

Phf14 Cas9-CKO Strategy

Designer: JiaYu

Reviewer: Xiaojing Li

Design Date: 2020-8-6

Project Overview



Project Name

Phf14

Project type

Cas9-CKO

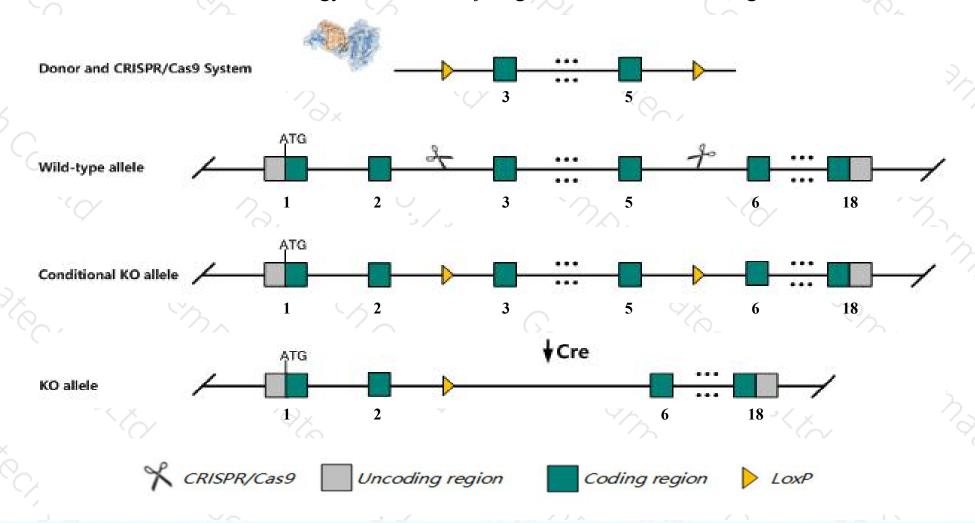
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Phf14* gene. The schematic diagram is as follows:



Technical routes



- The *Phf14* gene has 11 transcripts. According to the structure of *Phf14* gene, exon3-exon5 of *Phf14-203*(ENSMUST00000115511.8) transcript is recommended as the knockout region. The region contains 1072bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Phf14* 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



- > According to the existing MGI data, mice homozygous for a knock-out allele exhibit complete neonatal lethality due to respiratory failure, pulmonary wall hypertrophy, abnormal sternum ossification, and increased proliferation of bone marrow-derived mesenchymal cells and mouse embryonic fibroblasts.
- Transcript 205 CDS 3' incomplete the influences is unknown.
- > The *Phf14* gene is located on the Chr6. 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)



Phf14 PHD finger protein 14 [Mus musculus (house mouse)]

Gene ID: 75725, updated on 13-Mar-2020

Summary

☆ ?

Official Symbol Phf14 provided by MGI

Official Full Name PHD finger protein 14 provided by MGI

Primary source MGI:MGI:1923539

See related Ensembl: ENSMUSG00000029629

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 1110001C23Rik, 4932409F11Rik, 5730446A07Rik, AA623952, AV297001, mKIAA0783

Expression Broad expression in CNS E11.5 (RPKM 8.4), CNS E14 (RPKM 6.2) and 19 other tissuesSee more

Orthologs <u>human all</u>

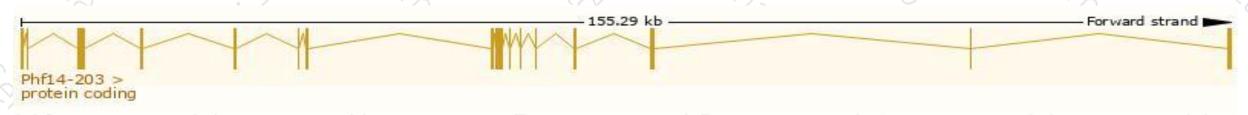
Transcript information (Ensembl)



The gene has 11 transcripts, all transcripts are shown below:

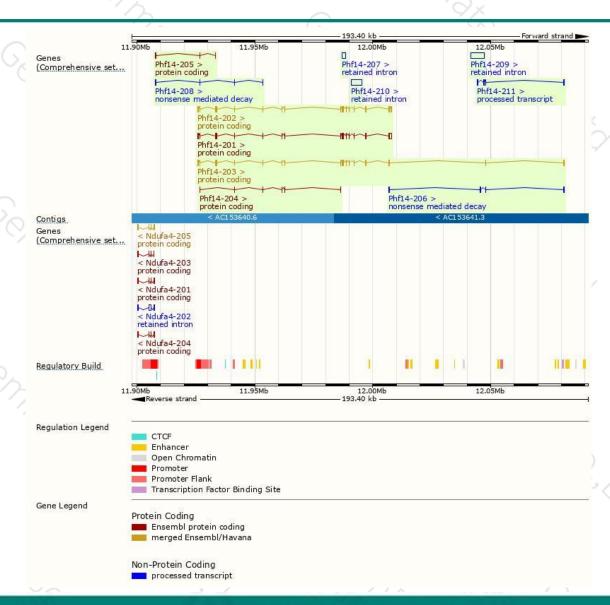
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Phf14-202	ENSMUST00000115510.7	3353	881aa	Protein coding	CCDS39428	G5E8S0	TSL:1 GENCODE basic
Phf14-203	ENSMUST00000115511.8	3336	<u>941aa</u>	Protein coding	CCDS51720	Q9D4H9	TSL:5 GENCODE basic APPRIS P
Phf14-201	ENSMUST00000090632.10	3985	<u>878aa</u>	Protein coding	828	Q9D4H9	TSL:1 GENCODE basic
Phf14-204	ENSMUST00000133776.1	727	243aa	Protein coding	-	F6TN92	CDS 5' and 3' incomplete TSL:3
Phf14-205	ENSMUST00000155037.3	478	<u>90aa</u>	Protein coding	-	A0A0N4SV73	CDS 3' incomplete TSL:2
Phf14-206	ENSMUST00000203045.1	566	<u>69aa</u>	Nonsense mediated decay	070	A0A0N4SWI1	CDS 5' incomplete TSL:3
Phf14-208	ENSMUST00000203459.2	483	<u>42aa</u>	Nonsense mediated decay	19 4 .	A0A0N4SW36	TSL:3
Phf14-211	ENSMUST00000214543.1	462	No protein	Processed transcript	12	(20	TSL:2
Phf14-209	ENSMUST00000204150.1	5905	No protein	Retained intron	ARM .	(30)	TSL:NA
Phf14-210	ENSMUST00000204565.1	4438	No protein	Retained intron	9±	940	TSL:NA
Phf14-207	ENSMUST00000203222.1	1592	No protein	Retained intron	825	828	TSL:NA
					1		Art V

The strategy is based on the design of *Phf14-203* transcript, the transcription is shown below:



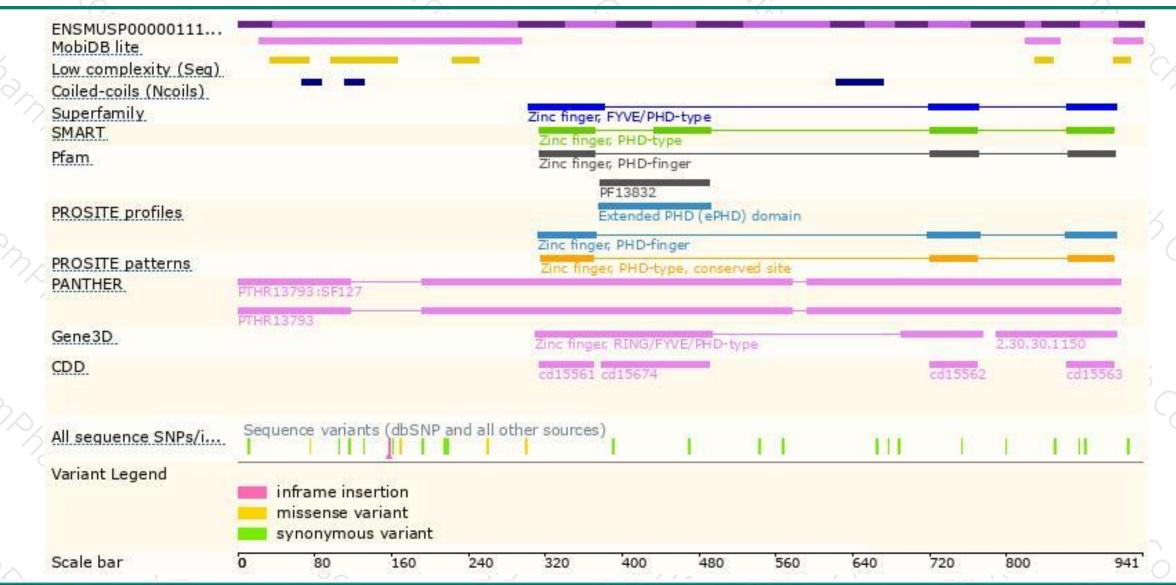
Genomic location distribution





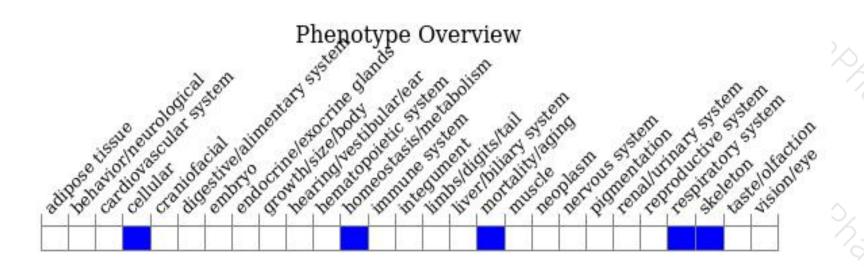
Protein domain





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 a knock-out allele exhibit complete neonatal lethality due to respiratory failure, pulmonary wall hypertrophy, abnormal sternum ossification, and increased proliferation of bone marrow-derived mesenchymal cells and mouse embryonic fibroblasts.



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





