

Ppih Cas9-CKO Strategy

Designer:

Reviewer:

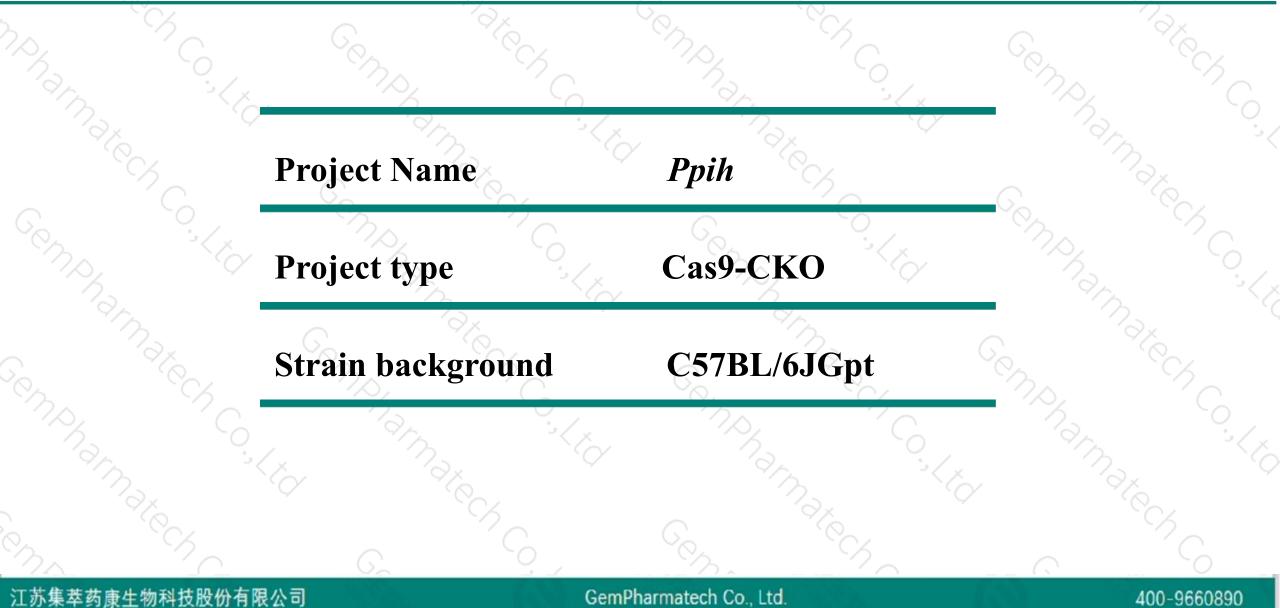
Design Date:

Xueting Zhang Yanhua Shen

2020-4-15

Project Overview

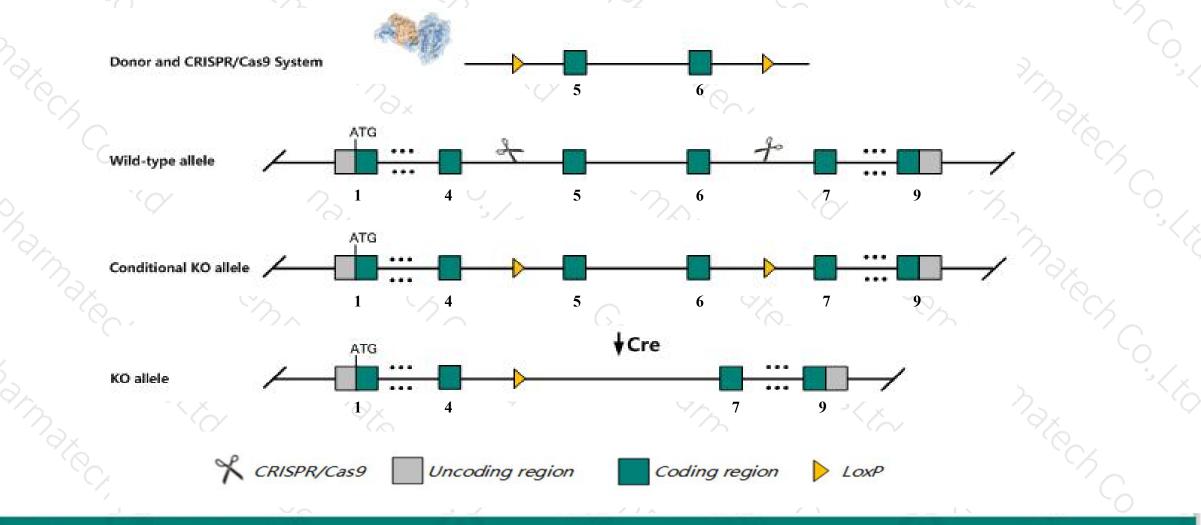




Conditional Knockout strategy



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



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The *Ppih* gene has 6 transcripts. According to the structure of *Ppih* gene, exon5-exon6 of *Ppih-202* (ENSMUST00000106317.1) transcript is recommended as the knockout region. The region contains 136bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Ppih* 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.



- > The N-terminal of *Ppih* gene will remain several amino acids ,it may remain the partial function of *Ppih* gene.
- The *Ppih* gene is located on the Chr4. 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)



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Ppih peptidyl prolyl isomerase H [Mus musculus (house mouse)]

Gene ID: 66101, updated on 13-Mar-2020

Summary

Official SymbolPpih provided by MGIOfficial Full Namepeptidyl prolyl isomerase H provided by MGIPrimary sourceMGI:MGI:106499See relatedEnsembl:ENSMUSG0000060288Gene typeprotein codingRefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
Muroidea; Murinae; Mus; MusAlso knownas110001J08Rik, 2010111B15Rik, 4833408F11Rik, Al464484, CYP-20, CYPH, D4Wsu43eOrthologhuman all

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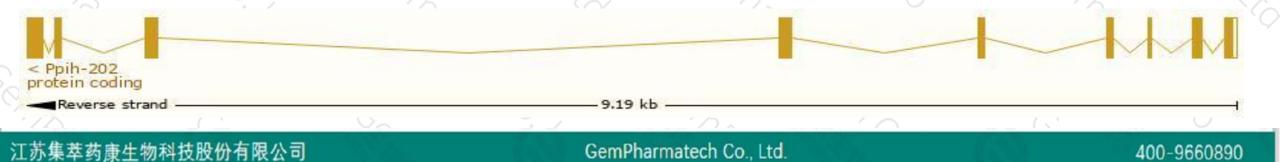
Transcript information (Ensembl)



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

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|----------|-----------------------|------|--------------|----------------|-----------|---------------|---|
| Ppih-205 | ENSMUST00000106321.8 | 1288 | <u>177aa</u> | Protein coding | CCDS18580 | Q4G0C5 Q9D868 | TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1 |
| Ppih-203 | ENSMUST00000106318.7 | 800 | <u>177aa</u> | Protein coding | CCDS18580 | Q4G0C5 Q9D868 | TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1 |
| Ppih-201 | ENSMUST00000056458.13 | 757 | <u>177aa</u> | Protein coding | CCDS18580 | Q4G0C5 Q9D868 | TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1 |
| Ppih-202 | ENSMUST00000106317.1 | 613 | <u>188aa</u> | Protein coding | CCDS51288 | <u>Q9D868</u> | TSL:1 GENCODE basic |
| Ppih-206 | ENSMUST00000147077.7 | 725 | <u>158aa</u> | Protein coding | 151 | A2BGI8 | CDS 5' incomplete TSL:5 |
| Ppih-204 | ENSMUST00000106319.7 | 622 | <u>134aa</u> | Protein coding | 143 | A2BGI9 | TSL:3 GENCODE basic |

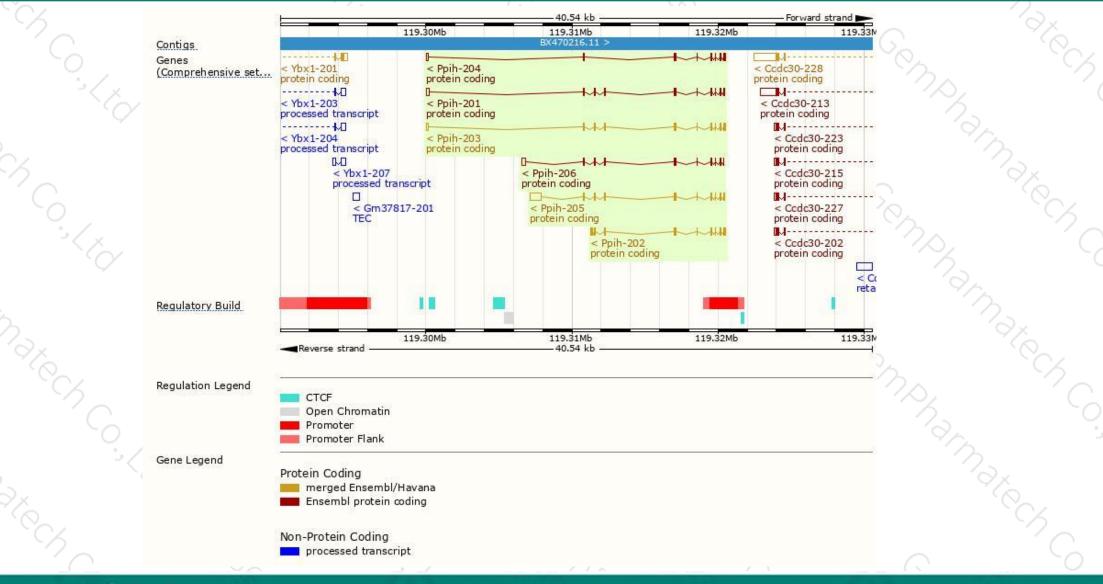
The strategy is based on the design of *Ppih-202* transcript, the transcription is shown below



Genomic location distribution



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Protein domain



| | ENSMUSP00000101 Superfamily | Cyclop hilin-like d | omain superfam | ily | | | | | | 6 | |
|---|--------------------------------|----------------------|------------------|-----------------|---------------------|-----------------|---------------|---------------|-----|--|-------------|
| 1 | Prints | | Cyclophilin-typ | e peptidyl-pro | olyl cis-trans ison | nerase domain | 3 | | | | ~O ., |
| | <u>Pfam</u> | Cyclop | hilin-type pepti | dyl-prolyl cis- | trans isomerase | domain | | | | | |
| | PROSITE profiles | Cyclophilin | -type peptidyl-p | rolyl cis-trans | isomerase doma | iin. | | | | | |
| | PROSITE patterns | | | Cycloph | ilin-type peptidyl | -prolyl cis-tra | ns isomerase, | conserved sit | | | |
| | PANTHER | PTHR11071 | | | | | | | | | |
| | | PTHR11071:SF443 | | | | | | | | | 9. / |
| | Gene3D | Oyclophilin-like dom | ain superfamily | | | | | | | | |
| | CDD | cd01926 | | | | | | | | | |
| | All sequence SNPs/i | Sequence variants | (dbSNP and a | II other sour | | н. | | | | | |
| | Variant Legend | missense var | | 1141111 | | | | | | | , ,, < , |
| | Scale bar | 0 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 | 188 | |
| | `Ч_С | G_ | 6 | _ | Gen a | 2 | °S _ | | 0 | ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ | |

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



