

Plekhd1 Cas9-CKO Strategy

Designer: Longyun Hu

Reviewer: Rui Xiong

Design Date: 2021-4-6

Project Overview



Project Name

Plekhd1

Project type

Cas9-CKO

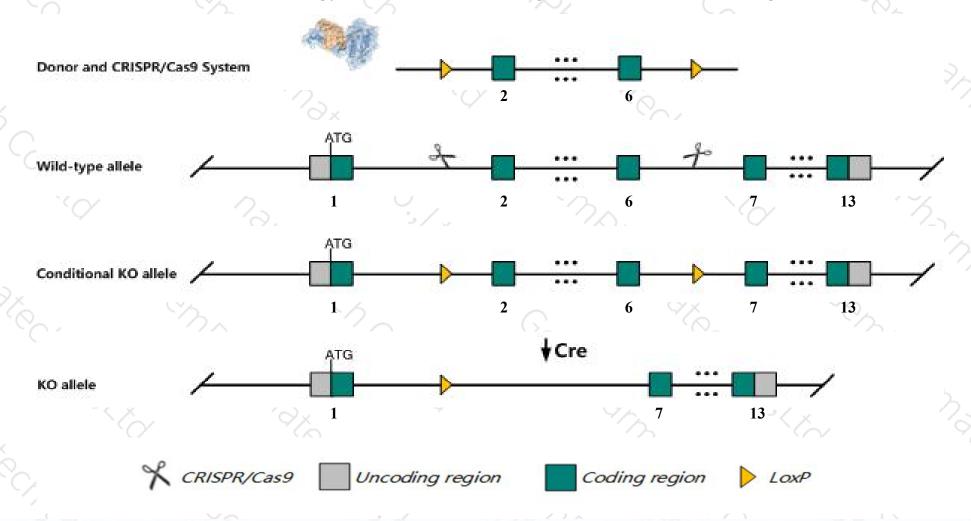
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Plekhd1* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Plekhd1* gene has 4 transcripts. According to the structure of *Plekhd1* gene, exon2-exon6 of *Plekhd1*201(ENSMUST00000140770.1) transcript is recommended as the knockout region. The region contains 406bp coding sequence.

 Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Plekhd1* 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



- > The *Plekhd1* gene is located on the Chr12. 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)



Plekhd1 pleckstrin homology domain containing, family D (with coiled-coil domains) member 1 [Mus musculus (house mouse)]

Gene ID: 217682, updated on 13-Mar-2020

Summary



Official Symbol Plekhd1 provided by MGI

Official Full Name pleckstrin homology domain containing, family D (with coiled-coil domains) member 1 provided by MGI

Primary source MGI:MGI:3036228

See related Ensembl:ENSMUSG00000066438

Gene type protein coding
RefSeq 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 3830431G21Rik

Expression Biased expression in cerebellum adult (RPKM 11.3), placenta adult (RPKM 7.7) and 12 other tissuesSee more

Orthologs <u>human</u> all

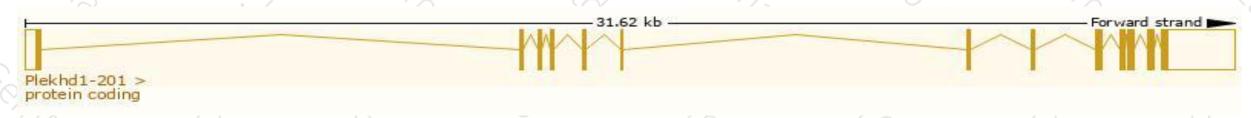
Transcript information (Ensembl)



The gene has 4 transcripts, all transcripts are shown below:

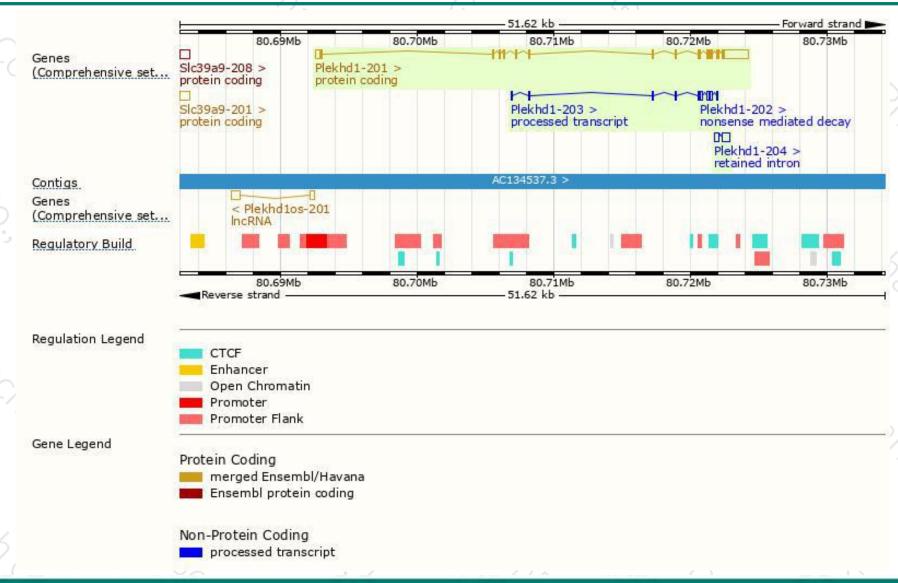
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Plekhd1-201	ENSMUST00000140770.1	3535	505aa	Protein coding	CCDS49100	B2RPU2	TSL:1 GENCODE basic APPRIS P1
Plekhd1-202	ENSMUST00000142760.1	451	3aa	Nonsense mediated decay	#8	8 9	CDS 5' incomplete TSL:3
Plekhd1-203	ENSMUST00000152465.1	428	No protein	Processed transcript	#2 #3	84	TSL:3
Plekhd1-204	ENSMUST00000153762.1	956	No protein	Retained intron	25	0.2	TSL:1

The strategy is based on the design of *Plekhd1-201* transcript, the transcription is shown below:



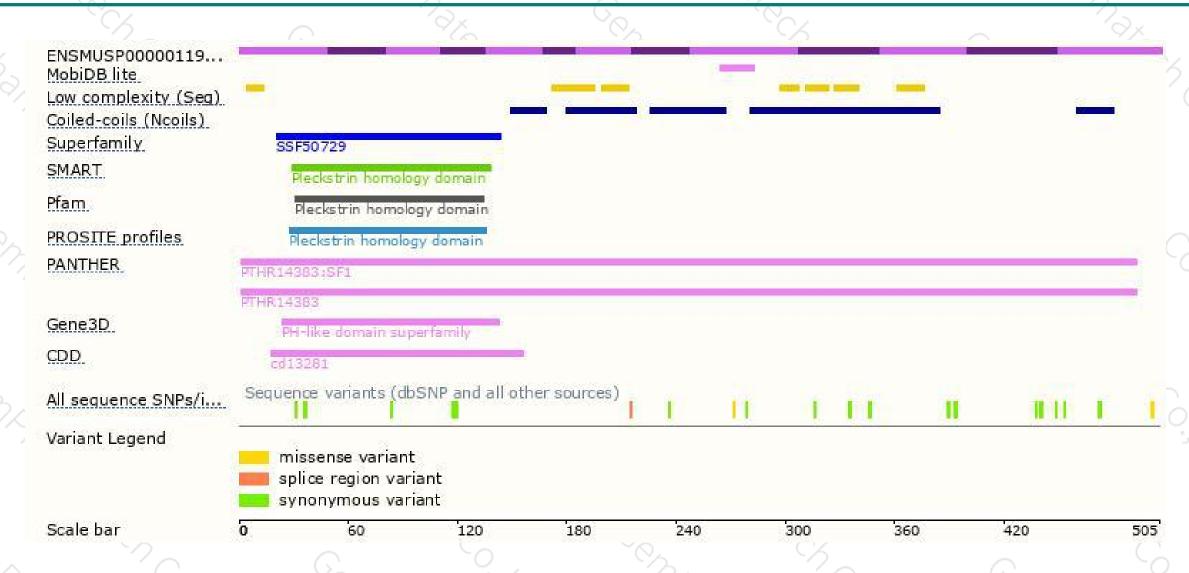
Genomic location distribution





Protein domain







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





