

Plekhh1 Cas9-CKO Strategy

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Reviewer: Xiaojing Li

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Project Overview



Project Name Plekhh1

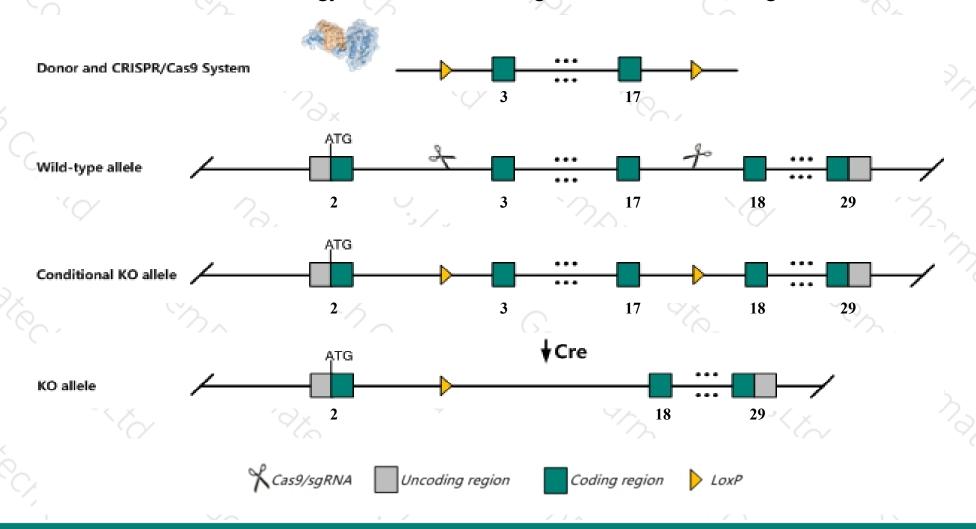
Project type Cas9-CKO

Strain background C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Plekhh1* gene. The schematic diagram is as follows:



Technical routes



- The *Plekhh1* gene has 4 transcripts. According to the structure of *Plekhh1* gene, exon3-exon17 of *Plekhh1-201*(ENSMUST00000039928.6) transcript is recommended as the knockout region. The region contains 2320bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Plekhh1* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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 *Plekhh1* 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)



Plekhh1 pleckstrin homology domain containing, family H (with MyTH4 domain) member 1 [Mus musculus (house mouse)]

Gene ID: 211945, updated on 13-Mar-2020

Summary



Official Symbol Plekhh1 provided by MGI

Official Full Name pleckstrin homology domain containing, family H (with MyTH4 domain) member 1 provided by MGI

Primary source MGI:MGI:2144989

See related Ensembl:ENSMUSG00000060716

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 AV308913, C77772, D630024D12Rik, mKIAA1200

Expression Broad expression in placenta adult (RPKM 18.0), colon adult (RPKM 15.8) and 20 other tissuesSee more

Orthologs <u>human</u> all

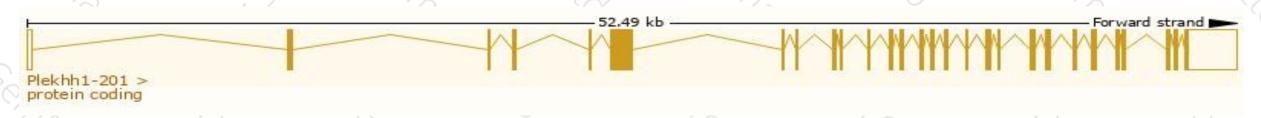
Transcript information (Ensembl)



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

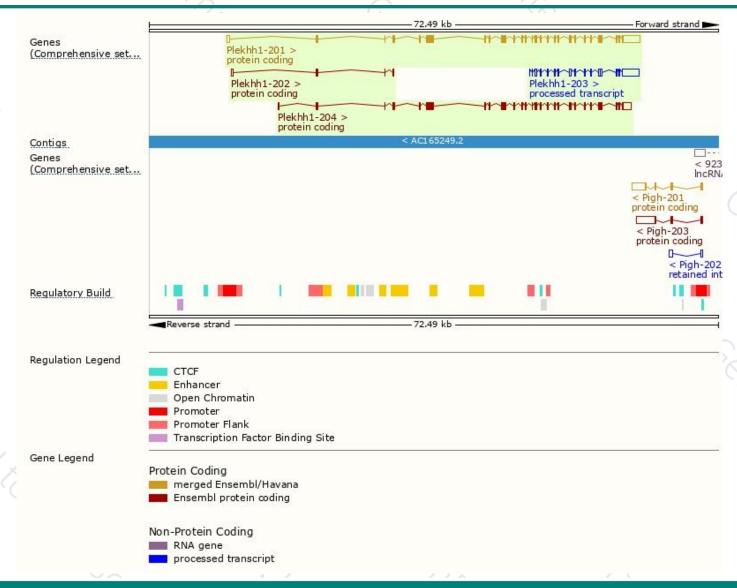
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Plekhh1-201	ENSMUST00000039928.6	6439	<u>1356aa</u>	Protein coding	CCDS49097	Q80TI1	TSL:1 GENCODE basic APPRIS P1
Plekhh1-204	ENSMUST00000219956.1	5229	<u>1356aa</u>	Protein coding	CCDS49097	Q80TI1	TSL:1 GENCODE basic APPRIS P1
Plekhh1-202	ENSMUST00000217954.1	430	<u>75aa</u>	Protein coding	-	A0A1W2P874	CDS 3' incomplete TSL:3
Plekhh1-203	ENSMUST00000219946.1	4106	No protein	Processed transcript	-	-	TSL:1
7 Pm							

The strategy is based on the design of *Plekhh1-201* transcript, the transcription is shown below:



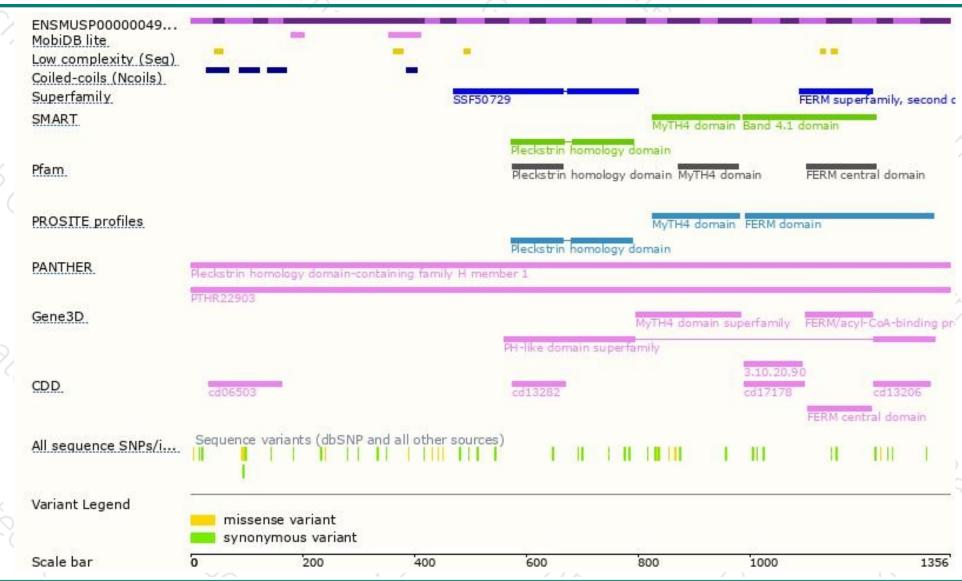
Genomic location distribution





Protein domain







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

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