

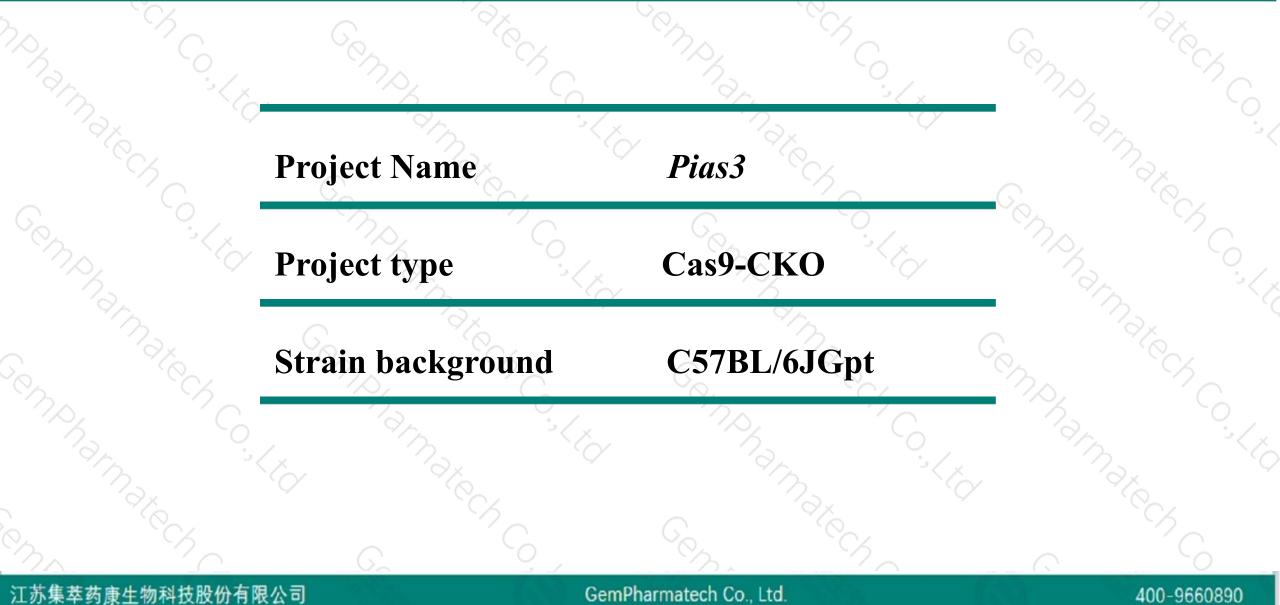
Pias3 Cas9-CKO Strategy

Designer: Reviewer: Design Date: Yang Zeng

2019-12-23

Project Overview

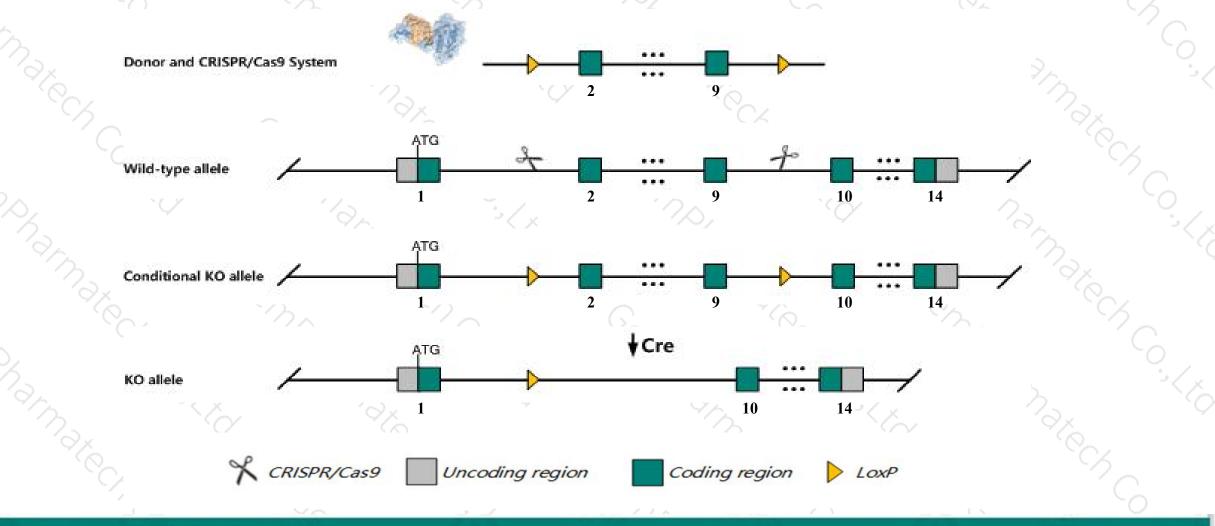




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Pias3 gene. The schematic diagram is as follows:



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400-9660890



The *Pias3* gene has 10 transcripts. According to the structure of *Pias3* gene, exon2-exon9 of *Pias3-201* (ENSMUST00000064900.15) transcript is recommended as the knockout region. The region contains 1121bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Pias3* 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, Double KO mice display a retinal phenotype reduced M-cone response at P21 and reduced S-cone and rod responses from 7 months.
- ≻Transcript *Pias3*-205 may be unaffected.
- The *Pias3* gene is located on the Chr3. 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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Pias3 protein inhibitor of activated STAT 3 [Mus musculus (house mouse)]

Gene ID: 229615, updated on 12-Aug-2019

Summary

- Official Symbol Pias3 provided by MGI Official Full Name protein inhibitor of ac
 - icial Full Name protein inhibitor of activated STAT 3 provided by MGI
 - Primary source MGI:MGI:1913126
 - See related Ensembl:ENSMUSG00000028101
 - 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 Pias3l
 - Expression Ubiquitous expression in limb E14.5 (RPKM 15.6), ovary adult (RPKM 8.7) and 28 other tissues See more
 - Orthologs human all

96666468 🕨	Chromosome 3 - NC_	000069.6	[96791155]>	0	and the	2
Rp121-ps11 + Ankrd35	Pias3 Gm22581 Nudt17 Rnf115 Polr3c		•			
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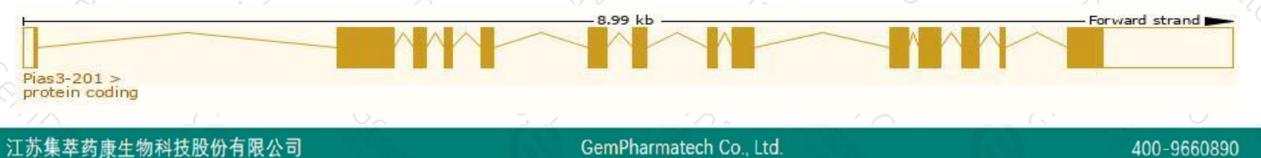
Transcript information (Ensembl)



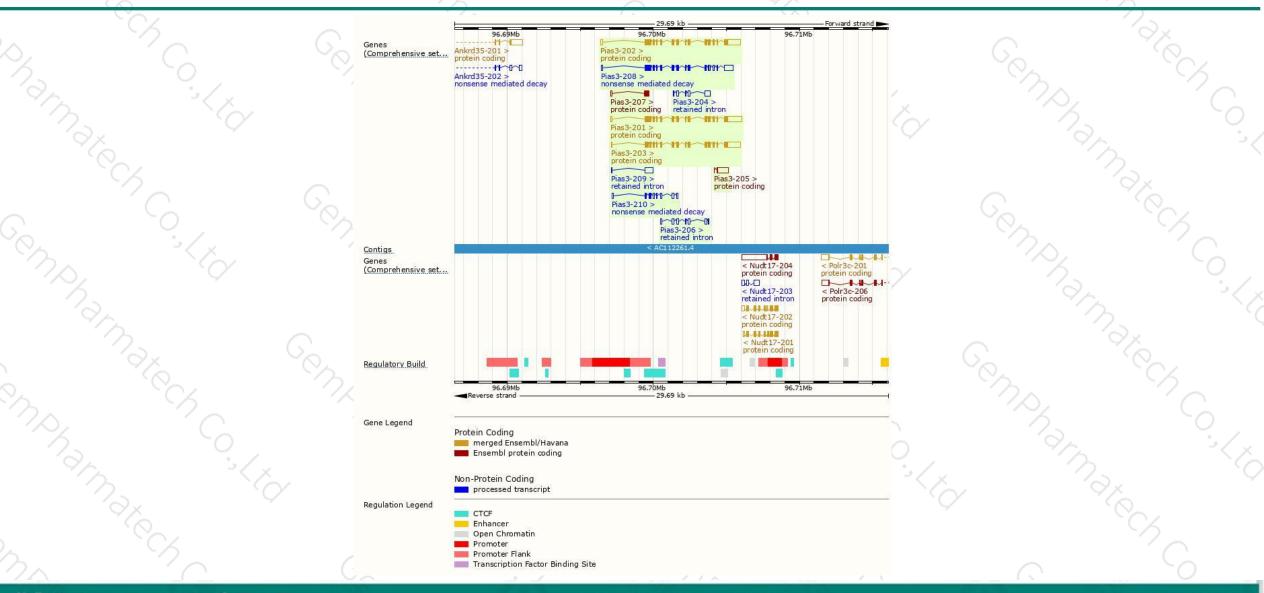
The gene has 10 transcripts, all transcripts are shown below:

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Name 🖕	Transcript ID 👙	bp 🖕	Protein 🖕	Translation ID	Biotype 🍦	CCDS 🍦	UniProt 🖕	Flags 🍦
Pias3-201	ENSMUST0000064900.15	2931	<u>628aa</u>	ENSMUSP0000069259.9	Protein coding	<u>CCDS51007</u> 교	<u>054714</u> @	TSL:1 GENCODE basic APPRIS ALT1
vias3-202	ENSMUST00000107076.9	2858	<u>619aa</u>	ENSMUSP00000102691.3	Protein coding	<u>CCDS38559</u> 률	<u>054714</u> @	TSL:1 GENCODE basic APPRIS P3
ias3-203	ENSMUST00000107077.3	2695	<u>593aa</u>	ENSMUSP00000102692.3	Protein coding	<u>CCDS17645</u> &	<u>054714</u> @	TSL:1 GENCODE basic
ias3-205	ENSMUST00000162156.2	812	<u>27aa</u>	ENSMUSP00000135843.1	Protein coding		H3BLM4@	CDS 5' incomplete TSL:3
as3-207	ENSMUST00000162778.2	439	<u>90aa</u>	ENSMUSP00000125377.2	Protein coding		E0CZH5@	CDS 3' incomplete TSL:3
ias3-208	ENSMUST00000162934.7	2281	<u>430aa</u>	ENSMUSP00000125747.1	Nonsense mediated decay		<u>E0CZ31</u> 团	TSL:1
ias3- <mark>21</mark> 0	ENSMUST00000176302.7	744	<u>52aa</u>	ENSMUSP00000134835.1	Nonsense mediated decay		<u>H3BJ41</u> @	TSL:5
ias3-206	ENSMUST00000162707.8	795	No protein	ā	Retained intron		8353	TSL:5
ias3-204	ENSMUST00000161296.1	770	No protein	ā.	Retained intron		8373	TSL:3
ias3-209	ENSMUST00000176288.1	627	No protein	ā. 5	Retained intron	ā	1953	TSL:2

The strategy is based on the design of *Pias3-201* transcript, The transcription is shown below



Genomic location distribution



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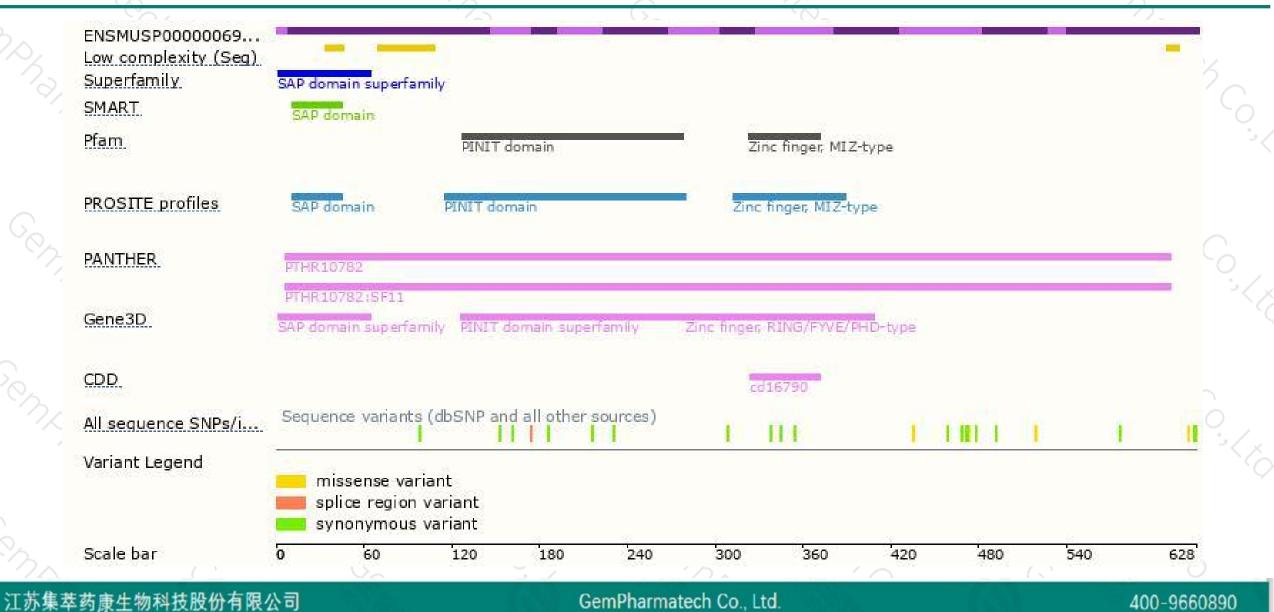
GemPharmatech Co., Ltd.

400-9660890

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Protein domain







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



