

# Cib4 Cas9-CKO Strategy

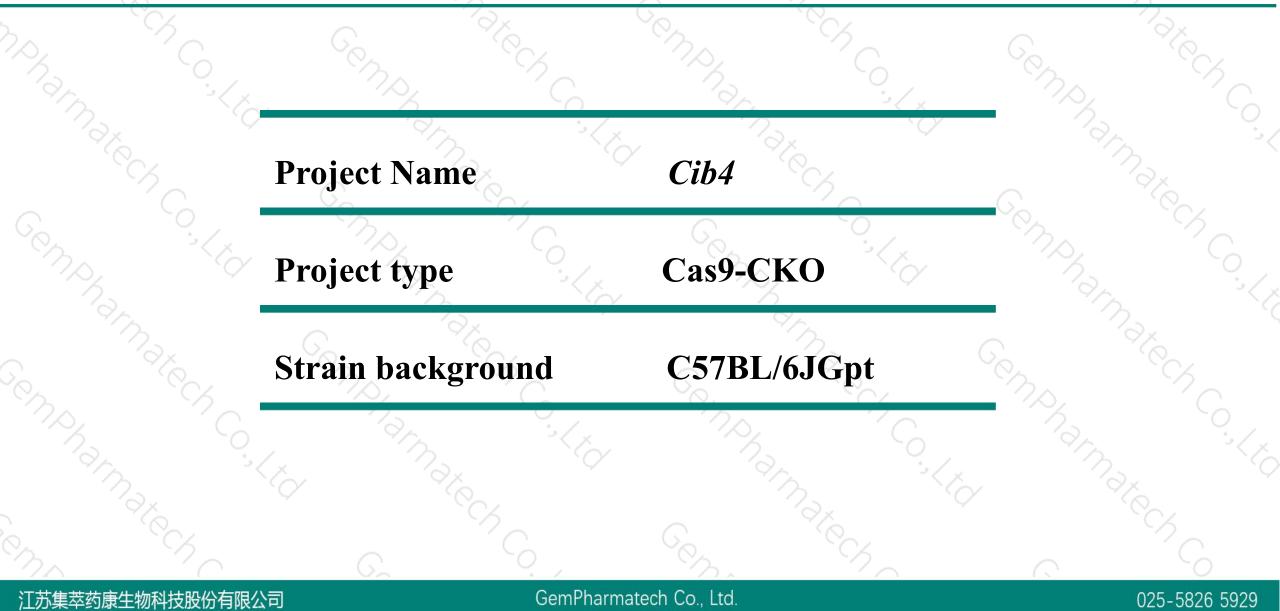
**Designer: Daohua Xu** 

**Reviewer: Xueting Zhang** 

Design Date: 2020-11-25

# **Project Overview**



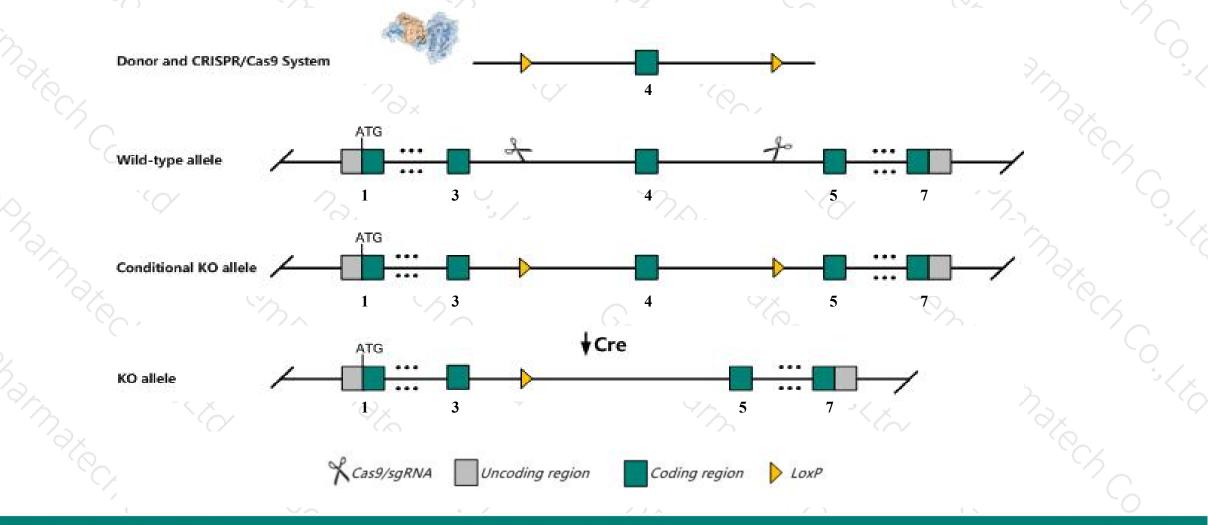


## **Conditional Knockout strategy**



025-5826 5929

This model will use CRISPR/Cas9 technology to edit the *Cib4* gene. The schematic diagram is as follows:



江苏集萃药康生物科技股份有限公司



025-5826 5929

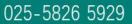
➤ The *Cib4* gene has 1 transcript. According to the structure of *Cib4* gene, exon4 of *Cib4-201*(ENSMUST00000065486.2) transcript is recommended as the knockout region. The region contains 142bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Cib4* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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 *Cib4* gene is located on the Chr5. 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)**



\$ ?

025-5826 5929

#### Cib4 calcium and integrin binding family member 4 [Mus musculus (house mouse)]

Gene ID: 73259, updated on 13-Mar-2020

#### Summary

Official Symbol	Cib4 provided by MGI
	calcium and integrin binding family member 4 provided by MGI
Primary source	MGI:MGI:1920509
See related	Ensembl:ENSMUSG0000053194
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
	Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1700041E20Rik
Expression	Restricted expression toward testis adult (RPKM 41.5)See more
Orthologs	human all



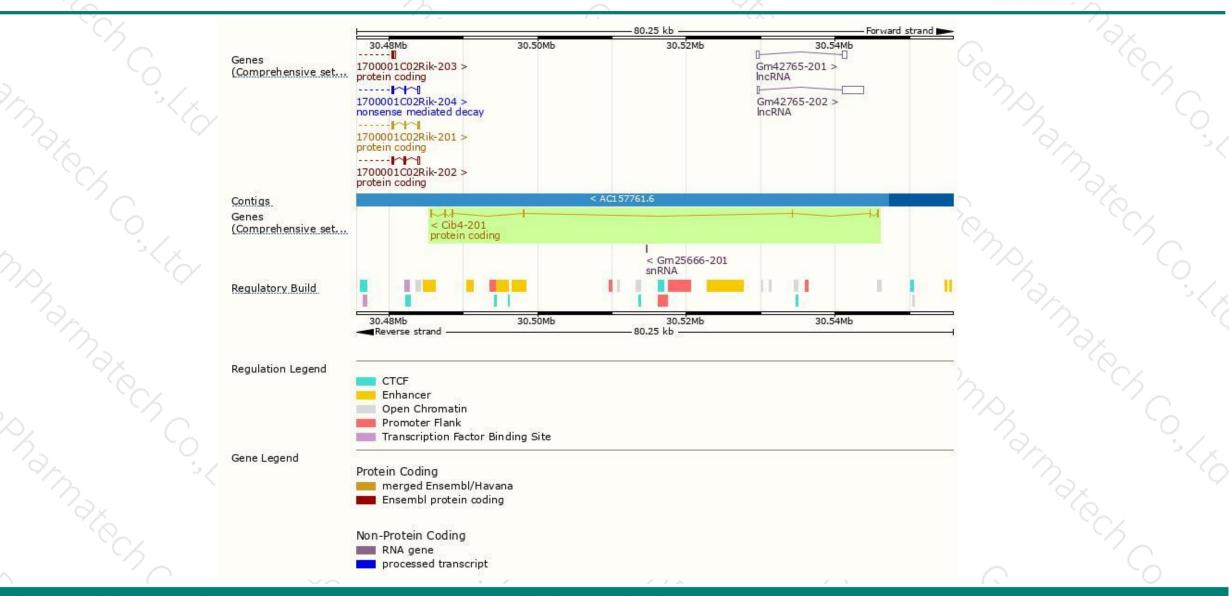
025-5826 5929

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

| || 苏集萃药康生物科技股份有限公司

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
ib4-201	ENSMUST0000065486.2	769	<u>185aa</u>	Protein coding	CCDS51453	Q9D9N5	TSL:1 GENCODE basic APPRIS P1
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	$\mathcal{X}_{\mathcal{C}}$		ALC CA			5	
	in the internet	3	$\sim \gamma$	0	Con a	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Charles of C
- Aller					- Charp		
	are ons						Construction
e strategy	y is based on the design of	f Cib4	-201 trans	cript,the transcr	iption is show	n below:	And C
ib4-201 tein coding							
Reverse stra				60.25 kb			

### **Genomic location distribution**



集萃药康 GemPharmatech

025-5826 5929

江苏集萃药康生物科技股份有限公司

## **Protein domain**



20	PROSITE profiles PROSITE patterns						EF-hand dor	and the second se	ium-binding sit	e	
1	PANTHER	PTHR45791: PTHR45791	SF4								
	Gene3D CDD		238,10				EF-hand (	domain			
	All sequence SNPs/i	Sequence v	ariants (	dbSNP and a	ll other sourc	es)		ġ			2
J.											
	Variant Legend	synon	/mous v	ariant							



If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



