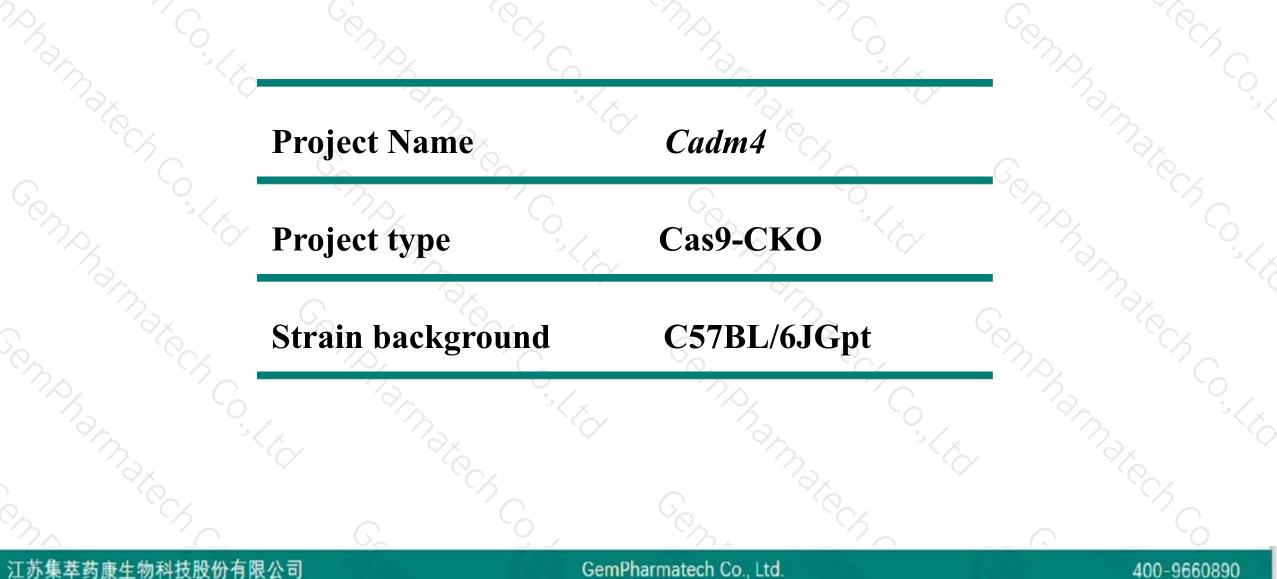


Cadm4 Cas9-CKO Strategy

Designer: Xueting Zhang Reviewer: Yanhua Shen Date:2020-02-24

Project Overview



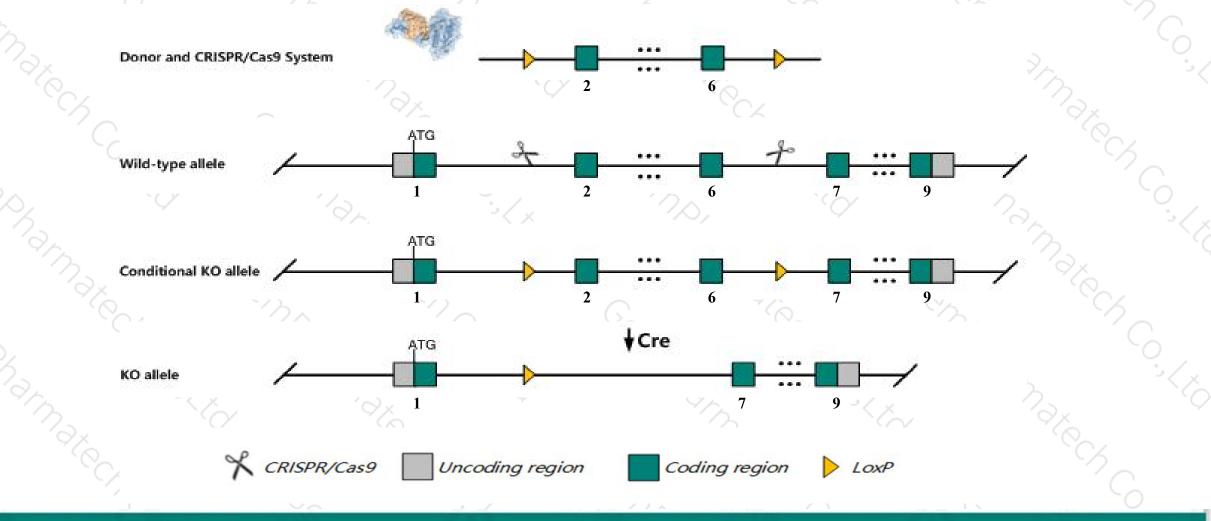


Conditional Knockout strategy



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This model will use CRISPR/Cas9 technology to edit the *Cadm4* gene. The schematic diagram is as follows:



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The Cadm4 gene has 2 transcripts. According to the structure of Cadm4 gene, exon2-exon6 of Cadm4-201 (ENSMUST00000068023.7) transcript is recommended as the knockout region. The region contains 691bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Cadm4* 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, Mice homozygous for one null allele do not display myelination abnormalities. Mice with ubiquitous conditional deletion of the gene show myelination abnormalities, decreased nerve conduction velocity, hindlimb rigidity, limb grasping, and impaired coordination.
- The floxed region is near to the N-terminal of Zfp428 gene, this strategy may influence the regulatory function of the N-terminal of Zfp428 gene.
- The *Cadm4* gene is located on the Chr7. 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)



Max Cr

Cadm4 cell adhesion molecule 4 [Mus musculus (house mouse)]

Gene ID: 260299, updated on 21-Aug-2019

Official Symbol Cadm4 provided by MGL

Summary

2 ?

| Official Symbol | Cadina provided by Mon |
|--------------------|--|
| Official Full Name | cell adhesion molecule 4 provided by MGI |
| Primary source | MGI:MGI:2449088 |
| See related | Ensembl:ENSMUSG00000054793 |
| 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; Mus; Mus |
| Also known as | Tsll2; lgdf4c; lgsf4c |
| Expression | Biased expression in CNS E18 (RPKM 63.0), cerebellum adult (RPKM 62.2) and 14 other tissues See more |
| Orthologs | human all |
| | |

Genomic context

☆ ?

See Cadm4 in Genome Data Viewer

Exon count: 9

Location: 7; 7 A3

| Annotation release | Status | Assembly | Chr | Location | 2. |
|--------------------|-------------------|------------------------------|-----|--------------------------------|----|
| <u>108</u> | current | GRCm38.p6 (GCF_000001635.26) | 7 | NC_000073.6 (2448202324504533) | 1 |
| Build 37.2 | previous assembly | MGSCv37 (GCF_000001635.18) | 7 | NC_000073.5 (2526704225289552) | |

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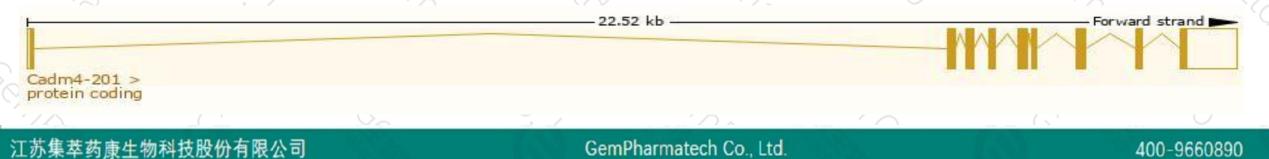
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The gene has 2 transcripts, all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|-----------|----------------------|------|--------------|----------------|-----------|---------------|-------------------------------|
| Cadm4-201 | ENSMUST0000068023.7 | 2161 | <u>388aa</u> | Protein coding | CCDS20951 | <u>Q8R464</u> | TSL:1 GENCODE basic APPRIS P1 |
| Cadm4-202 | ENSMUST00000205820.1 | 491 | No protein | IncRNA | 87 | | TSL:3 |

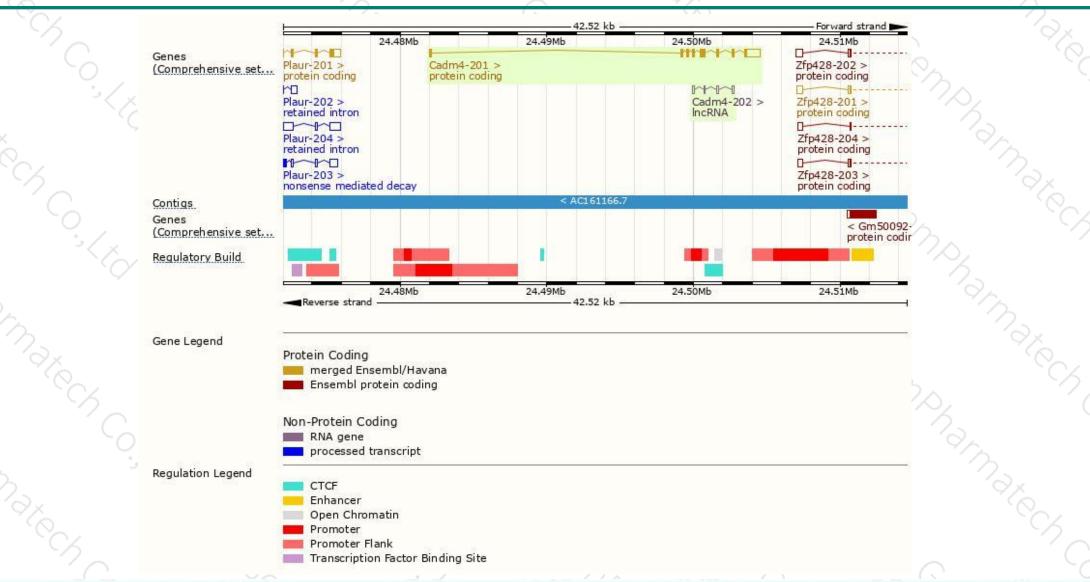
The strategy is based on the design of Cadm4-201 transcript, The transcription is shown below



Genomic location distribution



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Protein domain



| | | | | | | | | | (Ox |
|---|-----------------|--------------------|---------------|------------------|---------------|----------|------|-----|---------------------------------------|
| ENSMUSP00000066 Transmembrane heli SIFTS import Low complexity (Seg) | | | | | 114 | <u> </u> | | | 50 |
| Cleavage site (Sign | | | | | | | | | |
| Superfamily | Immunog | lobulin-like domai | in superfamil | Y | | | | | |
| SMART | Immunor | globulin subtype | | | | | - 20 | | Neurexin/synder |
| | 3 | | Im | munoglobulin s | ubtype 2 | | | | |
| Pfam | Immuno | globulin V-set dor | nain CD8 | 0-like, immunog | globulin C2-s | et | | | C |
| | | | | | | PF13927 | 2 | | 0 |
| PROSITE profiles | | | Immur | oglobulin-like d | lomain | | 5 | | |
| PANTHER | Cell adhesion r | nolecule 4 | | | | | | | |
| | PTHR45889 | | | | | | | | |
| Gene3D | Immunogl | obulin-like fold | 1 Alexandre | | | | | | _ |
| CDD | 12/10/10/10/201 | | | | | cd00096 | | | 6 |
| All sequence SNPs/i | Sequence variar | nts (dbSNP and | all other sou | irces) | | | | | · · · · · · · · · · · · · · · · · · · |
| All sequence SNPS/I | | 2 | | 1 | | | 1 | 11 | |
| Variant Legend | | | | | | | | | |
| | synonymou | | 28 | 10 | <u>65</u> | <i></i> | 102 | 622 | - |
| Scale bar | o 40 | 80 | 120 | 160 | 200 | 240 | 280 | 320 | 388 |
| 0 | | | | | | | | | |

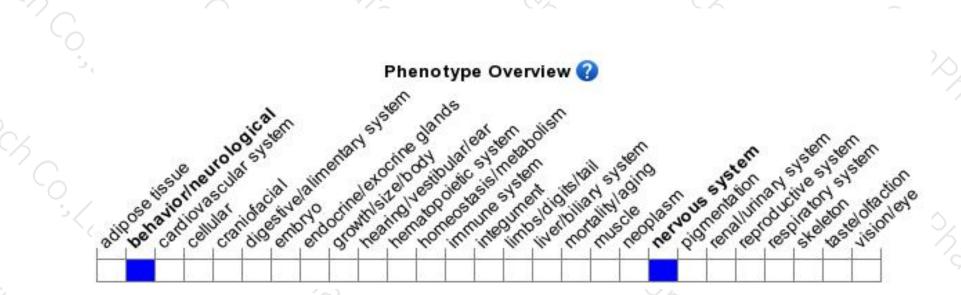
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Mouse phenotype description(MGI)





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 one null allele do not display myelination abnormalities. Mice with ubiquitous conditional deletion of the gene show myelination abnormalities, decreased nerve conduction velocity, hindlimb rigidity, limb grasping, and impaired coordination.

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



