

Plekhh3 Cas9-CKO Strategy

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Project Overview



Project Name Plekhh3

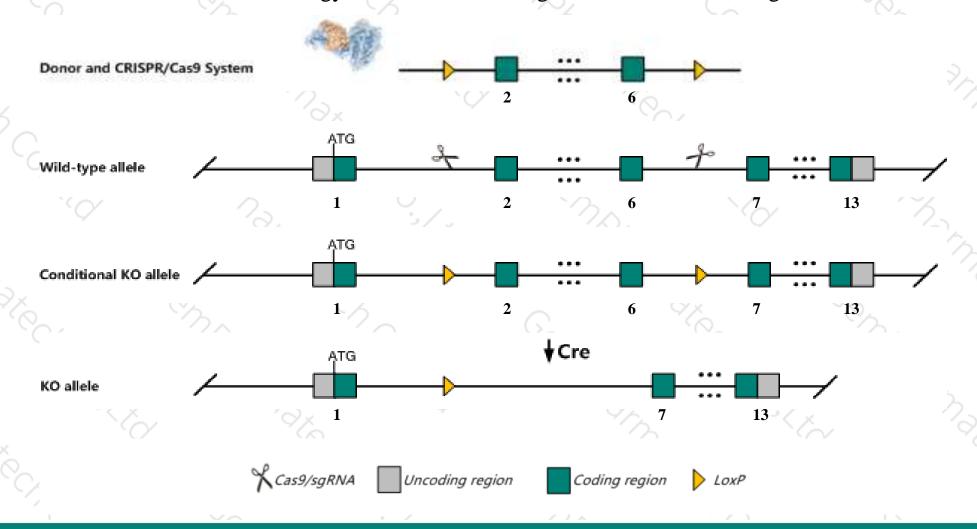
Project type Cas9-CKO

Strain background C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Plekhh3* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Plekhh3* gene has 6 transcripts. According to the structure of *Plekhh3* gene, exon2-exon6 of *Plekhh3*-201(ENSMUST00000043397.13) transcript is recommended as the knockout region. The region contains 607bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Plekhh3* 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



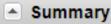
- ➤ The KO region is close to *Cntnap1* gene.Knockout the region may affect the function of *Cntnap1* gene.
- ➤ The *Plekhh3* gene is located on the Chr11. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on 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)



Plekhh3 pleckstrin homology domain containing, family H (with MyTH4 domain) member 3 [Mus musculus (house mouse)]

Gene ID: 217198, updated on 25-Sep-2020





Official Symbol Plekhh3 provided by MGI

Official Full Name pleckstrin homology domain containing, family H (with MyTH4 domain) member 3 provided by MGI

Primary source MGI:MGI:2384950

See related Ensembl: ENSMUSG00000035172

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 BC020025

Expression Broad expression in adrenal adult (RPKM 28.6), ovary adult (RPKM 23.4) and 23 other tissues See more

Orthologs <u>human</u> <u>all</u>

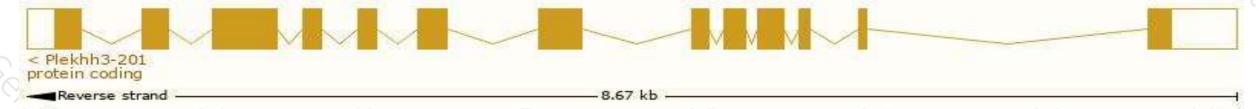
Transcript information (Ensembl)



The gene has 6 transcripts, all transcripts are shown below:

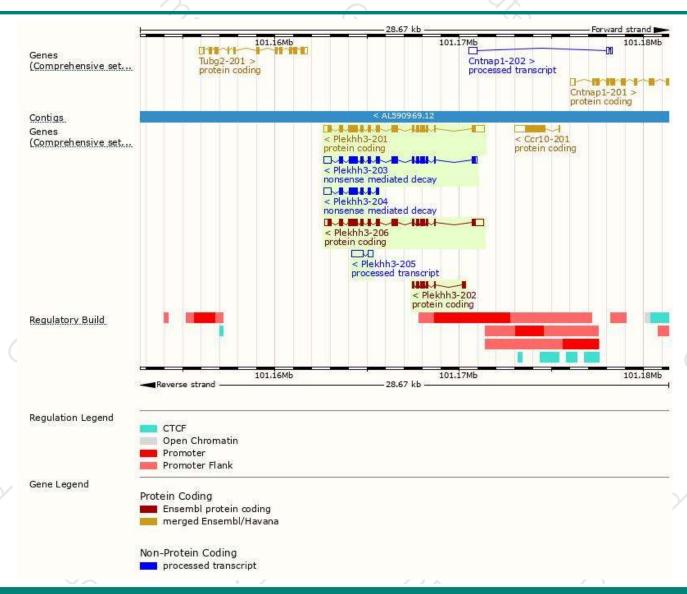
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Plekhh3-201	ENSMUST00000043397.13	3056	796aa	Protein coding	CCDS25453	Q8VCE9	TSL:1 GENCODE basic APPRIS P3	
Plekhh3-206	ENSMUST00000164474.7	3010	<u>793aa</u>	Protein coding	CCDS83899	Q8VCE9	TSL:1 GENCODE basic APPRIS ALT2	
Plekhh3-202	ENSMUST00000123864.1	785	247aa	Protein coding	9	A2A4L0	CDS 3' incomplete TSL:3	
Plekhh3-203	ENSMUST00000129895.7	2635	705aa	Nonsense mediated decay	=	Q8VCE9	TSL:5	
Plekhh3-204	ENSMUST00000139200.7	1410	320aa	Nonsense mediated decay	- 5	M0QWE8	CDS 5' incomplete TSL:1	
Plekhh3-205	ENSMUST00000156320.1	890	No protein	Processed transcript		S\$4	TSL:3	

The strategy is based on the design of *Plekhh3-201* transcript, the transcription is shown below:



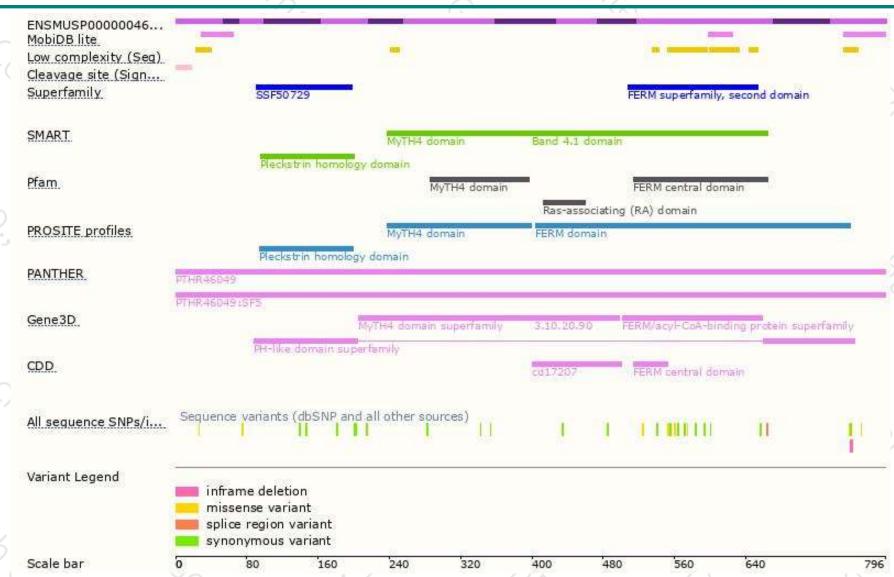
Genomic location distribution





Protein domain







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

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