Cacna1d Cas9-CKO Strategy

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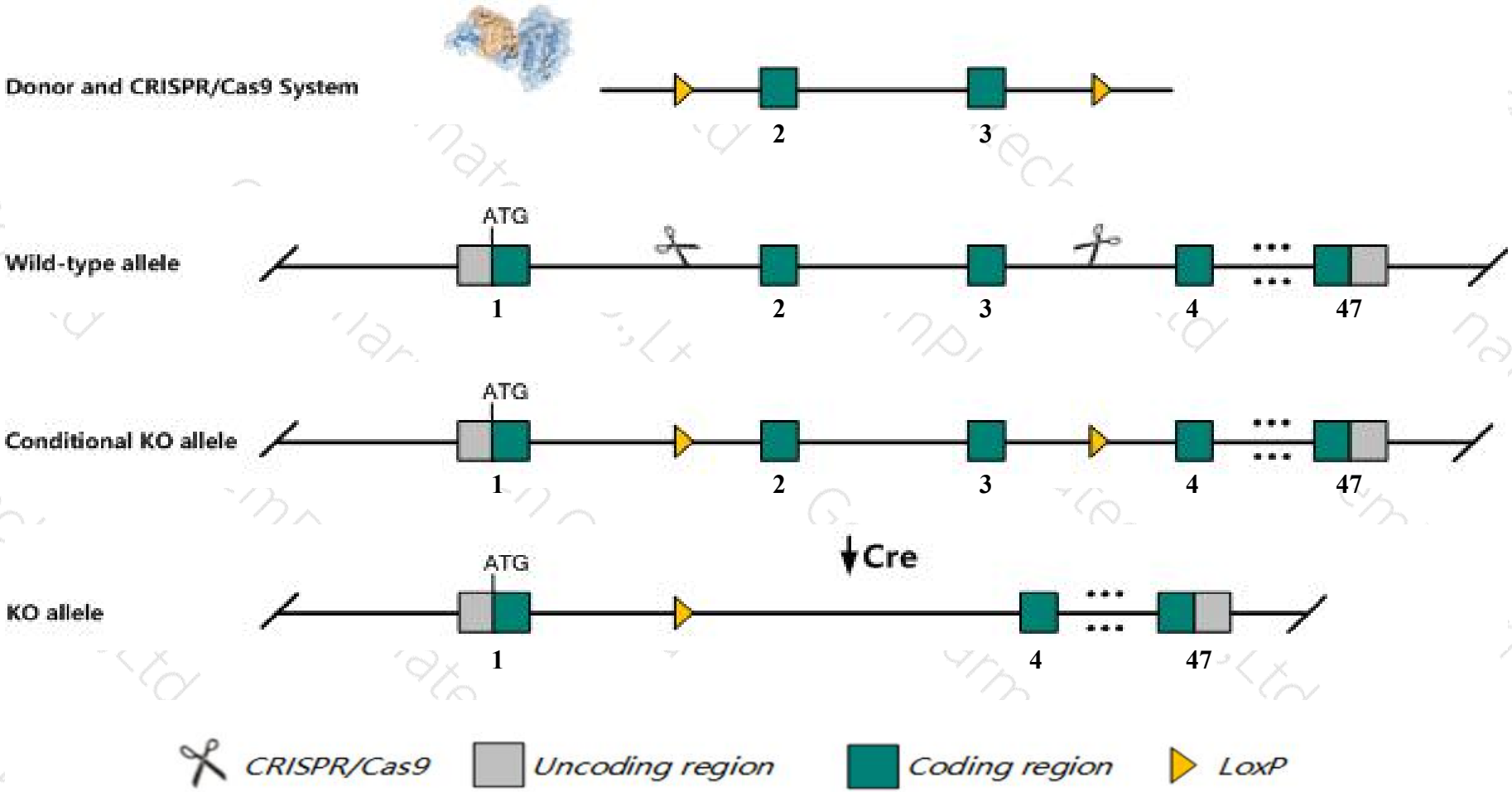
Project Overview

Project Name	***Cacna1d***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cacna1d* gene. The schematic diagram is as follows:



Technical routes

- The *Cacna1d* gene has 20 transcripts. According to the structure of *Cacna1d* gene, exon2-exon3 of *Cacna1d-202* (ENSMUST00000112250.5) transcript is recommended as the knockout region.The region contains 416bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Cacna1d* 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,Homozygotes for targeted mutations exhibit small size, hypoinsulinemia, glucose intolerance, decreased number and size of pancreatic islets, deafness with degeneration of hair cells, bradycardia, and arrhythmia.

- The *Cacna1d* gene is located on the Chr14. 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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Cacna1d calcium channel, voltage-dependent, L type, alpha 1D subunit [*Mus musculus* (house mouse)]

Gene ID: 12289, updated on 24-Oct-2019

Summary

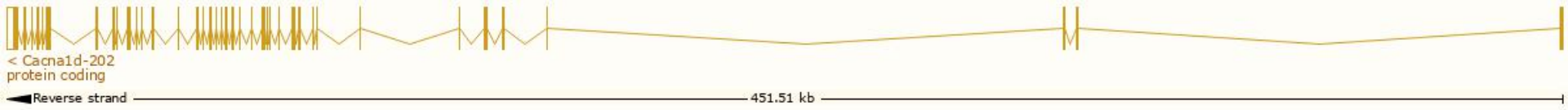
Official Symbol	Cacna1d provided by MGI
Official Full Name	calcium channel, voltage-dependent, L type, alpha 1D subunit provided by MGI
Primary source	MGI:MGI:88293
See related	Ensembl:ENSMUSG00000015968
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Cach3; Cacn4; C79217; Cav1.3; Cchl1a; D-LTCC; Cchl1a2; Cacnl1a2; 8430418G19Rik
Summary	This gene encodes a pore-forming subunit of the L-type, voltage-activated calcium channel family. These channels have been found to play a role in heart and smooth muscle contraction and in the transmission of auditory information. Homozygous knockout mice for this gene exhibit deafness and heart defects. These channels have also been linked to mitochondrial oxidative stress in a mouse model of Parkinson's disease. Alternative splicing results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Nov 2014]
Expression	Broad expression in frontal lobe adult (RPKM 8.2), CNS E18 (RPKM 6.5) and 26 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

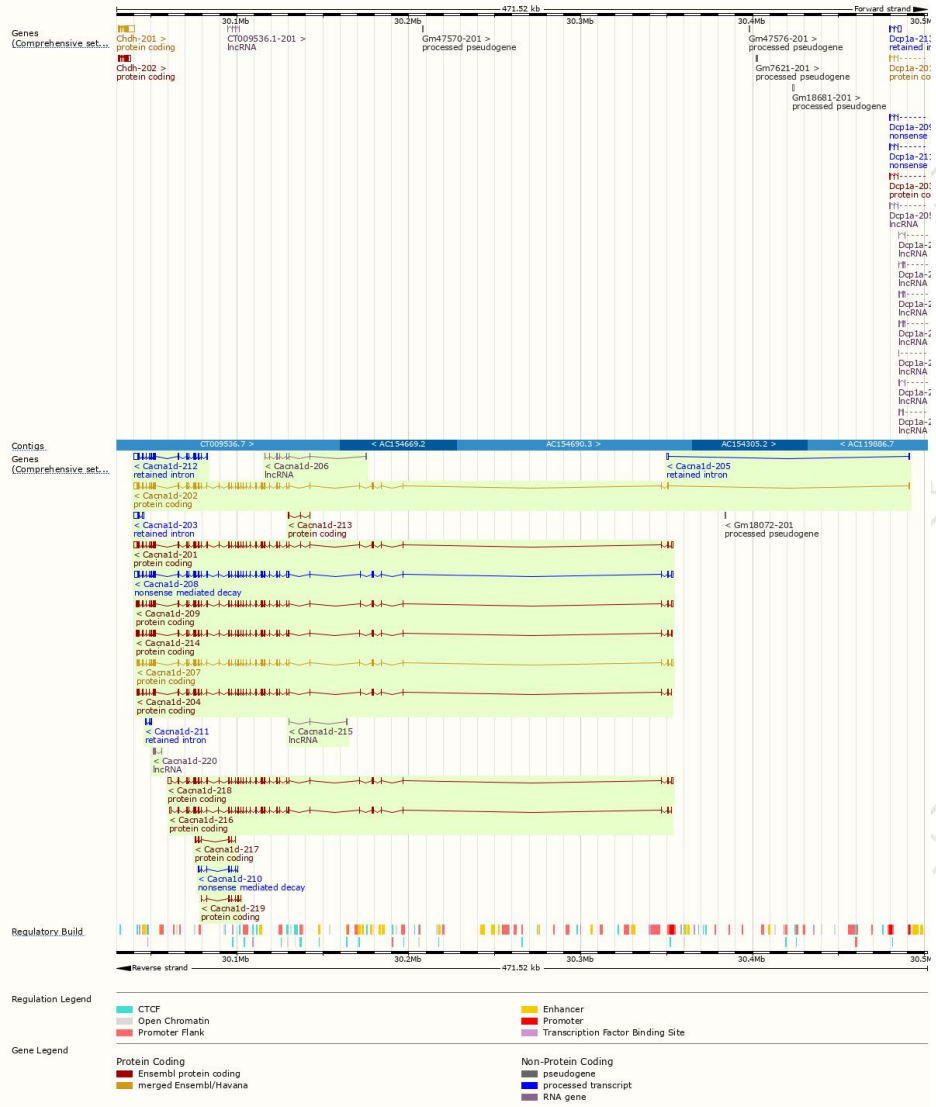
The gene has 20 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cacna1d-202	ENSMUST00000112250.5	8985	2166aa	Protein coding	CCDS36846	Q99246	TSL:1 GENCODE basic APPRIS P4
Cacna1d-207	ENSMUST00000224198.2	6928	2144aa	Protein coding	CCDS36847	Q99246	GENCODE basic APPRIS ALT2
Cacna1d-201	ENSMUST00000112249.9	8876	2187aa	Protein coding	-	A0A2C9F2E5	TSL:1 GENCODE basic APPRIS ALT2
Cacna1d-209	ENSMUST00000224785.2	7581	2159aa	Protein coding	-	A0A286YD72	GENCODE basic APPRIS ALT2
Cacna1d-218	ENSMUST00000238675.1	7285	1647aa	Protein coding	-	-	GENCODE basic
Cacna1d-214	ENSMUST00000238504.1	7128	2179aa	Protein coding	-	-	GENCODE basic APPRIS ALT2
Cacna1d-204	ENSMUST00000223803.2	6408	2135aa	Protein coding	-	Q99246	GENCODE basic APPRIS ALT2
Cacna1d-216	ENSMUST00000238568.1	5766	1643aa	Protein coding	-	-	CDS 5' incomplete
Cacna1d-219	ENSMUST00000238690.1	850	284aa	Protein coding	-	-	CDS 5' and 3' incomplete
Cacna1d-217	ENSMUST00000238571.1	669	223aa	Protein coding	-	-	CDS 5' and 3' incomplete
Cacna1d-213	ENSMUST00000238336.1	216	72aa	Protein coding	-	-	CDS 5' and 3' incomplete
Cacna1d-208	ENSMUST00000224395.1	9658	513aa	Nonsense mediated decay	-	A0A286YCE7	-
Cacna1d-210	ENSMUST00000224912.1	710	180aa	Nonsense mediated decay	-	A0A286YE45	CDS 5' incomplete
Cacna1d-212	ENSMUST00000225717.1	4918	No protein	Retained intron	-	-	-
Cacna1d-203	ENSMUST00000223573.1	3503	No protein	Retained intron	-	-	-
Cacna1d-205	ENSMUST00000223985.1	1223	No protein	Retained intron	-	-	-
Cacna1d-211	ENSMUST00000225353.1	635	No protein	Retained intron	-	-	-
Cacna1d-206	ENSMUST00000224073.1	1302	No protein	lncRNA	-	-	-
Cacna1d-220	ENSMUST00000238735.1	568	No protein	lncRNA	-	-	-
Cacna1d-215	ENSMUST00000238524.1	503	No protein	lncRNA	-	-	-

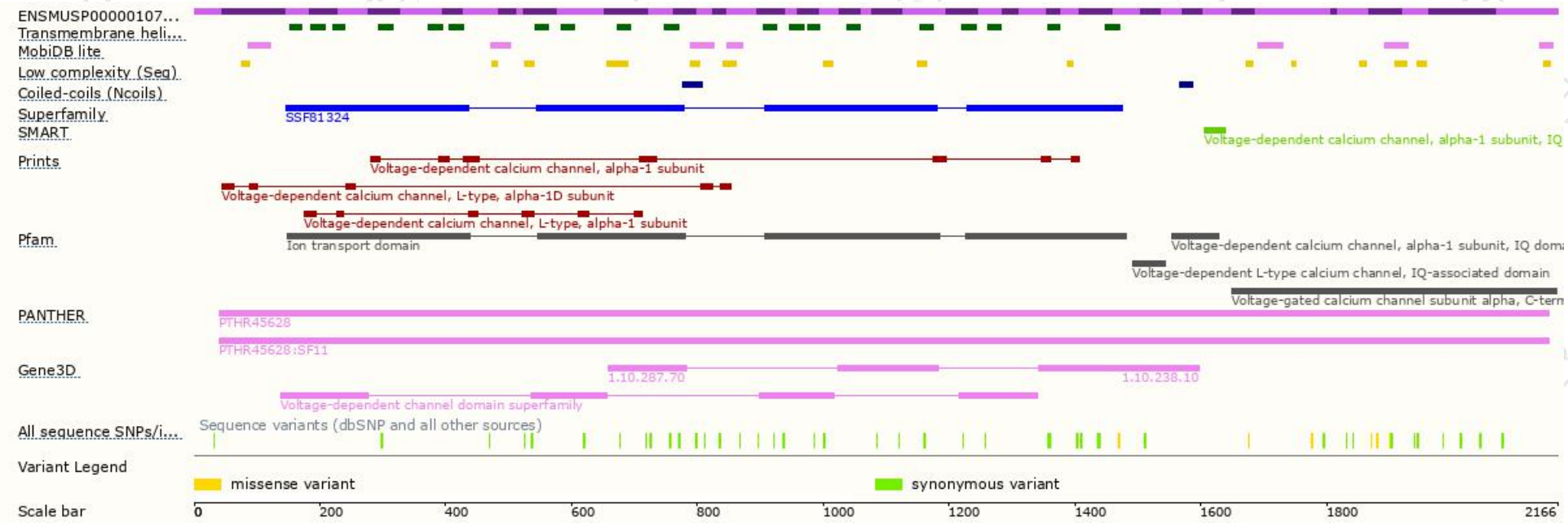
The strategy is based on the design of *Cacna1d-202* transcript,The transcription is shown below



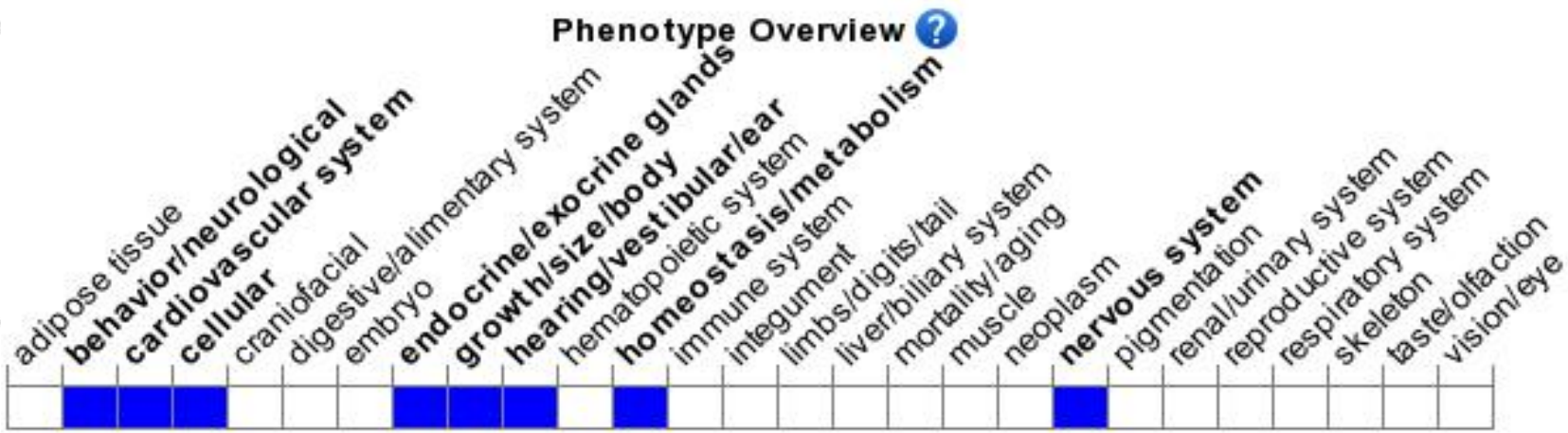
Genomic location distribution



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,Homozygotes for targeted mutations exhibit small size, hypoinsulinemia, glucose intolerance, decreased number and size of pancreatic islets, deafness with degeneration of hair cells, bradycardia, and arrhythmia.

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

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