

# *Cacnale* Cas9-KO Strategy

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# Project Overview

**Project Name**

*Cacna1e*

**Project type**

**Cas9-KO**

**Strain background**

**C57BL/6JGpt**

# Knockout strategy

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



- The *Cacnale* gene has 9 transcripts. According to the structure of *Cacnale* gene, exon2 of *Cacnale*-203 (ENSMUST00000187541.6) transcript is recommended as the knockout region. The region contains 106bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cacnale* gene. The brief process is as follows: CRISPR/Cas9 system

- According to the existing MGI data, Homozygotes for targeted null mutations exhibit altered R-type  $\text{Ca}^{2+}$  channels, increased timidity and body weight, impaired glucose tolerance, reduced locomotor activity, and lack of the cocaine stimulation of locomotor response.
- Transcript *Cacnale*-202&205 may not be affected.
- The effect on transcript *Cacnale*-208 is unknown.
- The *Cacnale* gene is located on the Chr1. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.



# Gene information (NCBI)

## Cacna1e calcium channel, voltage-dependent, R type, alpha 1E subunit [ *Mus musculus* (house mouse) ]

Gene ID: 12290, updated on 12-Aug-2019

### Summary

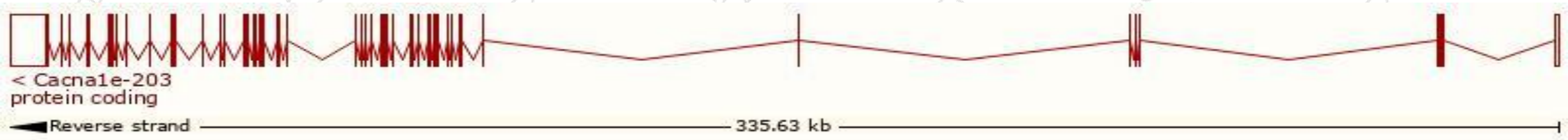
Official Symbol	Cacna1e provided by MGI
Official Full Name	calcium channel, voltage-dependent, R type, alpha 1E subunit provided by MGI
Primary source	MGI:MGI:106217
See related	Ensembl:ENSMUSG000000004110
Gene type	protein coding
RefSeq status	REVIEWED
Organism	<i>Mus musculus</i>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	BII; Cach6; Cav2.3; Cchra1; alpha1E; Cacn1a6; A430040I15
Summary	This gene encodes an integral membrane protein that belongs to the calcium channel alpha-1 subunits family. Voltage-sensitive calcium channels mediate the entry of calcium ions into excitable cells and are also involved in a variety of calcium-dependent processes. Voltage-dependent calcium channels are multi-subunit complexes, comprised of alpha-1, alpha-2, beta and delta subunits in a 1:1:1:1 ratio. The isoform alpha-1E gives rise to R-type calcium currents and belongs to the high-voltage activated group. Calcium channels containing the alpha-1E subunit may be involved in the modulation of neuronal firing patterns, an important component of information processing. [provided by RefSeq, Jul 2008]
Expression	Biased expression in frontal lobe adult (RPKM 6.4), cortex adult (RPKM 6.1) and 8 other tissues <a href="#">See more</a>
Orthologs	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

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

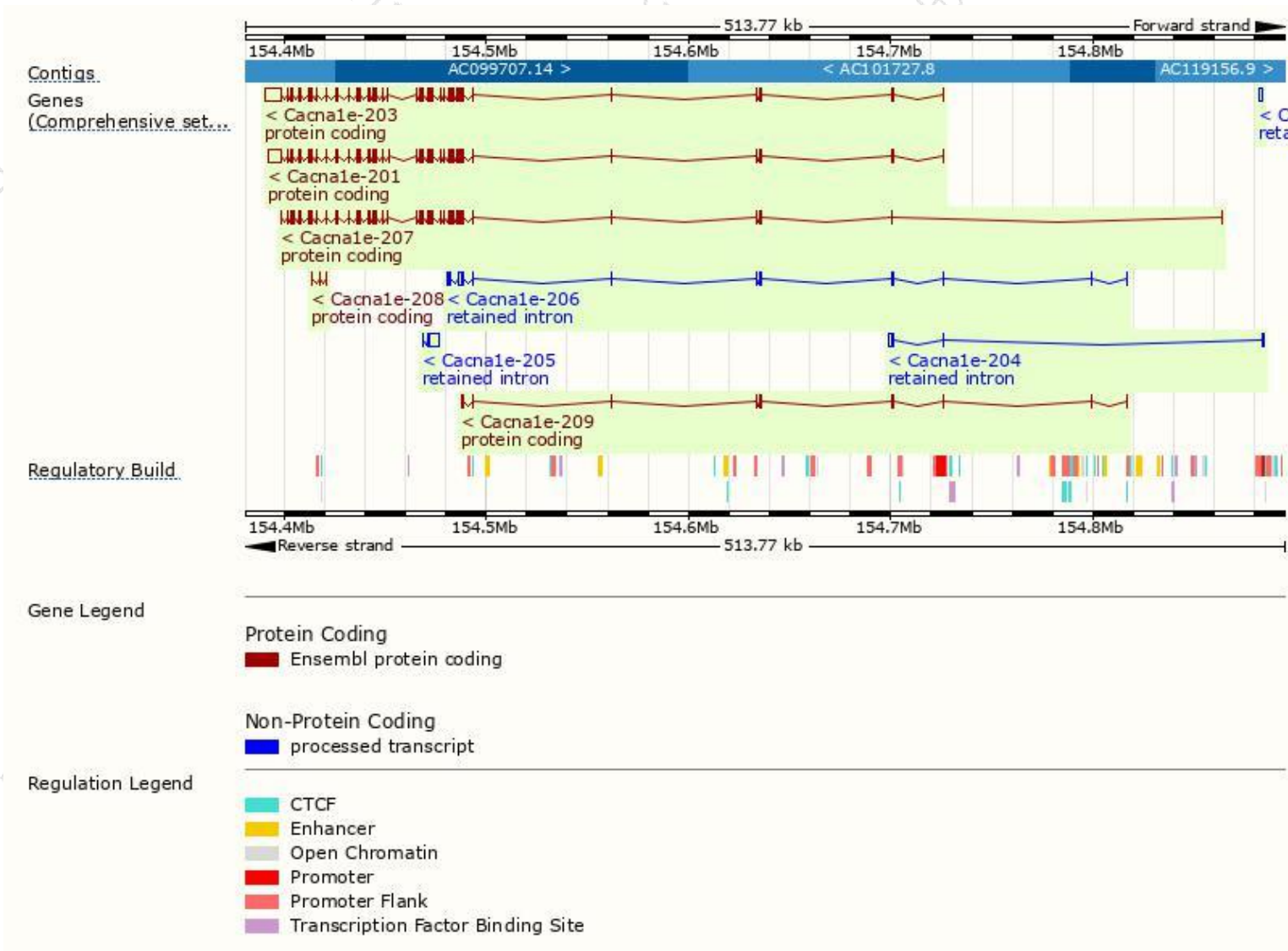
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cacna1e-203	<a href="#">ENSMUST00000187541.6</a>	14921	<a href="#">2273aa</a>	Protein coding	<a href="#">CCDS78710</a>	<a href="#">A0A087WS83</a>	TSL:1 GENCODE basic APPRIS P2
Cacna1e-201	<a href="#">ENSMUST00000004214.14</a>	12697	<a href="#">1965aa</a>	Protein coding	-	<a href="#">E9QK20</a>	TSL:5 GENCODE basic
Cacna1e-207	<a href="#">ENSMUST00000211821.1</a>	6765	<a href="#">2254aa</a>	Protein coding	-	<a href="#">A0A1D5RLU3</a>	TSL:5 GENCODE basic APPRIS ALT2
Cacna1e-209	<a href="#">ENSMUST00000238369.1</a>	1842	<a href="#">585aa</a>	Protein coding	-	-	CDS 3' incomplete
Cacna1e-208	<a href="#">ENSMUST00000226546.1</a>	331	<a href="#">110aa</a>	Protein coding	-	<a href="#">A0A2I3BPS0</a>	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete
Cacna1e-205	<a href="#">ENSMUST00000188384.1</a>	5001	No protein	Retained intron	-	-	TSL:1
Cacna1e-206	<a href="#">ENSMUST00000188965.6</a>	4056	No protein	Retained intron	-	-	TSL:1
Cacna1e-204	<a href="#">ENSMUST00000188321.1</a>	2356	No protein	Retained intron	-	-	TSL:1
Cacna1e-202	<a href="#">ENSMUST00000186256.1</a>	1859	No protein	Retained intron	-	-	TSL:NA

The strategy is based on the design of *Cacna1e-203* transcript,The transcription is shown below



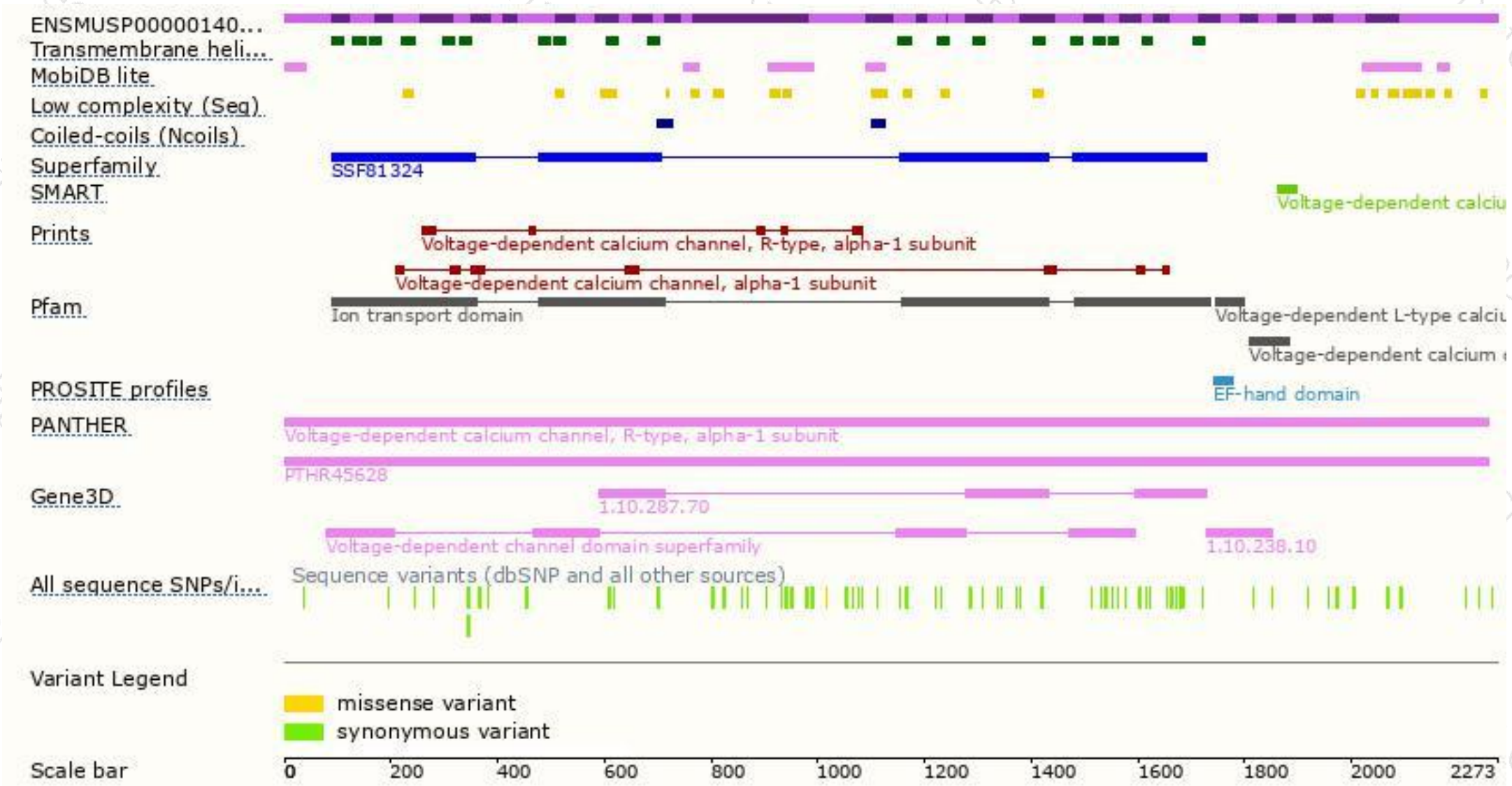


# Genomic location distribution

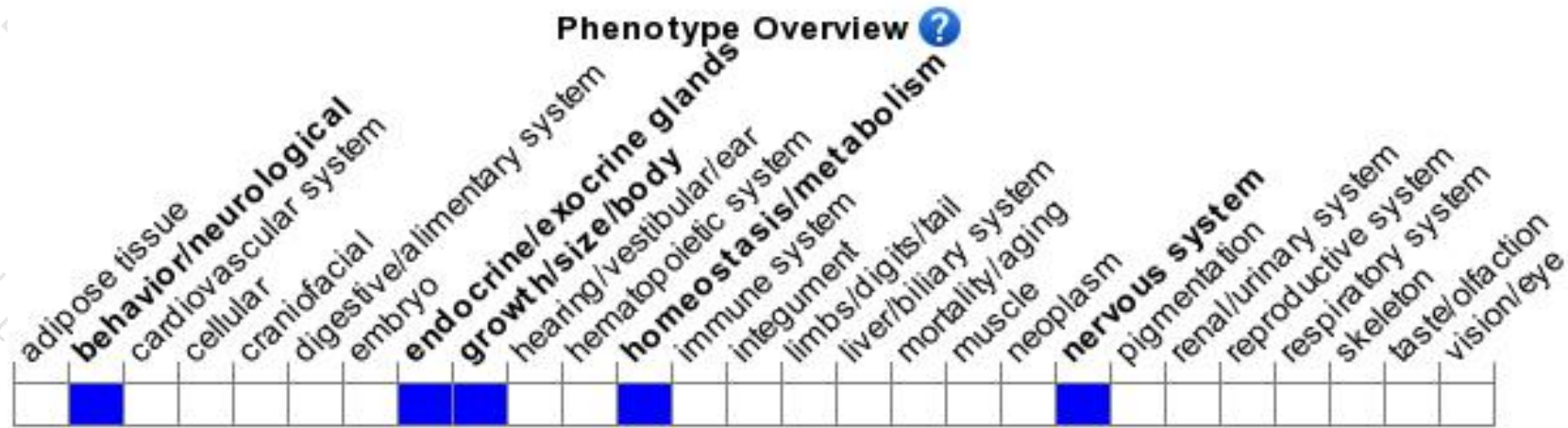




# Protein domain



# 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 null mutations exhibit altered R-type  $\text{Ca}^{2+}$  channels, increased timidity and body weight, impaired glucose tolerance, reduced locomotor activity, and lack of the cocaine stimulation of locomotor response.

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

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