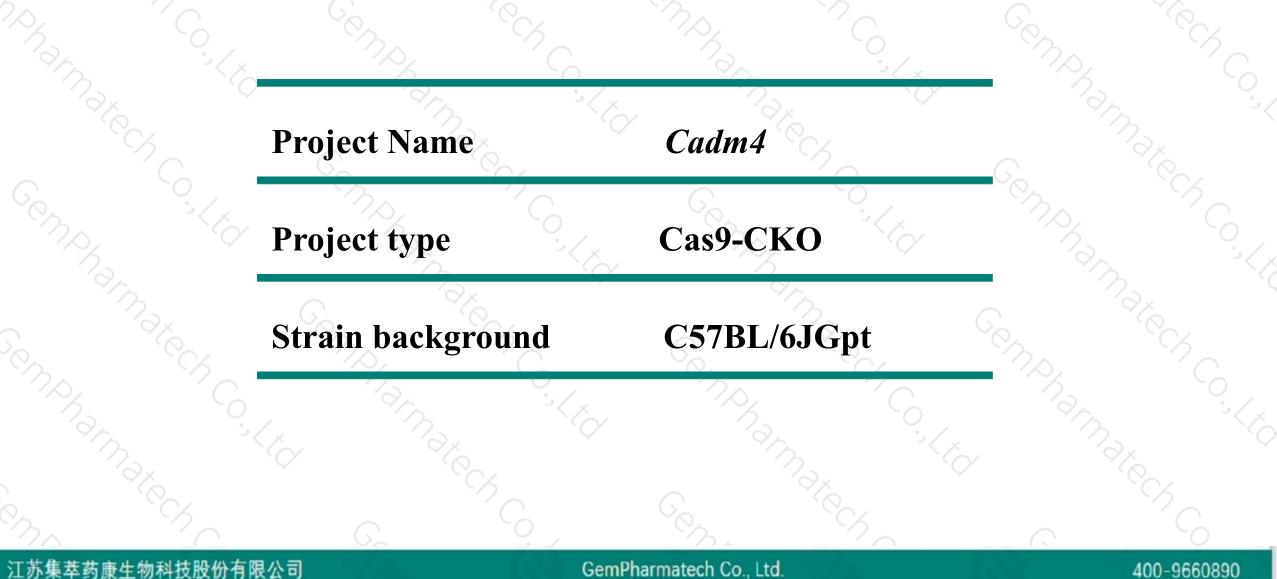


Cadm4 Cas9-CKO Strategy

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

Project Overview



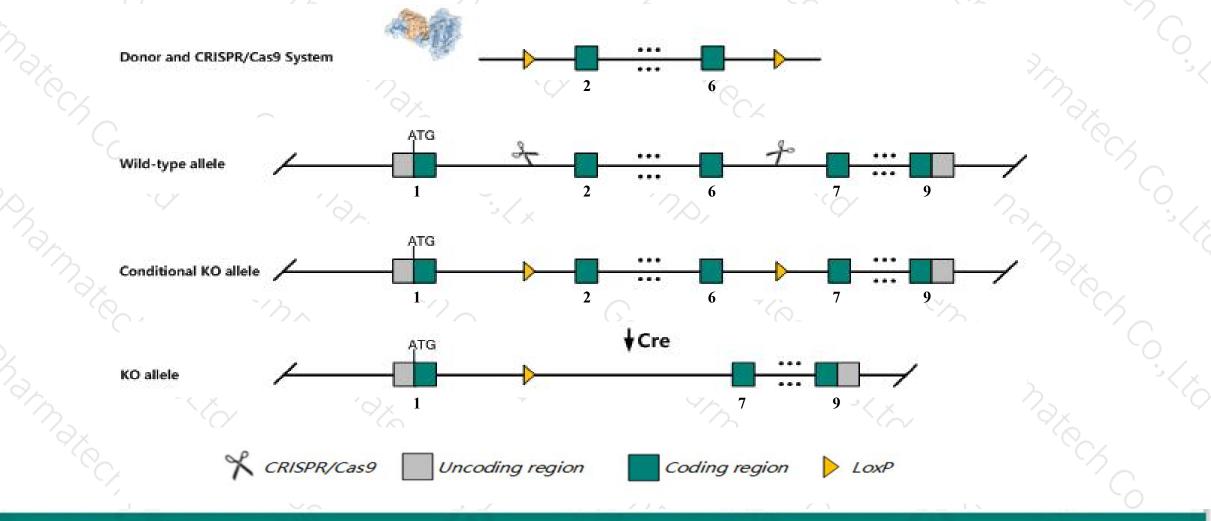


Conditional Knockout strategy



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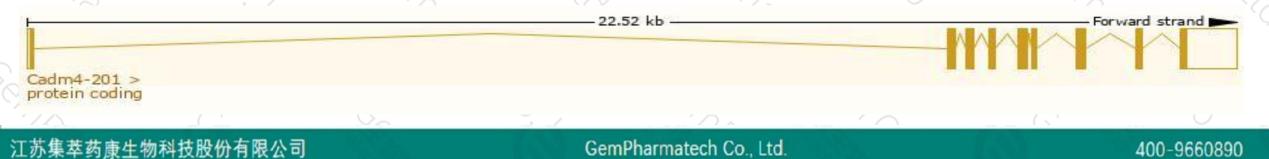
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The gene has 2 transcripts, all transcripts are shown below:

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

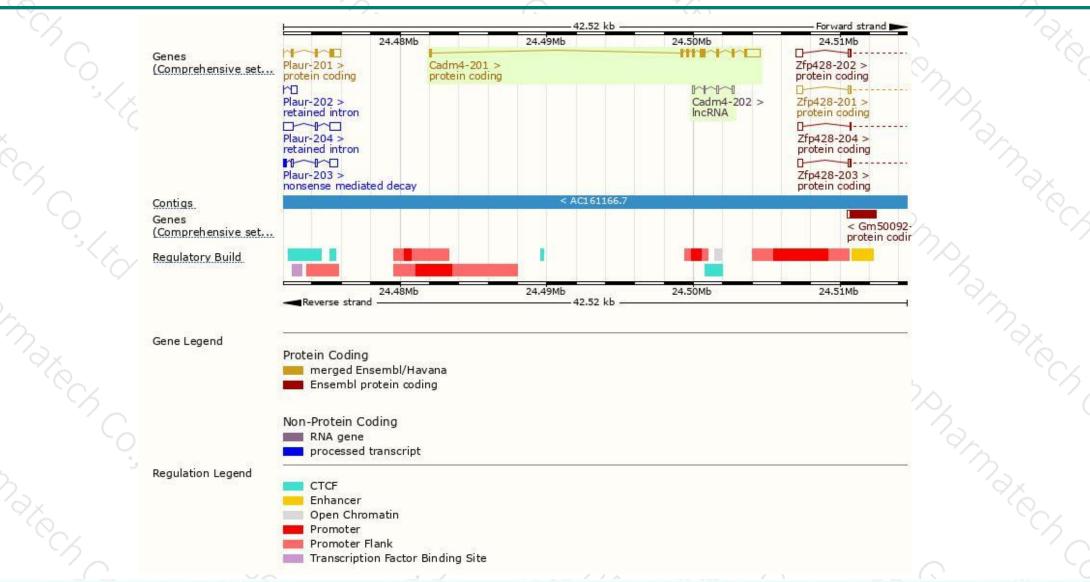
The strategy is based on the design of Cadm4-201 transcript, The transcription is shown below



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									

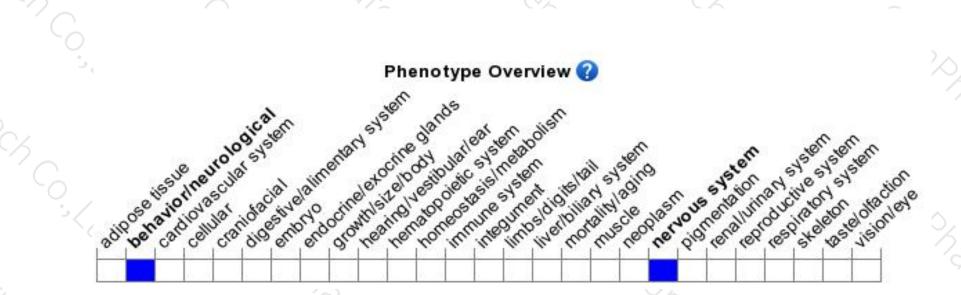
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Mouse phenotype description(MGI)





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



