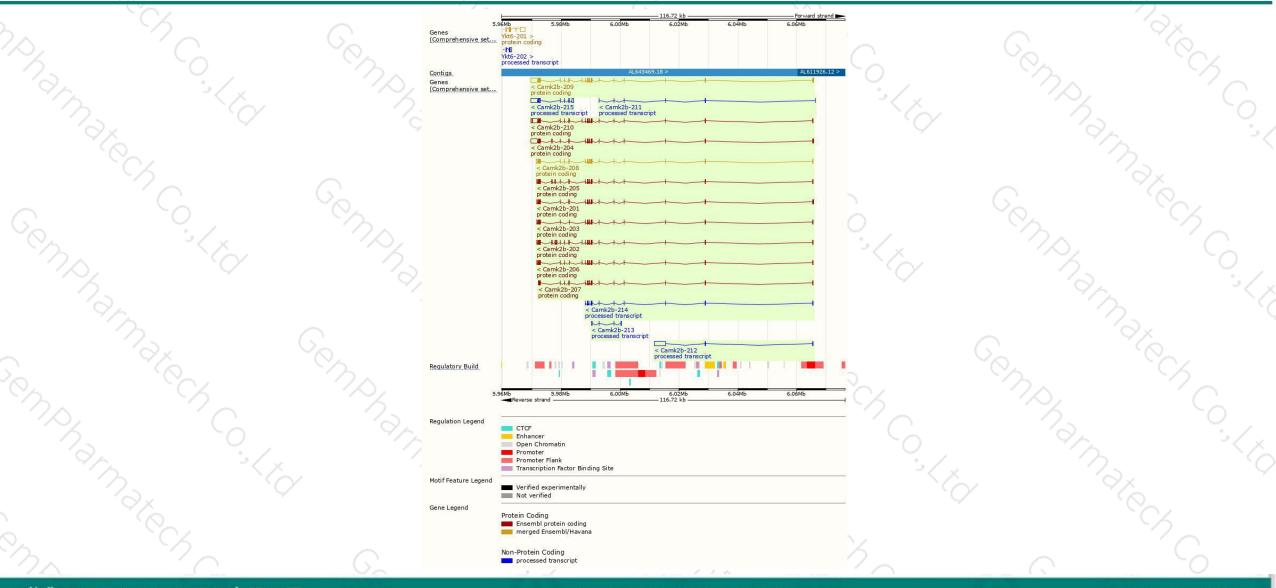
- 96.11 kb -

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Genomic location distribution



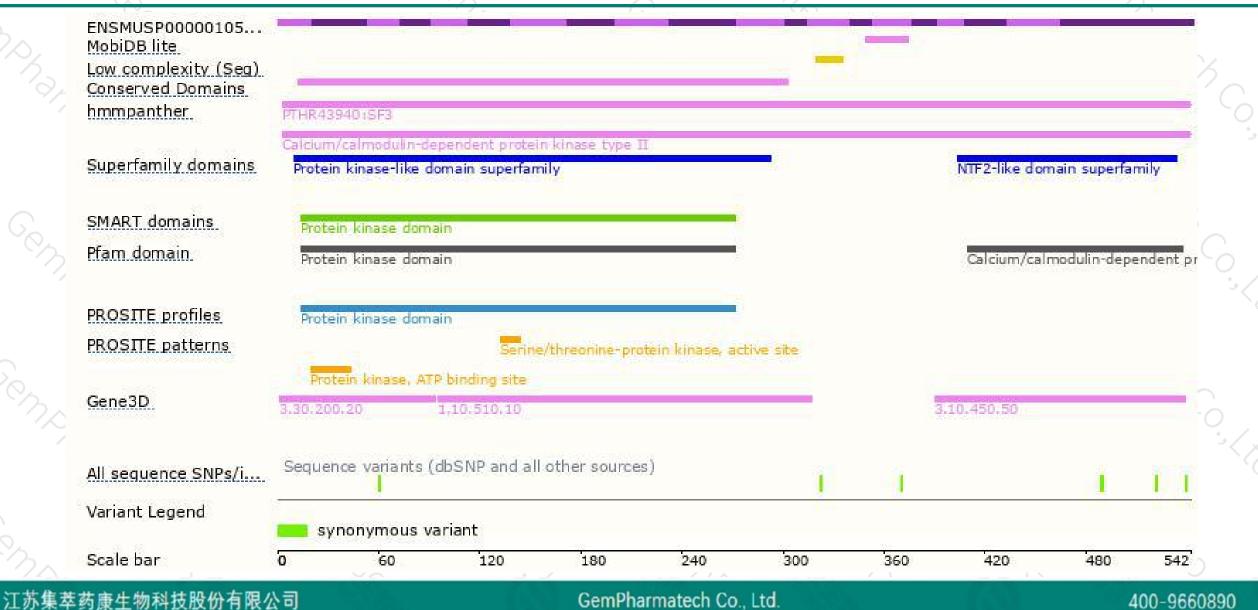


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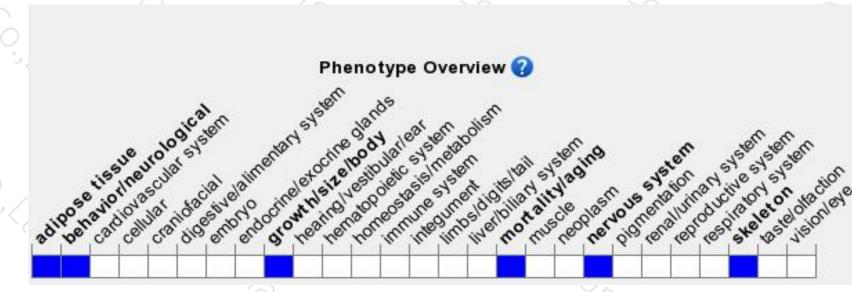
Protein domain





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 exhibit reversal of plasticity direction at parallel fiber-Purkinje cell synapses. Mice homozygous for a different null allele show motor impairments, including ataxia, altered body mass composition, a reduction in anxiety-related behavior, and cognitive deficits.

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



