

Cacna1f Cas9-CKO Strategy

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Design Date: 2020-2-11

Project Overview

Project Name

Cacna1f

Project type

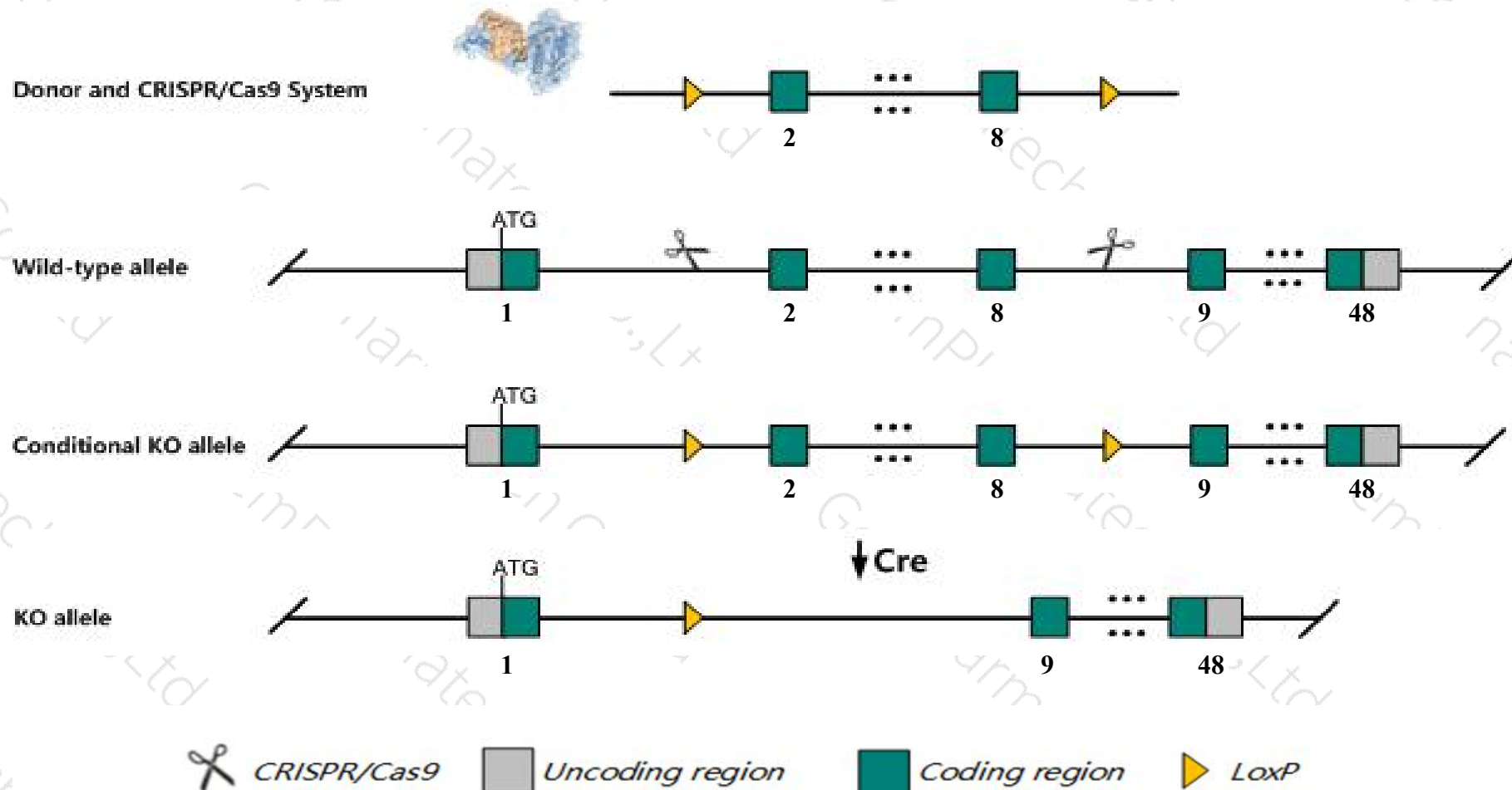
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cacna1f* gene. The schematic diagram is as follows:



- The *Cacnalf* gene has 11 transcripts. According to the structure of *Cacnalf* gene, exon2-exon8 of *Cacnalf*-202 (ENSMUST00000115726.8) transcript is recommended as the knockout region. The region contains 1093bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cacnalf* 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.

- According to the existing MGI data, Homozygous or hemizygous mutation of this gene results in impaired eye electrophysiology, abnormal retinal neuronal layer, bipolar cell, and horizontal cell morphology, and impaired retinal synapse morphology.
- This strategy has no effect on transcripts *Cacna1f*-204,210.
- The knockout area is about 2.5kb away from the 5-terminal of Ccdc22 and Gm36995, which may affect its 5-terminal regulation after knockout.
- The *Cacna1f* gene is located on the ChrX. 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)

Cacna1f calcium channel, voltage-dependent, alpha 1F subunit [*Mus musculus* (house mouse)]

Gene ID: 54652, updated on 27-Aug-2019

Summary

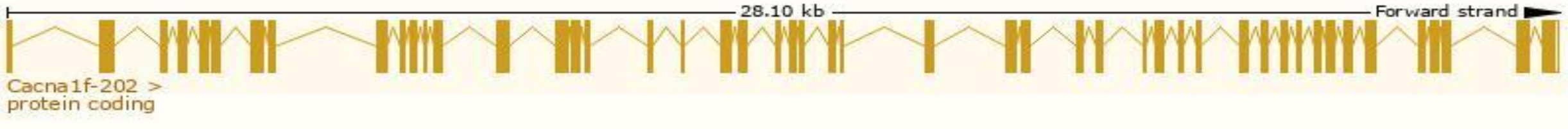
Official Symbol	Cacna1f provided by MGI
Official Full Name	calcium channel, voltage-dependent, alpha 1F subunit provided by MGI
Primary source	MGI:MGI:1859639
See related	Ensembl:ENSMUSG000000031142
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	nob2; Sfc17; nerg1; Cav1.4; A930034B14
Expression	Low expression observed in reference dataset See more
Orthologs	human all

Transcript information (Ensembl)

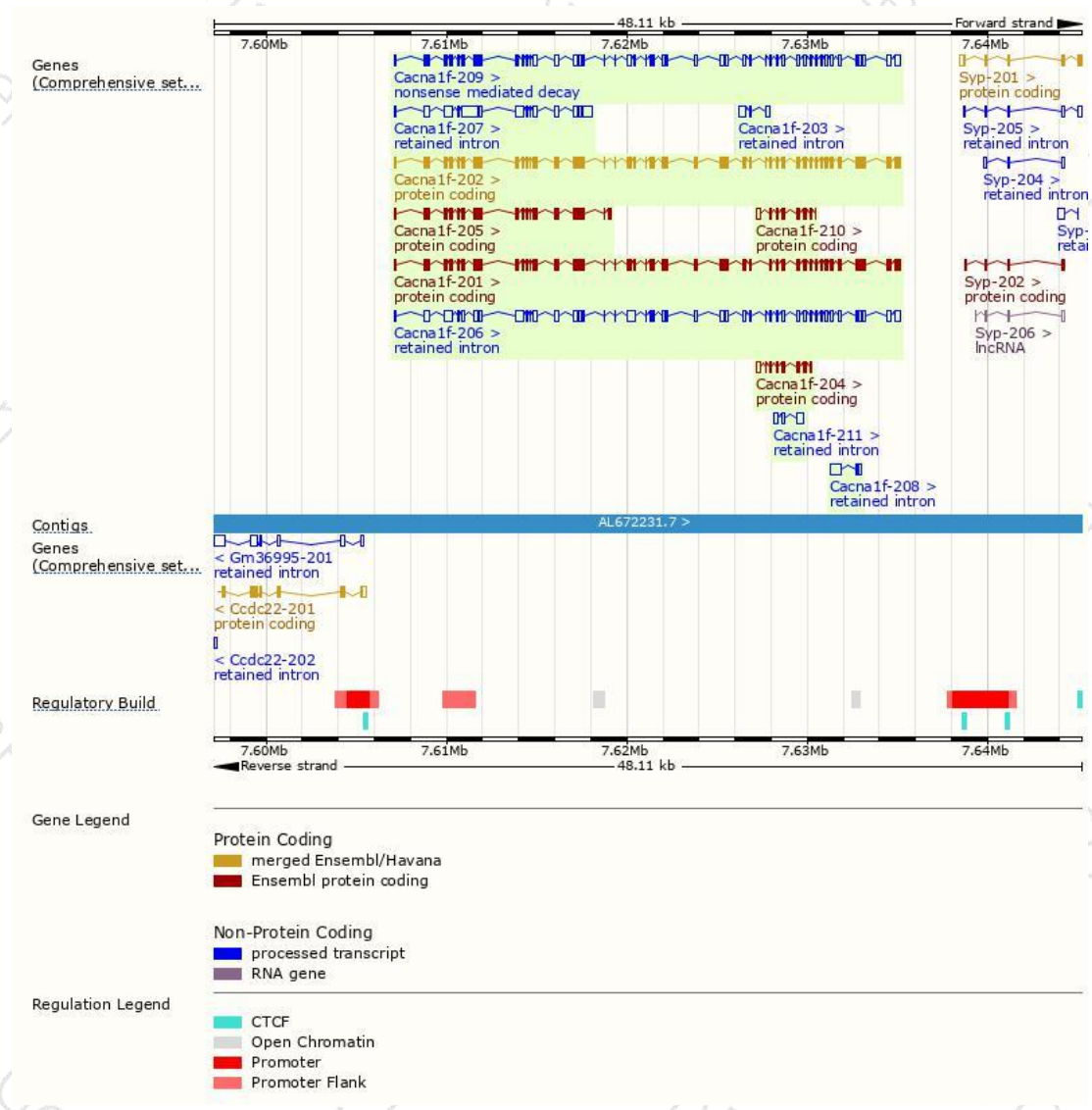
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cacna1f-202	ENSMUST00000115726.8	6079	1984aa	Protein coding	CCDS40840	Q7TNI3	TSL:1 GENCODE basic APPRIS P2
Cacna1f-201	ENSMUST00000115725.8	6028	1977aa	Protein coding	-	B1AVA4	TSL:5 GENCODE basic APPRIS ALT 2
Cacna1f-205	ENSMUST00000133637.7	2559	844aa	Protein coding	-	Q8C8Q4	CDS 3' incomplete TSL:1
Cacna1f-210	ENSMUST00000156047.1	921	247aa	Protein coding	-	B1AVA6	CDS 3' incomplete TSL:5
Cacna1f-204	ENSMUST00000128628.7	869	229aa	Protein coding	-	B1AVA5	CDS 3' incomplete TSL:3
Cacna1f-209	ENSMUST00000155090.7	6058	431aa	Nonsense mediated decay	-	S4R182	TSL:5
Cacna1f-206	ENSMUST00000141634.7	6622	No protein	Retained intron	-	-	TSL:2
Cacna1f-207	ENSMUST00000144522.7	3838	No protein	Retained intron	-	-	TSL:2
Cacna1f-208	ENSMUST00000151208.1	820	No protein	Retained intron	-	-	TSL:5
Cacna1f-203	ENSMUST00000123979.1	670	No protein	Retained intron	-	-	TSL:3
Cacna1f-211	ENSMUST00000157000.1	642	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Cacna1f-202* transcript,The transcription is shown below



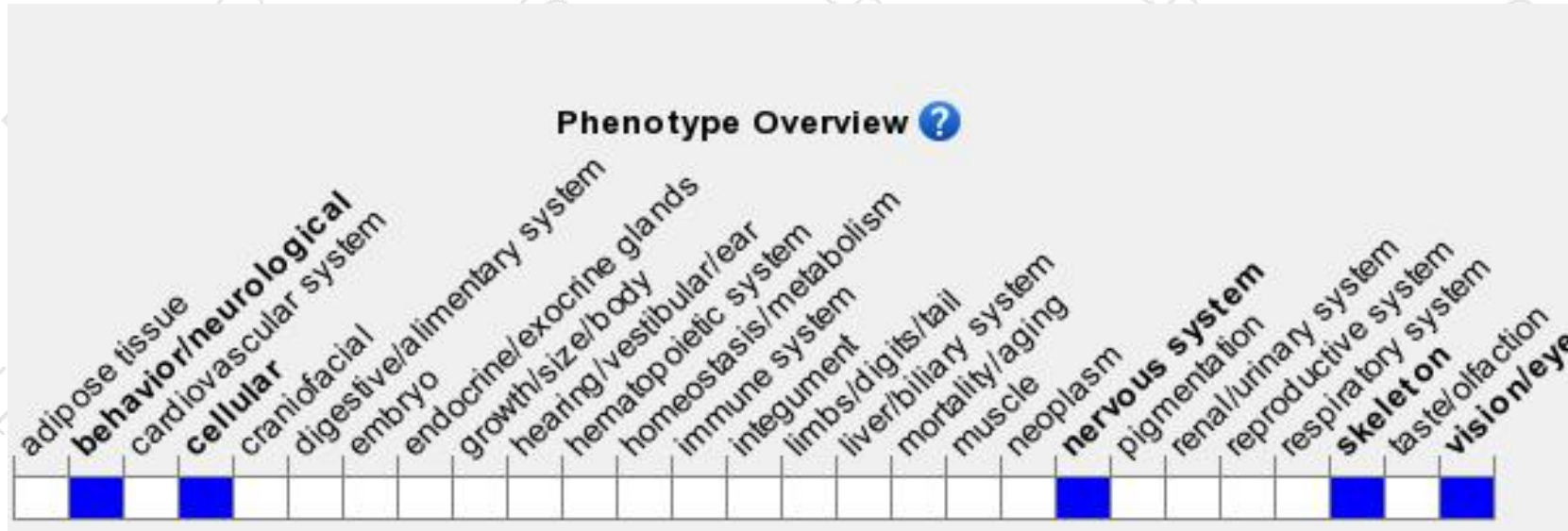
Genomic location distribution



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, Homozygous or hemizygous mutation of this gene results in impaired eye electrophysiology, abnormal retinal neuronal layer, bipolar cell, and horizontal cell morphology, and impaired retinal synapse morphology.

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

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