

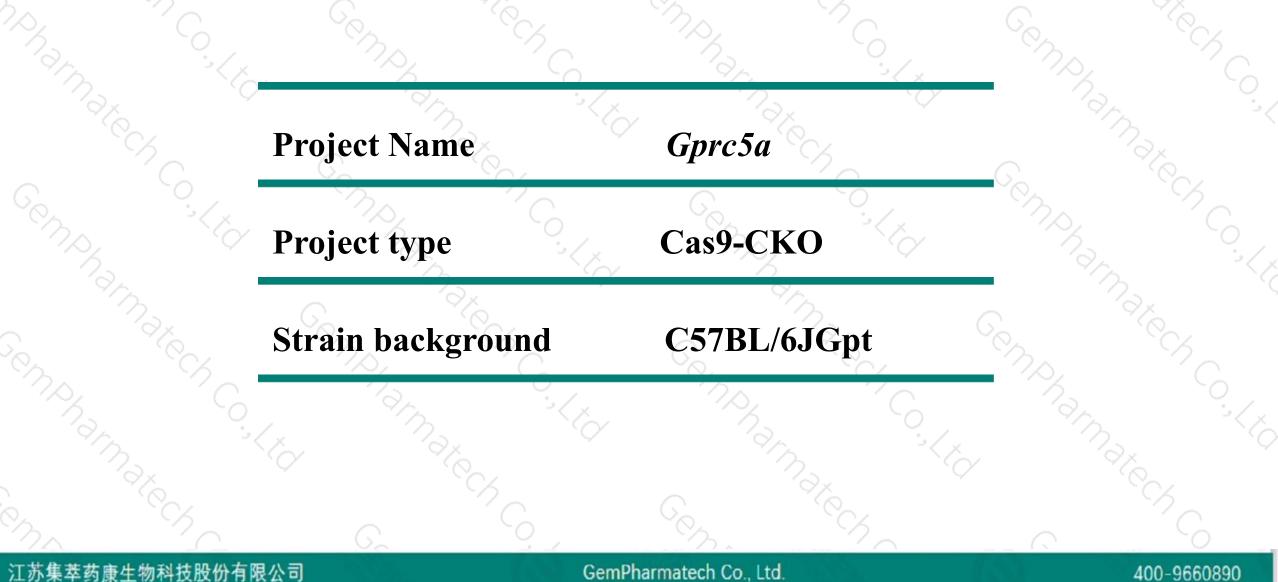
Gprc5a Cas9-CKO Strategy Annahamaters Co-1th

05

Cemphamatech, Designer: Yanhua Shen Design Date: 2019-08-06

Project Overview





江苏集萃药康生物科技股份有限公司

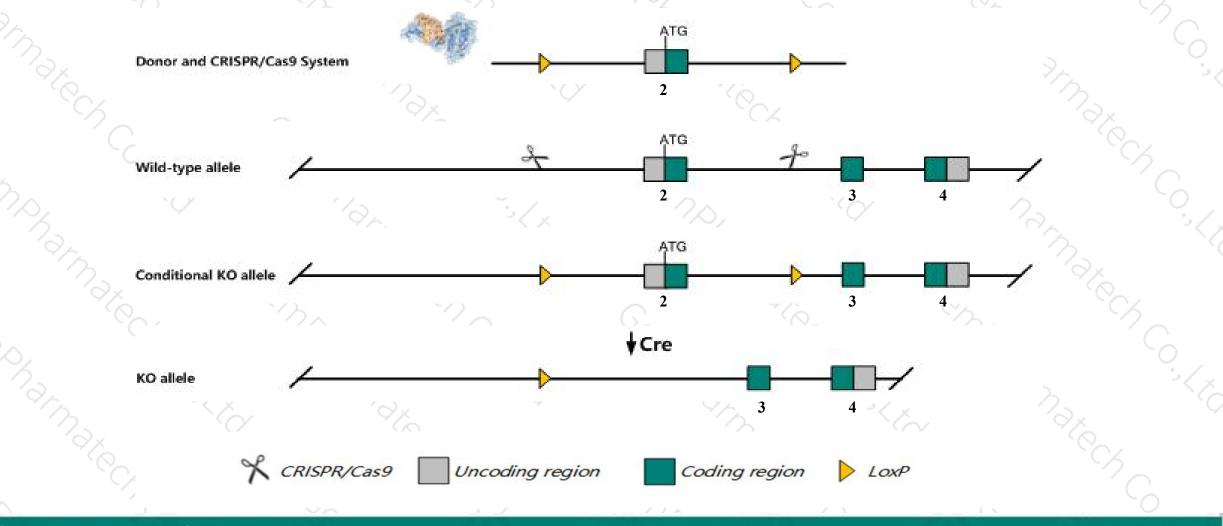
GemPharmatech Co., Ltd.

Conditional Knockout strategy



400-9660890

This model will use CRISPR/Cas9 technology to edit the *Gprc5a* gene. The schematic diagram is as follows:



江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.



The Gprc5a gene has 1 transcript. According to the structure of Gprc5a gene, exon2 of Gprc5a-201 (ENSMUST00000050104.7) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Gprc5a* 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 one knock-out allele are healthy and exhibit normal lung development and a normal life span. Mice homozygous for a different knock-out allele show a significantly increased incidence of acidophilic macrophage pneumonia and spontaneouslung tumors at 1-2 years of age.
- The Gprc5a 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)



☆ ?

Gprc5a G protein-coupled receptor, family C, group 5, member A [Mus musculus (house mouse)]

Gene ID: 232431, updated on 31-Jan-2019

Summary

Official Symbol	Gprc5a provided by MGI
Official Full Name	G protein-coupled receptor, family C, group 5, member A provided by MGI
Primary source	MGI:MGI:1891250
See related	Ensembl:ENSMUSG0000046733
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	Rai3, Raig1
Expression	Biased expression in lung adult (RPKM 130.8), colon adult (RPKM 45.8) and 4 other tissues See more
Orthologs	human all



The gene has 1 transcript, and the transcript is shown below:

	di kasi			prita				No.
	Flags	UniProt	CCDS	Biotype	Protein	bp	Transcript ID	Name
PPRIS P1	TSL:1 GENCODE basic APPRIS	G5E8C3	CCDS20644	Protein coding	<u>356aa</u>	2083	ENSMUST0000050104.7	prc5a-201
Nu.				<u> </u>	5	\sim	· · · · · · · · · · · · · · · · · · ·	No.
		>						
					`Ч		C C	
		· . /					-36x (2)	
	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~				<u> </u>		$\sim q \qquad \gamma$	
	19 Mar		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~					
			19 m					- Ar
							x Go	10
					$\gamma_{\alpha}$		$\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}}}}}}}}}}$	
6								
	12	own below	scription is sh	script,The trans	-201 trans	Sprc5a	is based on the design of (	strategy i
d at so at end			0.				Y. /	
	Conpland Conpland Forward	own below	scription is sh	script,The trans	- <i>201</i> trans	Sprc5a	is based on the design of C	The strategy i

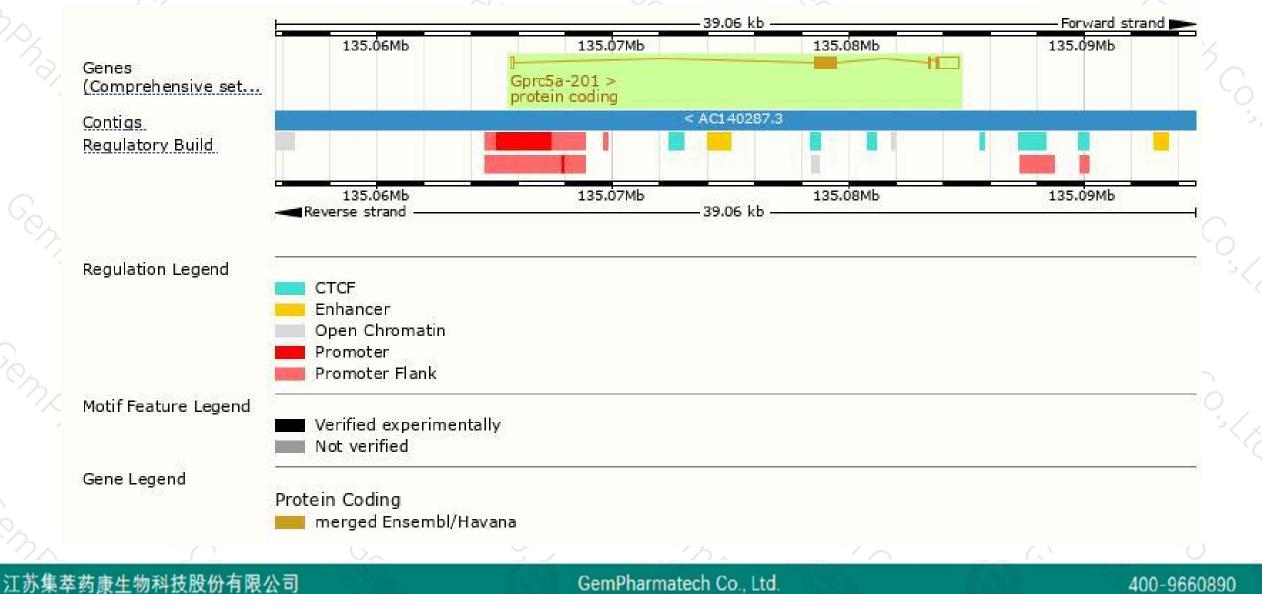
#### 江苏集萃药康生物科技股份有限公司

#### GemPharmatech Co., Ltd.

#### 400-9660890

## **Genomic location distribution**





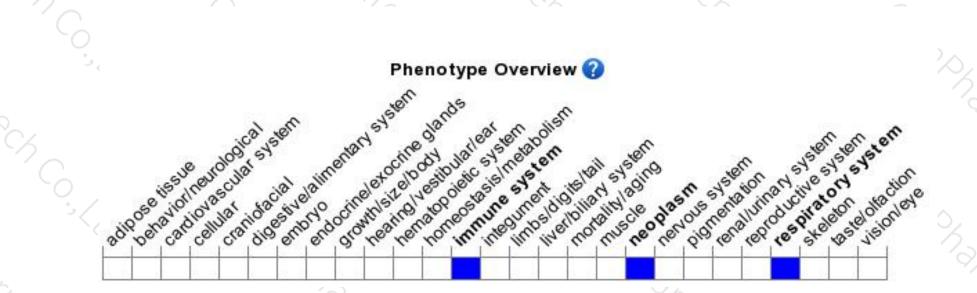
## **Protein domain**





## 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 one knock-out allele are healthy and exhibit normal lung development and a normal life span. Mice homozygous for a different knock-out allele show a significantly increased incidence of acidophilic macrophage pneumonia and spontaneouslung tumors at 1-2 years of age.

#### 江苏集萃药康生物科技股份有限公司

#### GemPharmatech Co., Ltd.

#### 400-9660890



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



