GemPharmatech™

Cacna1a Cas9-CKO Strategy

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Overview

Target Gene Name

- Cacna1a

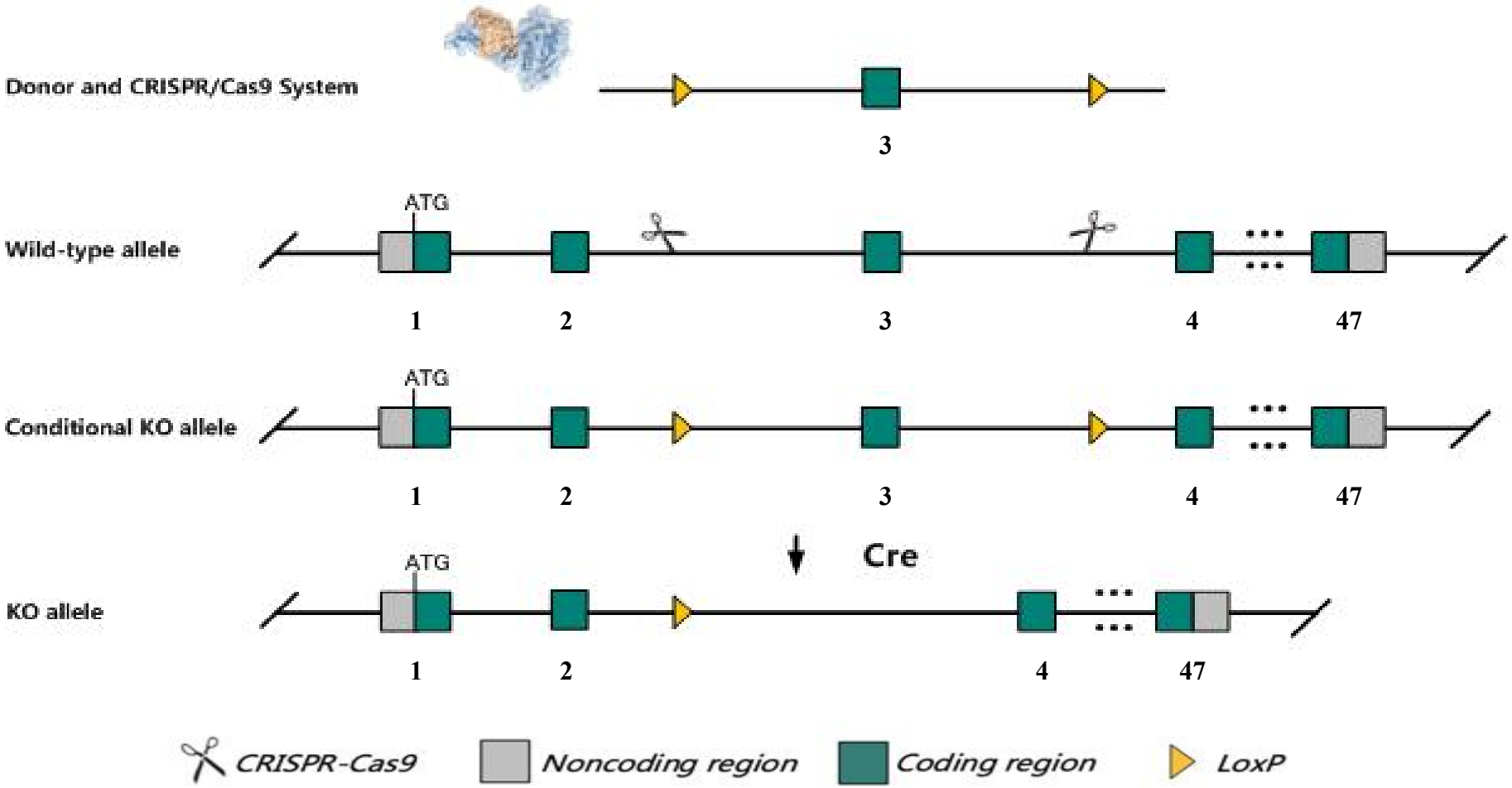
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Cacna1a* gene.

Technical Information

- The *Cacna1a* gene has 13 transcripts. According to the structure of *Cacna1a* gene, exon3 of *Cacna1a*-201 (ENSMUST00000121390.8) transcript is recommended as the knockout region. The region contains 140bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Cacna1a* 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- 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.

Gene Information

Cacna1a calcium channel, voltage-dependent, P/Q type, alpha 1A subunit [Mus musculus (house mouse)]

Gene ID: 12286, updated on 13-Mar-2020

Summary

Official Symbol	Cacna1a provided by MGI
Official Full Name	calcium channel, voltage-dependent, P/Q type, alpha 1A subunit provided by MGI
Primary source	MGI:MGI:109482
See related	Ensembl:ENSMUSG00000034656
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	APCA, Bl, Caca1a, Cacnl1a4, Cav2.1, Ccha1a, EA2, FHM, HPCA, MHP, MHP1, SCA6, alpha1A, la, nmf352, rkr, smrl, tg
Expression	Biased expression in cerebellum adult (RPKM 34.5), cortex adult (RPKM 15.3) and 11 other tissues See more
Orthologs	human all

Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

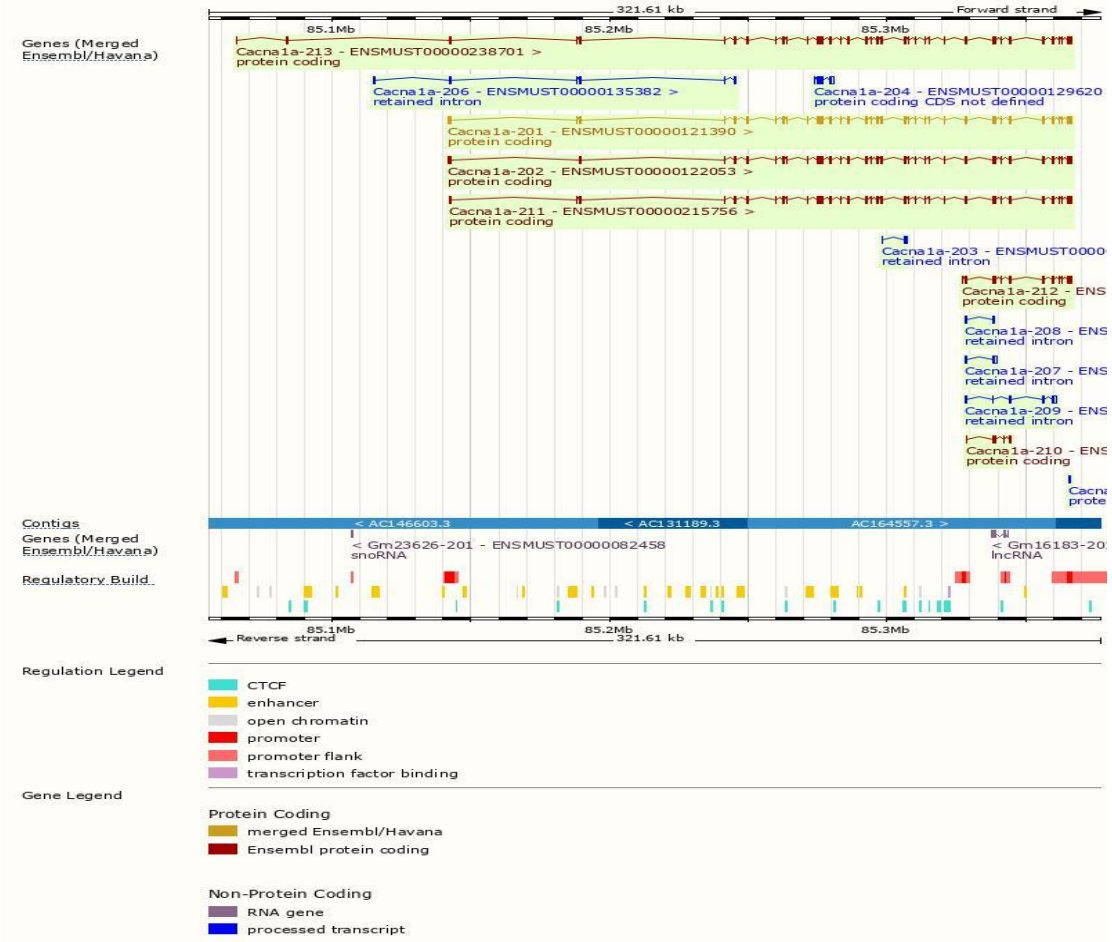
The gene has 13 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cacna1a-201	ENSMUST00000121390.7	7926	2368aa	Protein coding	CCDS52618	P97445	TSL:1 GENCODE basic APPRIS P3
Cacna1a-202	ENSMUST00000122053.1	7589	2321aa	Protein coding	CCDS57628	E9Q1R5	TSL:1 GENCODE basic APPRIS ALT2
Cacna1a-213	ENSMUST00000238701.1	8370	2457aa	Protein coding	-	-	GENCODE basic APPRIS ALT2
Cacna1a-211	ENSMUST00000215756.1	7503	2321aa	Protein coding	-	A0A1L1SQZ2	CDS 5' incomplete TSL:5
Cacna1a-212	ENSMUST00000238337.1	2941	776aa	Protein coding	-	-	GENCODE basic
Cacna1a-210	ENSMUST00000153691.1	549	183aa	Protein coding	-	-	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
Cacna1a-204	ENSMUST00000129620.1	1639	No protein	Processed transcript	-	-	TSL:1
Cacna1a-205	ENSMUST00000130507.1	249	No protein	Processed transcript	-	-	TSL:1
Cacna1a-209	ENSMUST00000144879.7	1742	No protein	Retained intron	-	-	TSL:1
Cacna1a-207	ENSMUST00000141981.7	1153	No protein	Retained intron	-	-	TSL:2
Cacna1a-206	ENSMUST00000135382.1	1150	No protein	Retained intron	-	-	TSL:1
Cacna1a-208	ENSMUST00000143215.7	740	No protein	Retained intron	-	-	TSL:3
Cacna1a-203	ENSMUST00000126302.1	597	No protein	Retained intron	-	-	TSL:5

The strategy is based on the design of *Cacna1a*-201 transcript, the transcription is shown below:

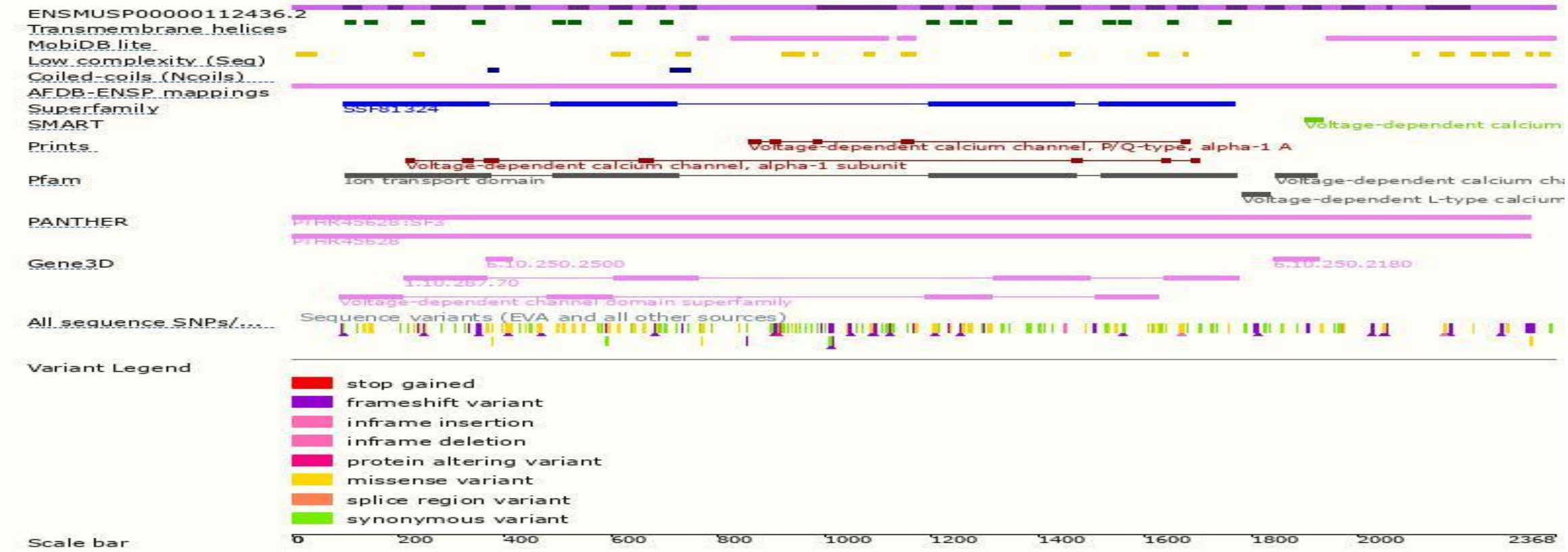
Source: https://www.ensembl.org

Genomic Information



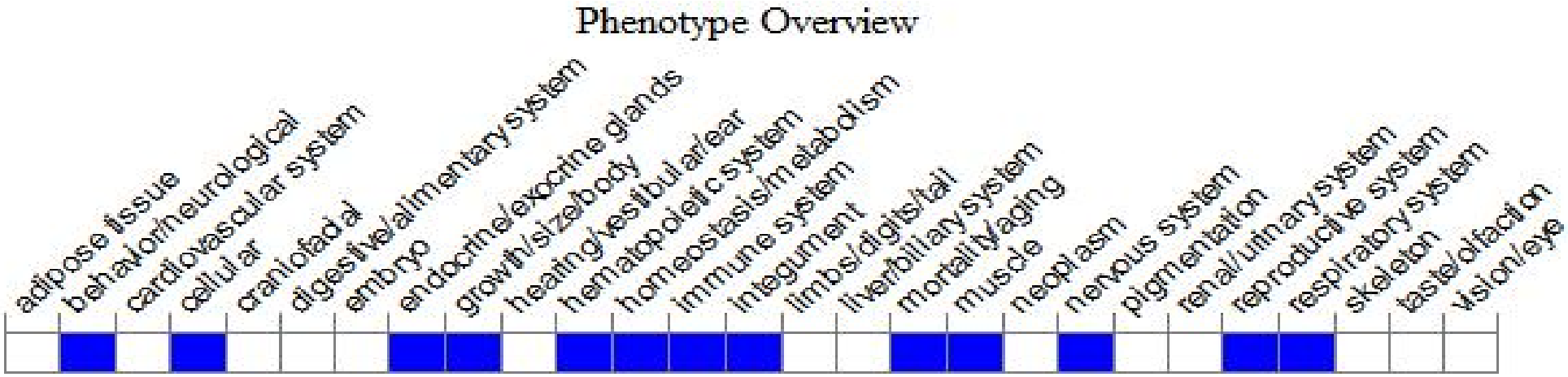
Source: : https://www.ensembl.org

Protein Information



Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



- Homozygotes for different mutant alleles are characterized by variably severe wobbly gait beginning prior to weaning, ataxia, episodic dyskinesia, cerebellar atrophy, and absence epilepsy.

Source: https://www.informatics.jax.org

Important Information

- *Cacna1a* is located on Chr8. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is 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 the existing technology level.
- Transcripts 212 maybe unaffected.
- Transcript 210 CDS 5' incomplete the influences is unknown.