

Cacna2d3 Cas9-CKO Strategy

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Design Date:

2020-3-12

Project Overview

Project Name

Cacna2d3

Project type

Cas9-CKO

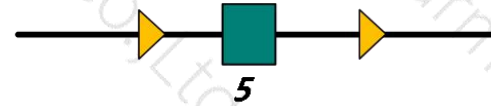
Strain background

C57BL/6JGpt

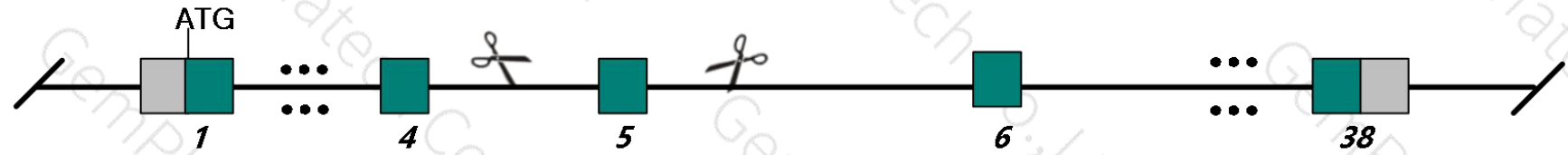
Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cacna2d3* gene. The schematic diagram is as follows:

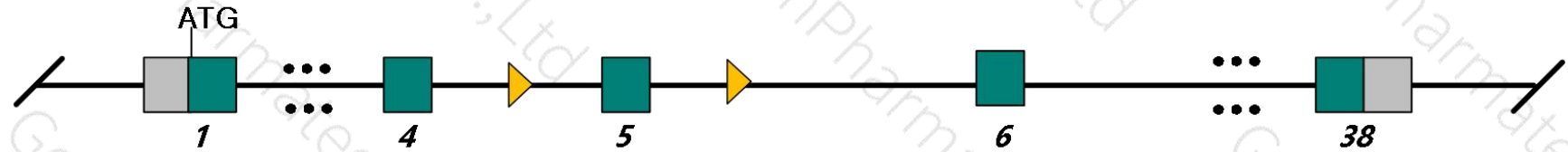
Donor and CRISPR/Cas9 System



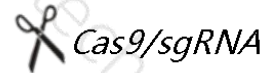
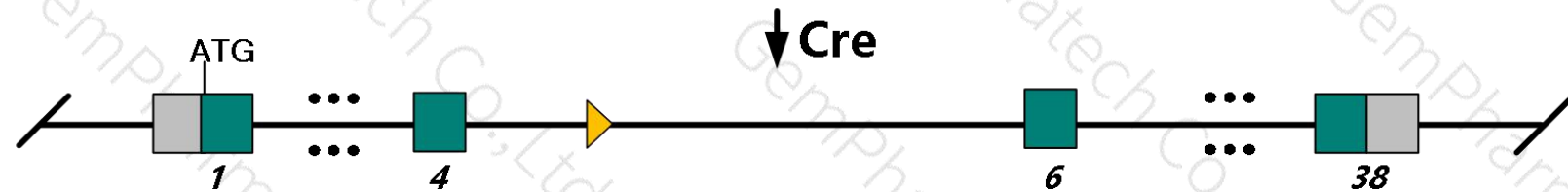
Wild-type allele



Conditional KO allele



KO allele



Cas9/sgRNA



Uncoding region



Coding region



LoxP

- The *Cacna2d3* gene has 9 transcripts. According to the structure of *Cacna2d3* gene, exon5 of *Cacna2d3-201* (ENSMUST00000022567.8) transcript is recommended as the knockout region. The region contains 163bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cacna2d3* 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 MGI data, mice homozygous for disruptions in this gene have a decreased startle reflex and occasional animals show increased aggression and hyperactivity.
- The transcript *Cacna2d3-208* may not be affected.
- The *Cacna2d3* 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)

Cacna2d3 calcium channel, voltage-dependent, alpha2/delta subunit 3 [*Mus musculus* (house mouse)]

Gene ID: 12294, updated on 12-Aug-2019

Summary

Official Symbol Cacna2d3 provided by [MGI](#)
Official Full Name calcium channel, voltage-dependent, alpha2/delta subunit 3 provided by [MGI](#)
Primary source [MGI:MGI:1338890](#)
See related [Ensembl:ENSMUSG00000021991](#)
Gene type protein coding
RefSeq status PROVISIONAL
Organism [Mus musculus](#)
Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as Cacna2d3; alpha2delta3
Expression Biased expression in cortex adult (RPKM 15.3), CNS E18 (RPKM 9.9) and 8 other tissues [See more](#)
Orthologs [human](#) [all](#)

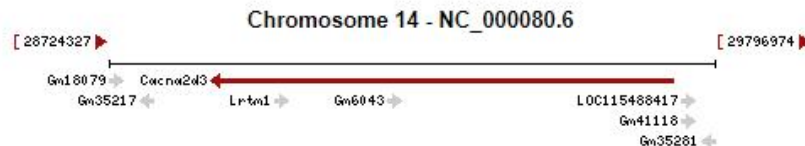
Genomic context

Location: 14; 14 A3

See Cacna2d3 in [Genome Data Viewer](#)

Exon count: 39

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	14	NC_000080.6 (28904943..29722775, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	14	NC_000080.5 (29718129..30535050, complement)



Transcript information (Ensembl)

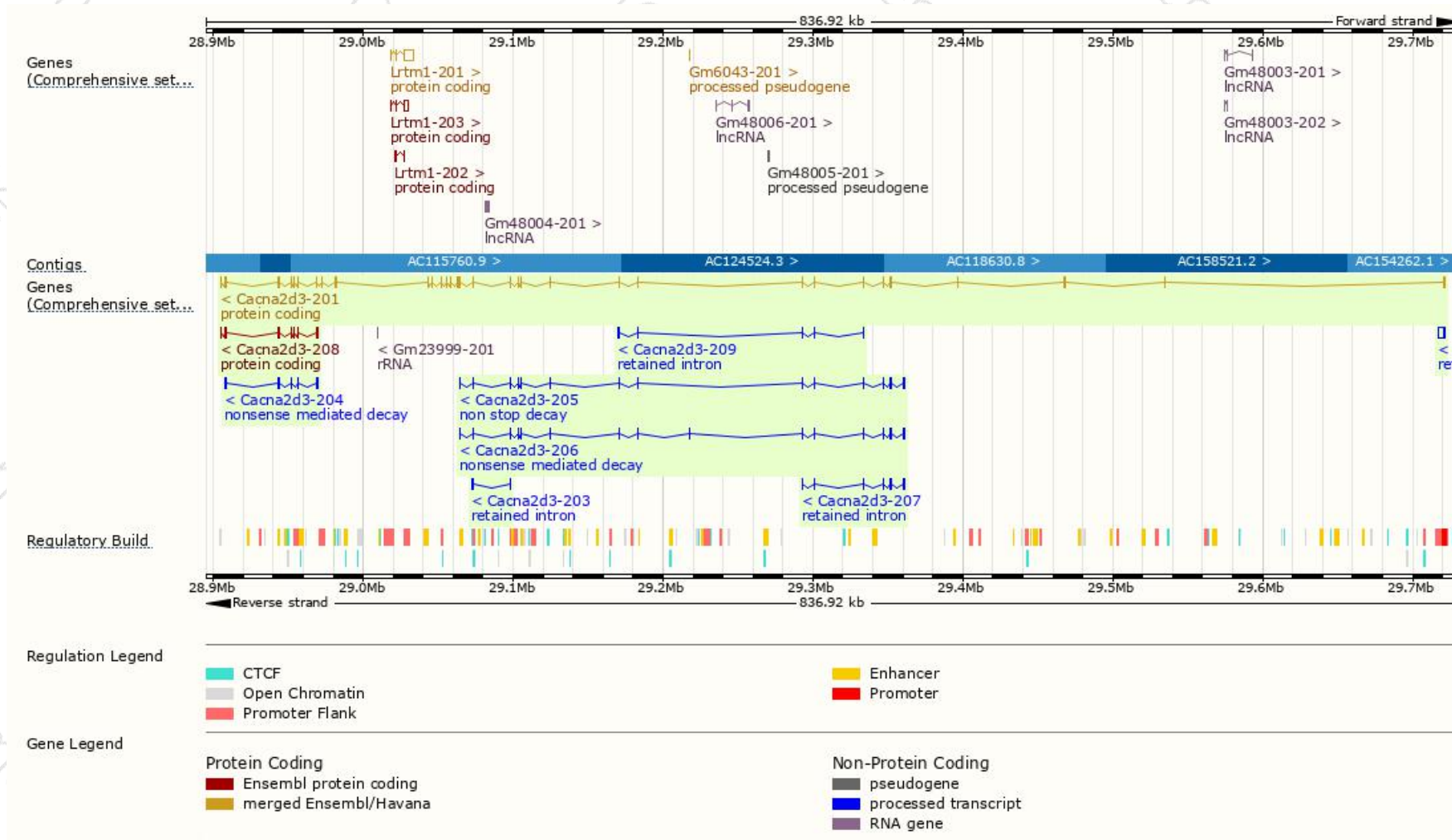
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cacna2d3-201	ENSMUST00000022567.8	3695	1091aa	Protein coding	-	A0A411ACY3 Q9Z1L5	TSL:1 GENCODE basic APPRIS P1
Cacna2d3-208	ENSMUST000000225985.1	918	264aa	Protein coding	-	A0A286YCF6	GENCODE basic
Cacna2d3-205	ENSMUST000000225733.1	1458	359aa	Non stop decay	-	A0A286YCT5	-
Cacna2d3-206	ENSMUST000000225863.1	1588	134aa	Nonsense mediated decay	-	A0A286YE06	-
Cacna2d3-204	ENSMUST000000225668.1	632	100aa	Nonsense mediated decay	-	A0A286YCK6	-
Cacna2d3-202	ENSMUST000000224128.1	3637	No protein	Retained intron	-	-	-
Cacna2d3-209	ENSMUST000000226048.1	892	No protein	Retained intron	-	-	-
Cacna2d3-207	ENSMUST000000225953.1	830	No protein	Retained intron	-	-	-
Cacna2d3-203	ENSMUST000000224950.1	605	No protein	Retained intron	-	-	-

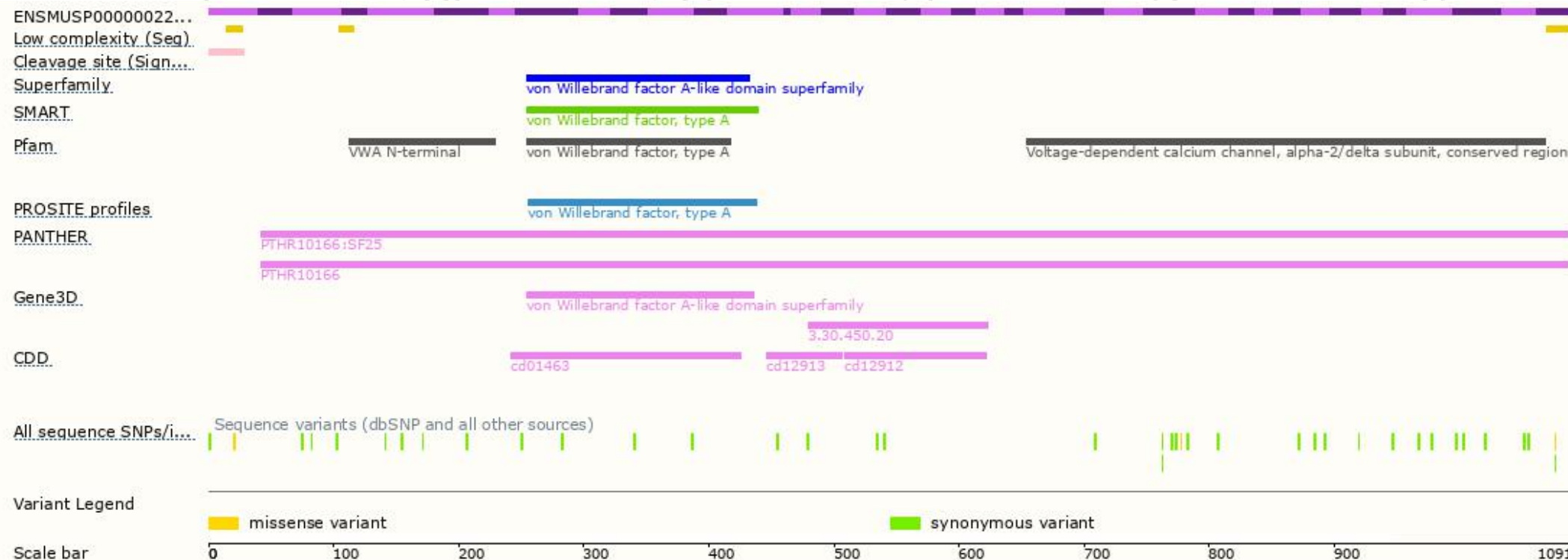
The strategy is based on the design of *Cacna2d3-201* transcript, the transcription is shown below



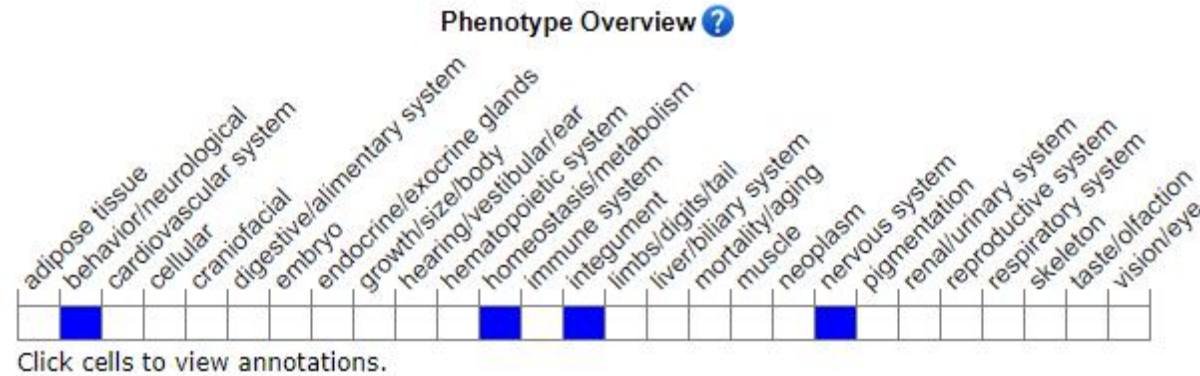
Genomic location distribution



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 homozygous for disruptions in this gene have a decreased startle reflex and occasional animals show increased aggression and hyperactivity.

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

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