

Pi4ka Cas9-CKO Strategy

Designer:

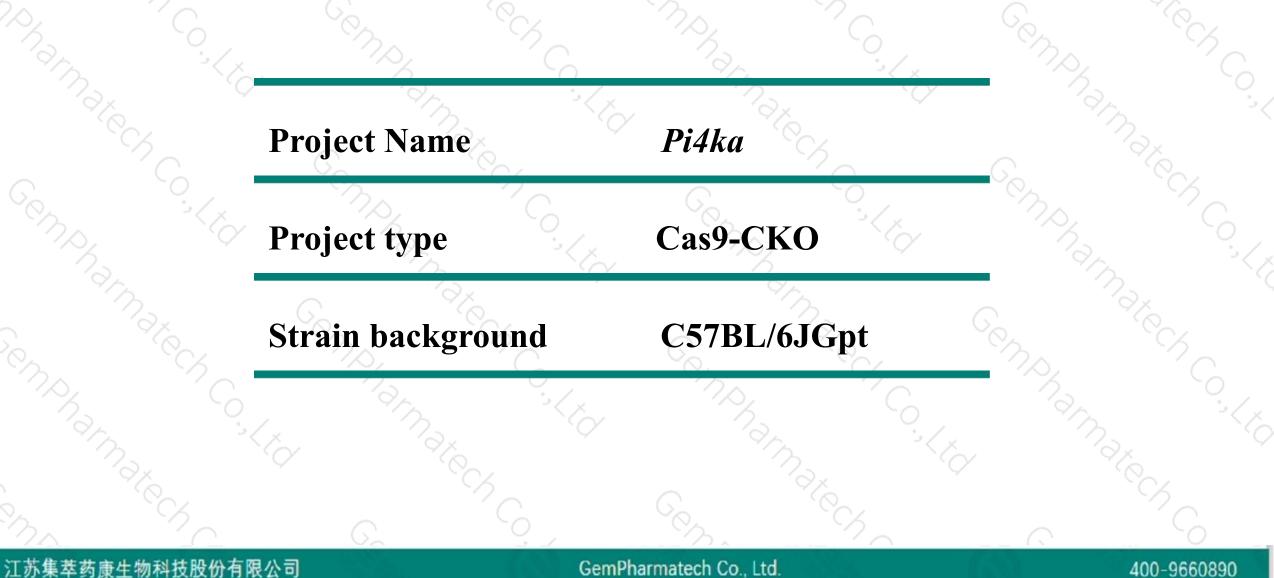
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Design Date:

2019-8-19

Project Overview





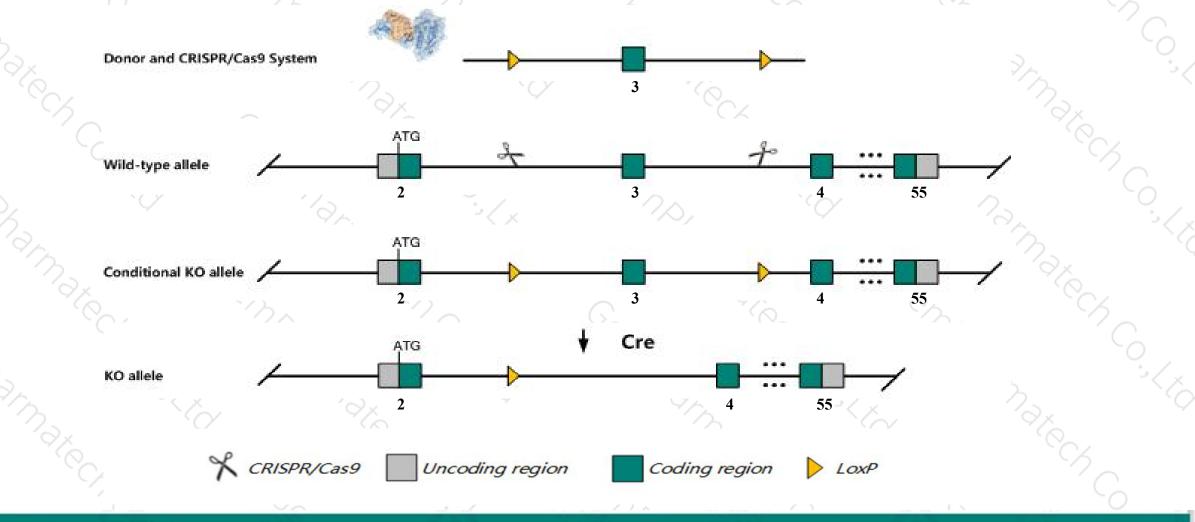
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Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Pi4ka* gene. The schematic diagram is as follows:



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The *Pi4ka* gene has 11 transcripts. According to the structure of *Pi4ka* gene, exon3 of *Pi4ka-209* (ENSMUST00000232232.1) transcript is recommended as the knockout region. The region contains 94bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Pi4ka* 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.



- According to the existing MGI data, Mice homozygous for a targeted knock-out or knock-in conditionally activated exhibit premature death associated with degeneration of mucosal cells in the stomach and intestines. Mice homozygous for a knock-out allele exhibit early embryonic lethality.
- > Transcript *Pi4ka-210* may not be affected.
- The *Pi4ka* gene is located on the Chr16. 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)



\$?

Pi4ka phosphatidylinositol 4-kinase alpha [Mus musculus (house mouse)]

Gene ID: 224020, updated on 19-Feb-2019

Summary

Official Symbol	Pi4ka provided by MGI
Official Full Name	phosphatidylinositol 4-kinase alpha provided by MGI
Primary source	MGI:MGI:2448506
See related	Ensembl:ENSMUSG00000041720
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	Pik4ca
Expression	Ubiquitous expression in cortex adult (RPKM 25.4), frontal lobe adult (RPKM 25.2) and 28 other tissues See more
Orthologs	human all

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Transcript information (Ensembl)



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

Name 🍦	Transcript ID	bp 🖕	Protein 🖕	Biotype 🖕	CCDS 🖕	UniProt 🝦	Flags
Pi4ka-209	ENSMUST00000232232.1	6630	<u>2044aa</u>	Protein coding	CCDS37271@	<u>A0A140T8l9</u> &	GENCODE basic APPRIS P1
Pi4ka-201	ENSMUST0000036161.11	6431	<u>2044aa</u>	Protein coding	CCDS37271	<u>A0A140T8I9</u> &	TSL:1 GENCODE basic APPRIS P1
Pi4ka-210	ENSMUST00000232404.1	958	<u>319aa</u>	Protein coding	(1)	<u>A0A338P7D3</u> 译	CDS 5' and 3' incomplete
Pi4ka-204	ENSMUST00000231651.1	888	<u>74aa</u>	Nonsense mediated decay	-	<u>A0A338P6F9</u> &	CDS 5' incomplete
Pi4ka-205	ENSMUST00000231683.1	803	<u>153aa</u>	Nonsense mediated decay		<u>A0A338P6S0</u> &	CDS 5' incomplete
Pi4ka-202	ENSMUST00000231419.1	3855	No protein	Retained intron	1.21	<u></u>	127
Pi4ka-206	ENSMUST00000231914.1	1504	No protein	Retained intron	199	<u>4</u>	120
Pi4ka-211	ENSMUST00000232631.1	979	No protein	Retained intron	1.52	65	170
Pi4ka-203	ENSMUST00000231529.1	932	No protein	Retained intron		-	
Pi4ka-207	ENSMUST00000231917.1	669	No protein	Retained intron		-	-
Pi4ka-208	ENSMUST00000231961.1	608	No protein	Retained intron		27	1.00

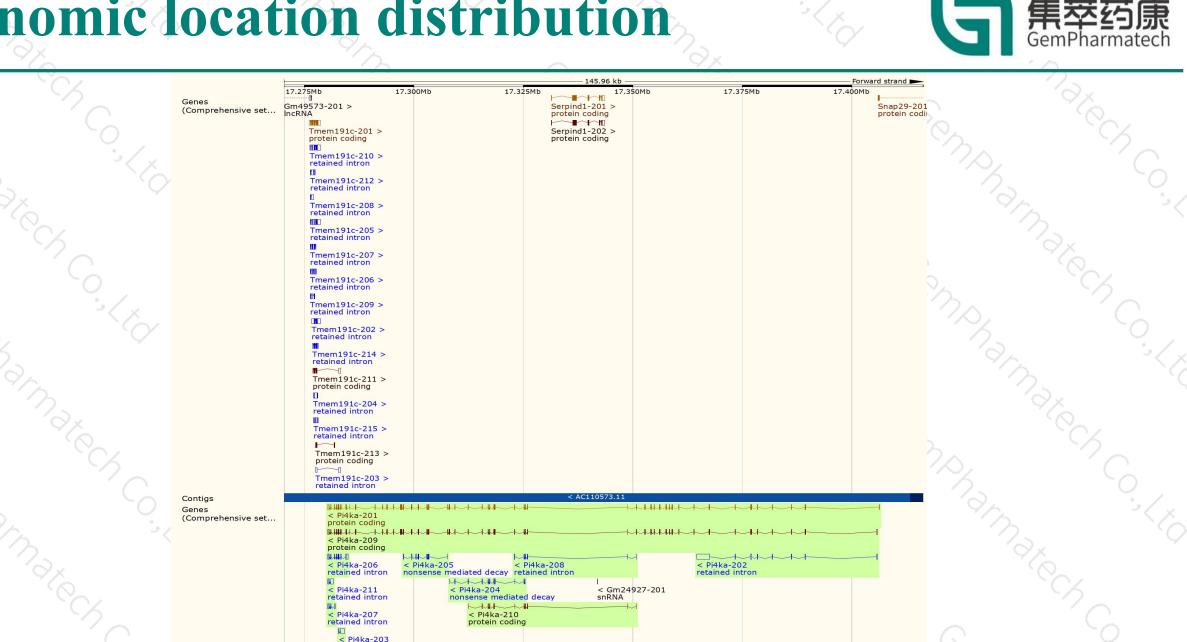
The strategy is based on the design of *Pi4ka-209* transcript, The transcription is shown below

< Pi4ka-209 protein coding

Reverse strand

— 125.54 kb —

Genomic location distribution



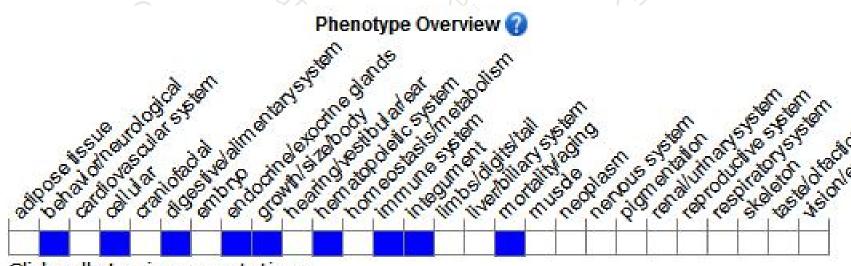
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Protein domain





Mouse phenotype description(MGI)



Click cells to view annotations.

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 targeted knock-out or knock-in conditionally activated exhibit premature death associated with degeneration of mucosal cells in the stomach and intestines. Mice homozygous for a knock-out allele exhibit early embryonic lethality.

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



