

Cacna1b Cas9-CKO Strategy

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Project Overview

Project Name

Cacna1b

Project type

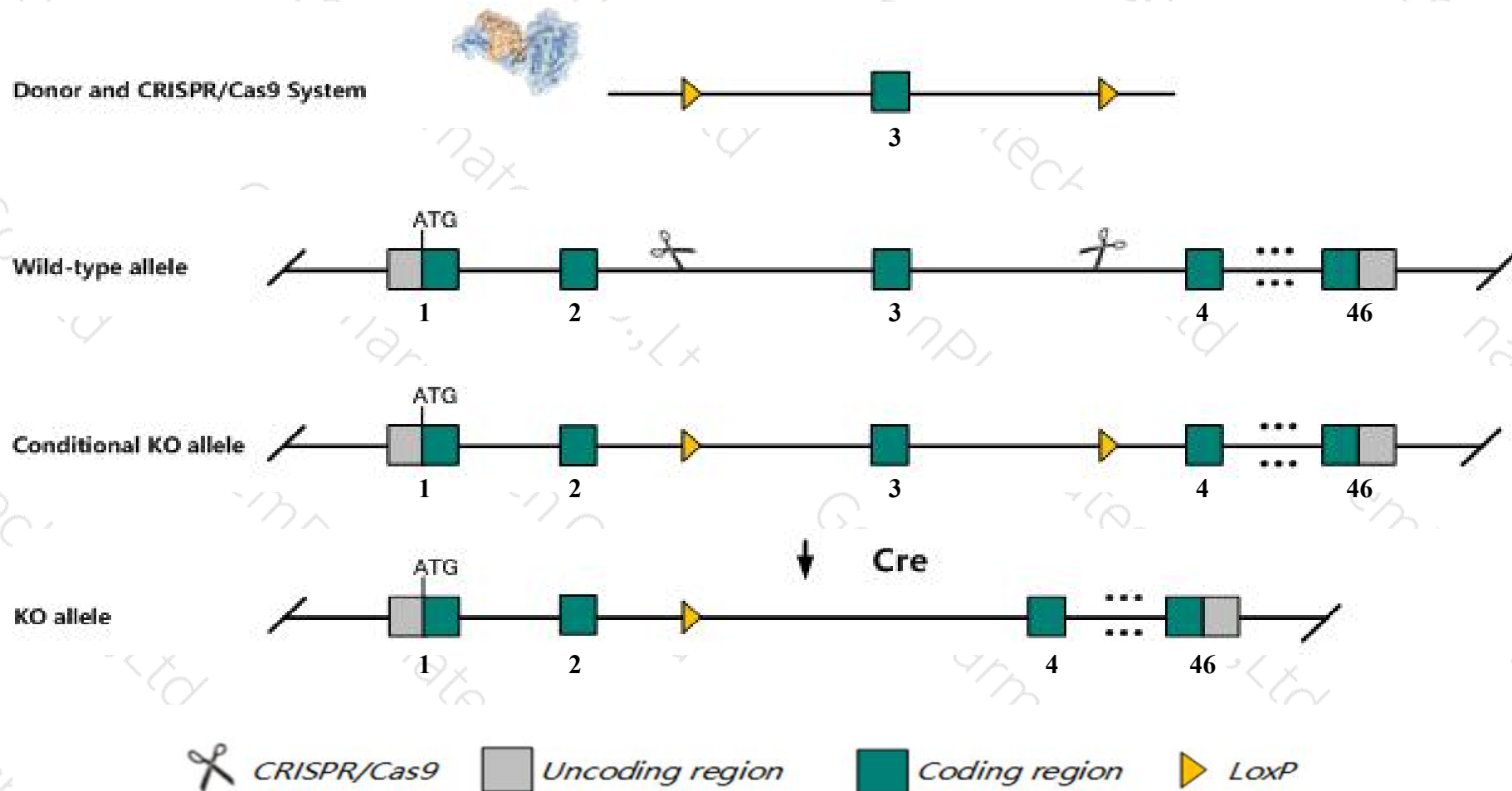
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cacna1b* gene. The schematic diagram is as follows:



- The *Cacnalb* gene has 10 transcripts. According to the structure of *Cacnalb* gene, exon3 of *Cacnalb-201* (ENSMUST00000041342.11) transcript is recommended as the knockout region. The region contains 140bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cacnalb* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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.

- According to the existing MGI data, Mice deficient in this gene exhibit defects in nociception, memory and learning. They also exhibit hyperactive and hyperaggressive behaviors as well as defects in the sleep-wake cycle. Deficits in the sympathetic nervous system results in defects in circulatory regulation.
- The N-terminal of *Cacna1b* gene will remain 130aa, it may remain the partial function of *Cacna1b* gene.
- The effect on transcript *Cacna1b*-206&207&208&209&210 is unknown in this strategy.
- The *Cacna1b* gene is located on the Chr2. 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)

Cacna1b calcium channel, voltage-dependent, N type, alpha 1B subunit [Mus musculus (house mouse)]

Gene ID: 12287, updated on 31-Jan-2019

Summary



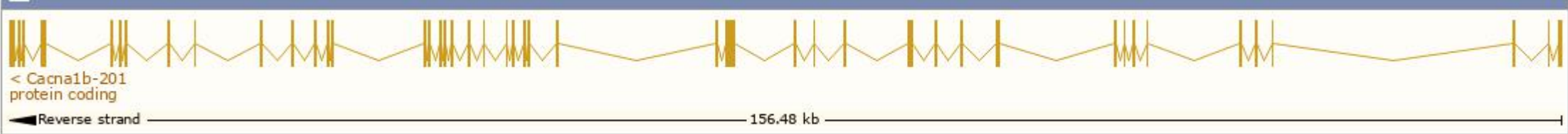
Official Symbol	Cacna1b provided by MGI
Official Full Name	calcium channel, voltage-dependent, N type, alpha 1B subunit provided by MGI
Primary source	MGI:MGI:88296
See related	Ensembl:ENSMUSG000000004113
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW050276, AW060892, AW822256, Blll, Cav2.2, Cchn1a, alpha(1B)
Expression	Biased expression in CNS E18 (RPKM 11.3), whole brain E14.5 (RPKM 9.4) and 8 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

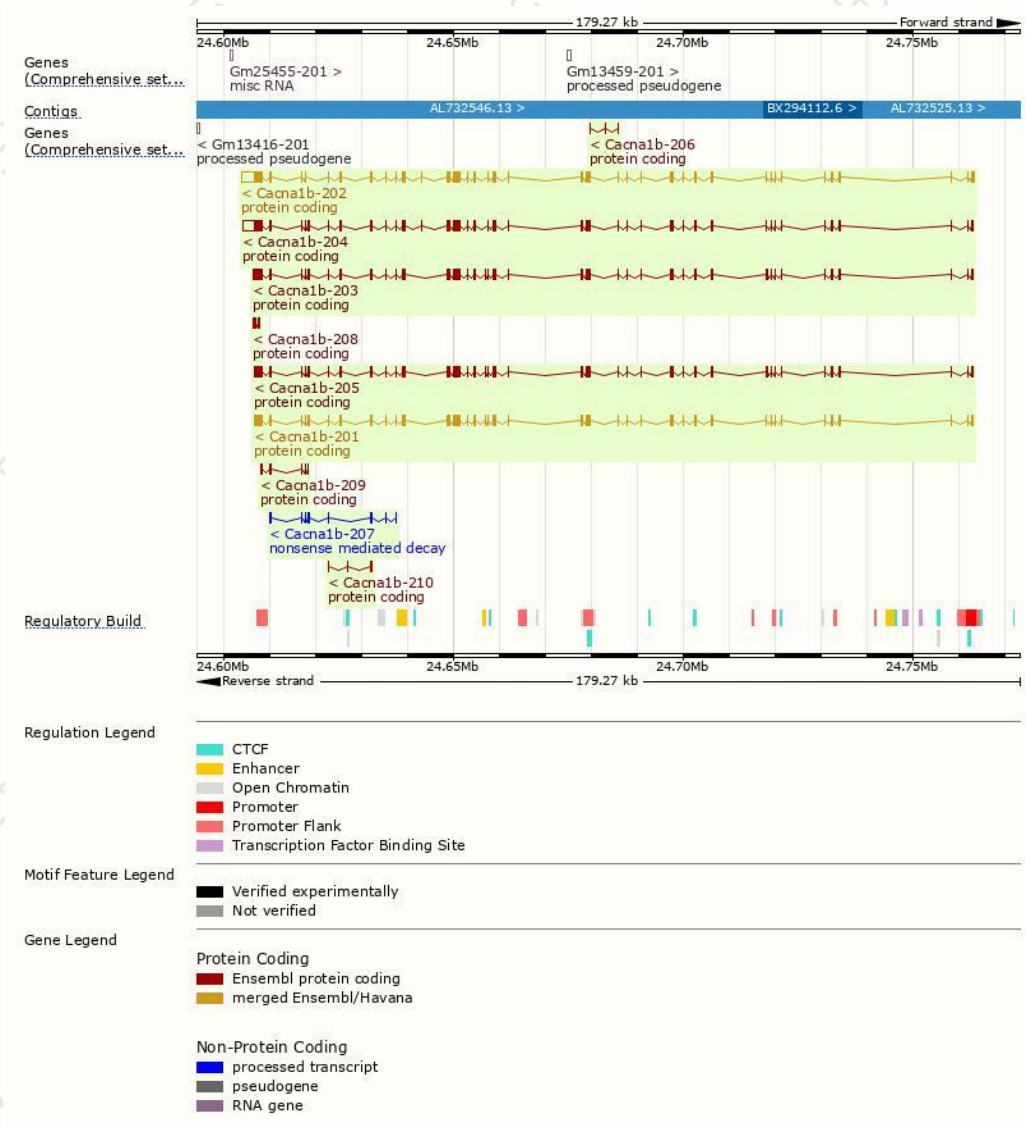
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cacna1b-202	ENSMUST00000070864.13	9655	2288aa	Protein coding	CCDS15739	A2AIS0	TSL:5 GENCODE basic APPRIS P3
Cacna1b-201	ENSMUST00000041342.11	6984	2327aa	Protein coding	CCDS38065	Q55017	TSL:1 GENCODE basic APPRIS ALT2
Cacna1b-204	ENSMUST00000102939.8	9736	2325aa	Protein coding	-	A2AIR9	TSL:5 GENCODE basic APPRIS ALT2
Cacna1b-203	ENSMUST00000100348.9	7182	2328aa	Protein coding	-	A2AIR7	TSL:5 GENCODE basic APPRIS ALT2
Cacna1b-205	ENSMUST00000114447.7	6987	2328aa	Protein coding	-	A2AIR7	TSL:5 GENCODE basic APPRIS ALT2
Cacna1b-209	ENSMUST00000133892.1	684	228aa	Protein coding	-	A2AIZ9	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Cacna1b-208	ENSMUST00000131861.1	523	168aa	Protein coding	-	A0A0A6YWR0	CDS 5' incomplete TSL:5
Cacna1b-210	ENSMUST00000155356.3	332	111aa	Protein coding	-	F7C9S8	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Cacna1b-206	ENSMUST00000124183.1	308	102aa	Protein coding	-	A2AJ01	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Cacna1b-207	ENSMUST00000125798.2	949	114aa	Nonsense mediated decay	-	A0A0A6YWZ5	CDS 5' incomplete TSL:5

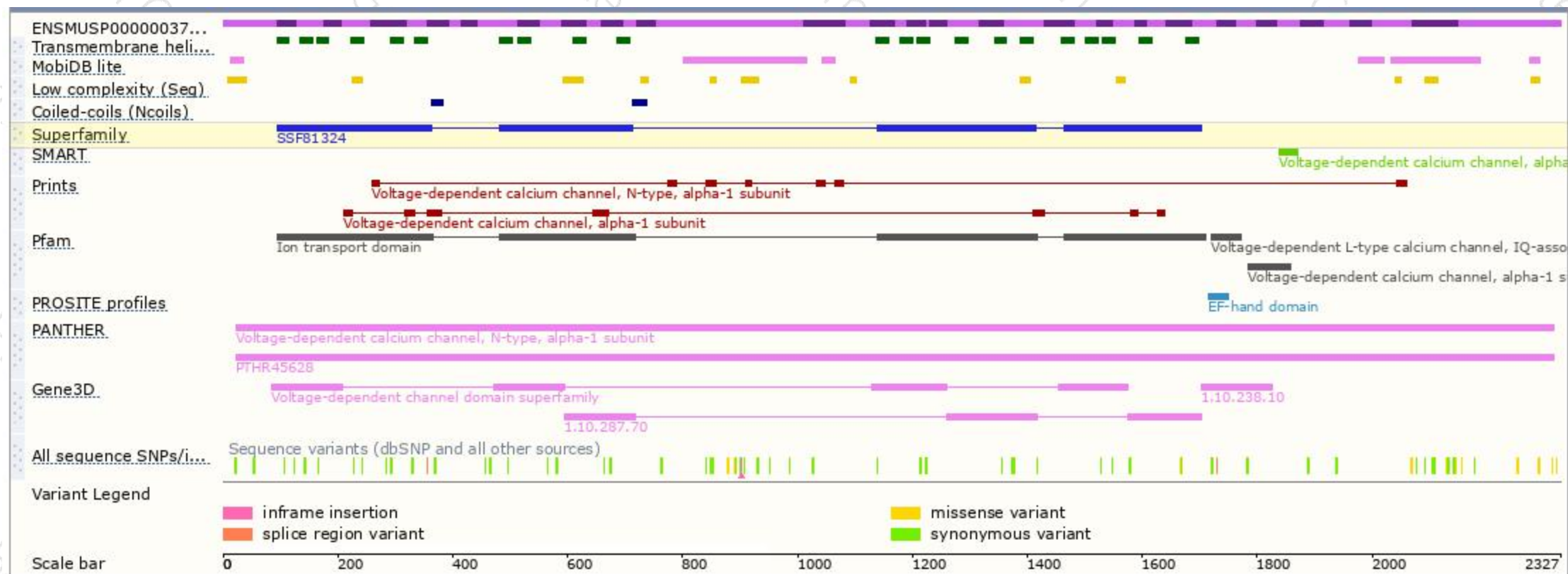
The strategy is based on the design of *Cacna1b-201* transcript,The transcription is shown below



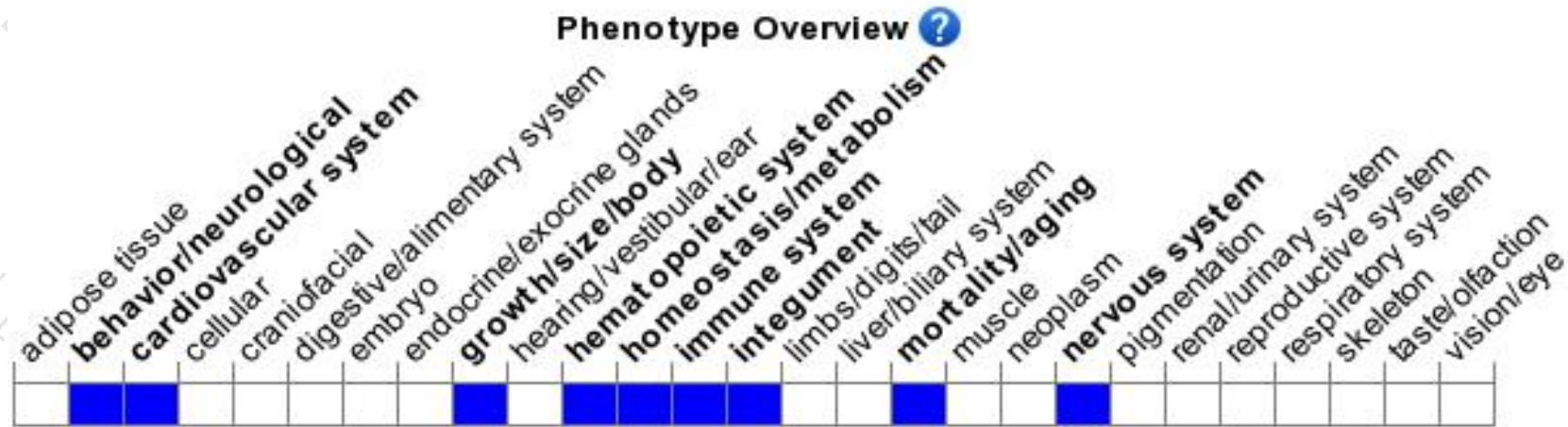
Genomic location distribution



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 deficient in this gene exhibit defects in nociception, memory and learning.

They also exhibit hyperactive and hyperaggressive behaviors as well as defects in the the sleep-wake cycle. Deficits in the sympathetic nervous system results in defects in circulatory regulation.

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

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