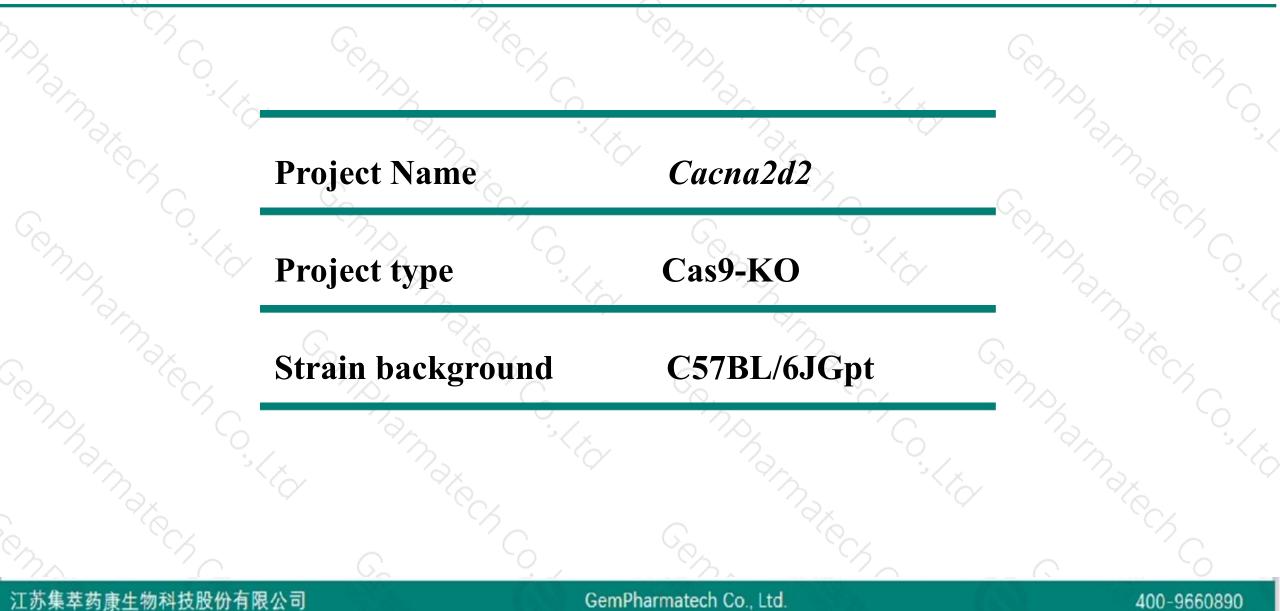


# Cacna2d2 Cas9-KO Strategy

Designer:Xueting Zhang Reviewer:Yanhua Shen Date:2019-12-23

## **Project Overview**

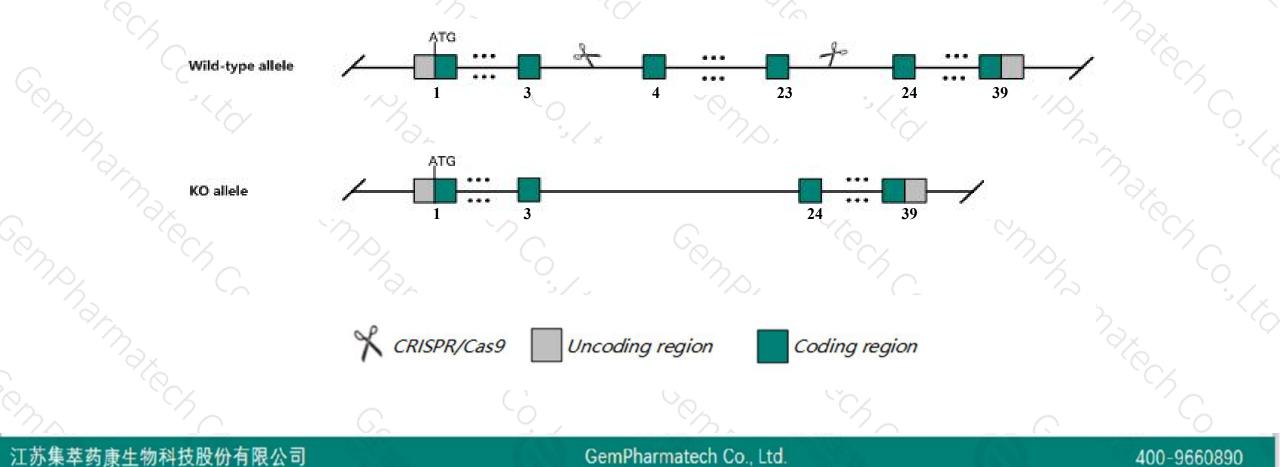




# **Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the Cacna2d2 gene. The schematic diagram is as follows:





- The Cacna2d2 gene has 10 transcripts. According to the structure of Cacna2d2 gene, exon4-exon23 of Cacna2d2-205 (ENSMUST00000168532.7) transcript is recommended as the knockout region. The region contains 1600bp coding sequence. Knock out the region will result in disruption of protein function.
- > In this project we use CRISPR/Cas9 technology to modify Cacna2d2 gene. The brief process is as follows: CRISPR/Cas9 sys

- According to the existing MGI data, Homozygotes for different mutant alleles show variable movement abnormalities including waddling, reeling or very slow gait, ataxia, and mild spike-wave seizures. While gross CNS abnormalities and demyelination are present in some mutant lines, they are not observed in others.
- ➤ Transcript *Cacna2d2*-206&210 may not be affected.
- The Cacna2d2 gene is located on the Chr9. 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.

Notice

## **Gene information** (NCBI)



Cacna2d2 calcium channel, voltage-dependent, alpha 2/delta subunit 2 [ Mus musculus (house mouse) ] Gene ID: 56808, updated on 11-Sep-2019 2 ? Summary Official Symbol Cacna2d2 provided by MGI Official Full Name calcium channel, voltage-dependent, alpha 2/delta subunit 2 provided by MGI Primary source MGI:MGI:1929813 Ensembl:ENSMUSG00000010066 See related Gene type protein coding RefSeg 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 du; td; a2d2; torpid; Cacna2d; mKIAA0558 Expression Biased expression in cerebellum adult (RPKM 24.9), whole brain E14.5 (RPKM 22.0) and 10 other tissues See more Orthologs human all Genomic context ☆ ? Location: 9 F1: 9 58.02 cM See Cacna2d2 in Genome Data Viewer

Exon count: 39

 Annotation release
 Status
 Assembly
 Chr
 Location

 108
 current
 GRCm38.p6 (GCF\_000001635.26)
 9
 NC\_000075.6 (107399733..107529346)

 Build 37.2
 previous assembly
 MGSCv37 (GCF\_000001635.18)
 9
 NC\_000075.5 (107302211..107431674)

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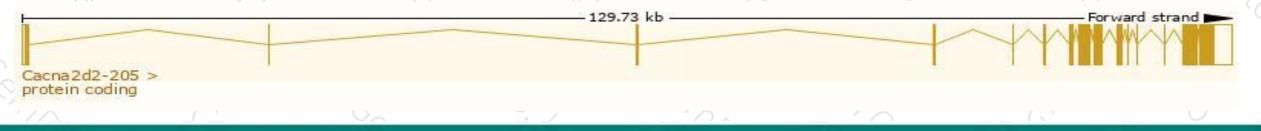
# **Transcript information (Ensembl)**



#### The gene has 10 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cacna2d2-205	ENSMUST00000168532.7	5807	<u>1156aa</u>	Protein coding	CCDS72304	Q6PHS9	TSL:1 GENCODE basic APPRIS ALT2
Cacna2d2-201	ENSMUST00000010210.12	5783	<u>1148aa</u>	Protein coding	CCDS52917	Q6PHS9	TSL:1 GENCODE basic APPRIS ALT2
Cacna2d2-208	ENSMUST00000170737.2	5332	<u>1150aa</u>	Protein coding	CCDS72305	Q6PHS9	TSL:1 GENCODE basic APPRIS ALT2
Cacna2d2-202	ENSMUST0000085092.11	5183	<u>1154aa</u>	Protein coding	CCDS52916	Q6PHS9	TSL:5 GENCODE basic APPRIS P4
Cacna2d2-203	ENSMUST00000164988.8	5542	<u>1157aa</u>	Protein coding	1.5	E9Q683	TSL:5 GENCODE basic APPRIS ALT2
Cacna2d2-204	ENSMUST00000166799.7	3474	<u>1157aa</u>	Protein coding		Q6PHS9	TSL:5 GENCODE basic APPRIS ALT2
Cacna2d2-210	ENSMUST00000194842.1	1975	No protein	Retained intron	8 <b>4</b>	140	TSL:NA
Cacna2d2-206	ENSMUST00000168959.1	604	No protein	Retained intron	<u>.</u>	1920	TSL:3
Cacna2d2-209	ENSMUST00000171809.1	727	No protein	IncRNA	17	(15)	TSL:3
Cacna2d2-207	ENSMUST00000169354.7	671	No protein	IncRNA	-	( <b>.</b>	TSL:5

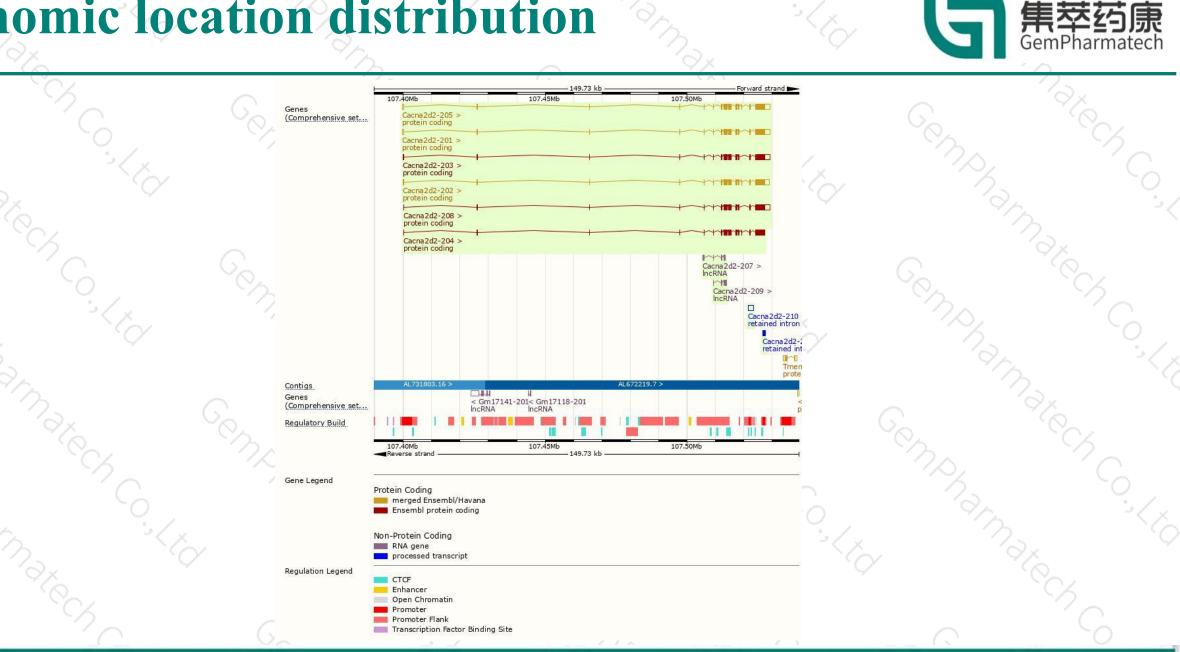
The strategy is based on the design of Cacna2d2-205 transcript, The transcription is shown below



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### **Genomic location distribution**

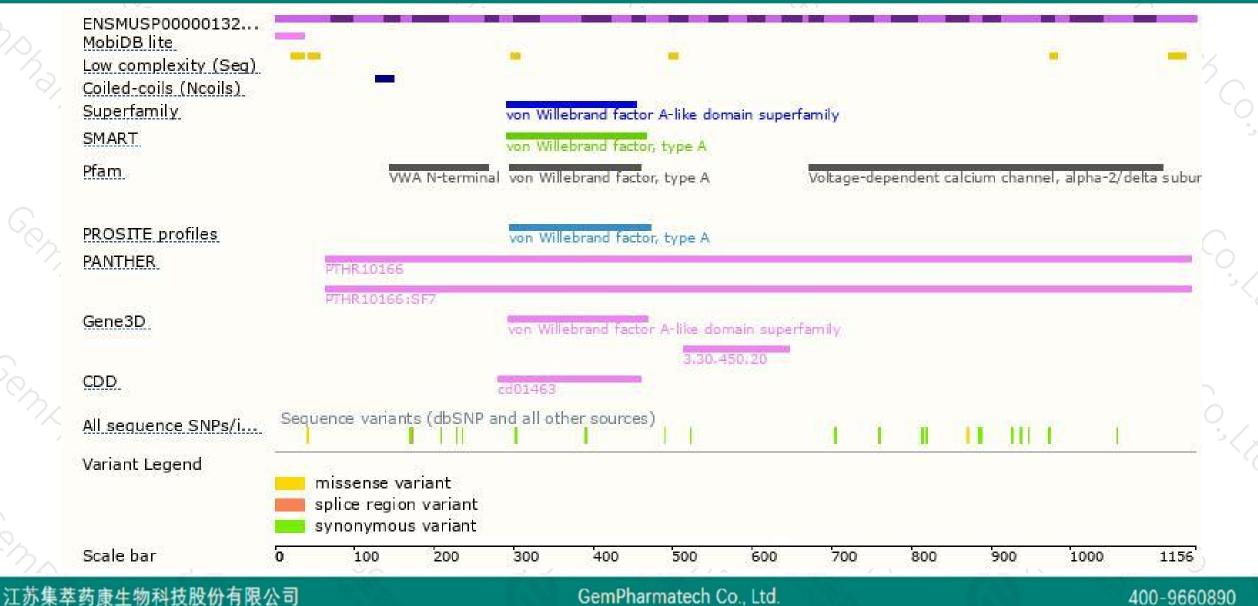


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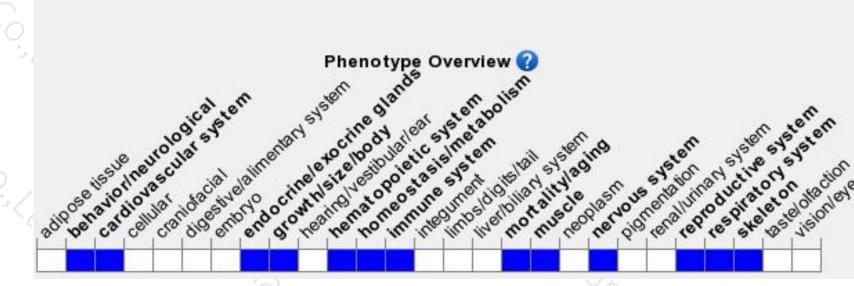
### **Protein domain**





### 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 different mutant alleles show variable movement abnormalities including waddling, reeling or very slow gait, ataxia, and mild spike-wave seizures. While gross CNS abnormalities and demyelination are present in some mutant lines, they are not observed in others.

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If you have any questions, you are welcome to inquire. Tel: 400-9660890



