

# Rab3d Cas9-CKO Strategy

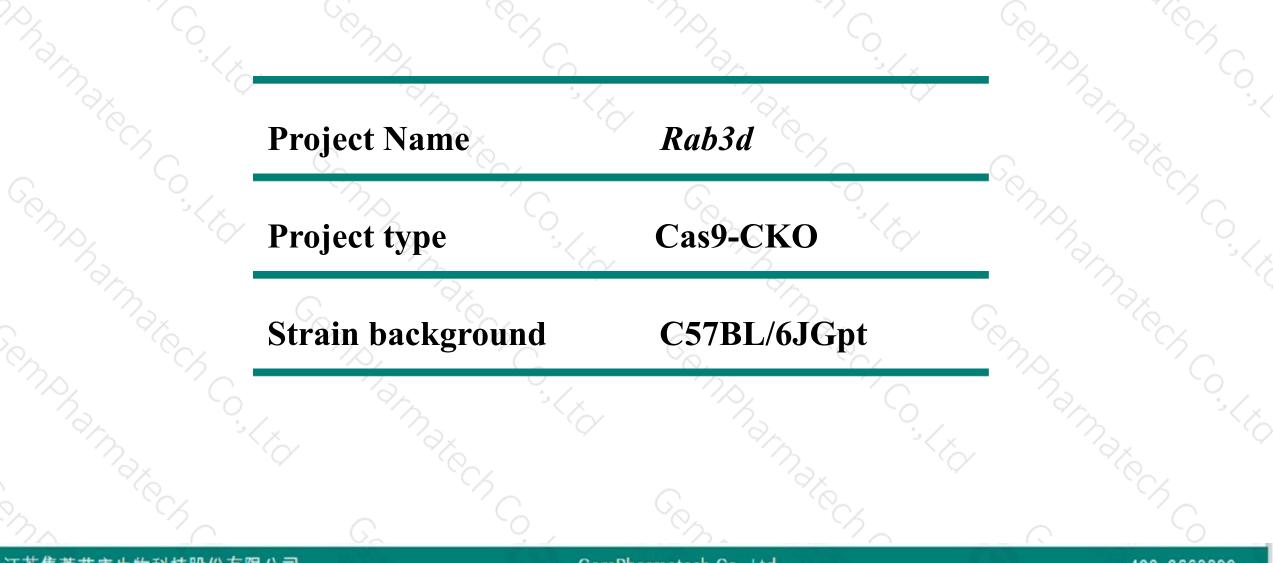
Designer: Reviewer:

**Design Date:** 

Daohua Xu Huimin Su 2020-1-22

# **Project Overview**





江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

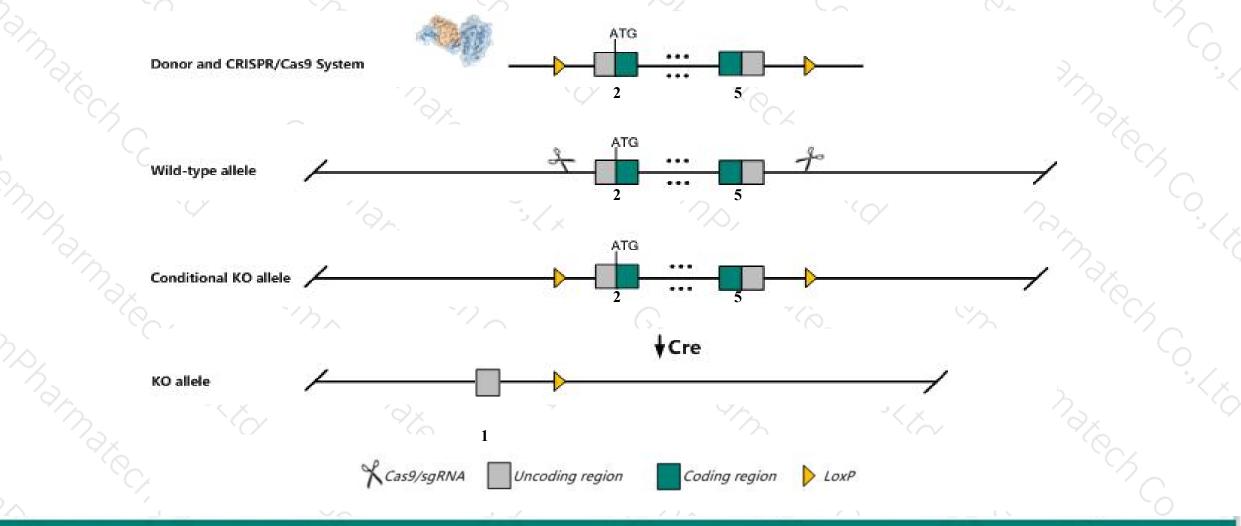
400-9660890

### **Conditional Knockout strategy**



400-9660890

This model will use CRISPR/Cas9 technology to edit the *Rab3d* 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 Rab3d gene has 5 transcripts. According to the structure of Rab3d gene, exon2-exon5 of Rab3d-203 (ENSMUST00000122211.7) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Rab3d* 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 disruptions in this gene show no obvious phenotypic changes. Secretory granules in mast cells and some exocrine glands are double in volume however.
  - The Rab3d gene is located on the Chr9. 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)**



\$ ?

#### Rab3d RAB3D, member RAS oncogene family [Mus musculus (house mouse)]

Gene ID: 19340, updated on 24-Mar-2019

#### Summary

Official Symbol	Rab3d provided by MGI
Official Full Name	RAB3D, member RAS oncogene family provided by MGI
<b>Primary source</b>	MGI:MGI:97844
See related	Ensembl:ENSMUSG0000019066
Gene type	protein coding
<b>RefSeq status</b>	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	C130057E11Rik
Expression	Broad expression in colon adult (RPKM 51.1), stomach adult (RPKM 49.7) and 23 other tissues See more
Orthologs	human all

#### 江苏集萃药康生物科技股份有限公司

#### GemPharmatech Co., Ltd.

#### 400-9660890

# **Transcript information (Ensembl)**



The gene has 5 transcripts, all transcripts are shown below:

Name Transcript ID   Rab3d-203 ENSMUST00000122211.7		bp	Protein	Biotype	CCDS	UniProt	Flags	
		3886	<u>219aa</u>	Protein coding	CCDS22912	P35276 Q543Q4	TSL:1 GENCODE basic APPRIS P1	
Rab3d-201	ENSMUST00000115351.9	3835	<u>219aa</u>	Protein coding	CCDS22912	P35276 Q543Q4	TSL:1 GENCODE basic APPRIS P1	
Rab3d-202	ENSMUST00000119055.7	694	<u>136aa</u>	Protein coding	19	D3YW33	TSL:3 GENCODE basic	
Rab3d-204	ENSMUST00000128442.1	545	<u>106aa</u>	Protein coding	20	D3YWL1	CDS 3' incomplete TSL:5	
Rab3d-205	d-205 ENSMUST00000154019.1 429		No protein	Retained intron		17	TSL:2	

The strategy is based on the design of Rab3d-203 transcript, The transcription is shown below

#### < Rab3d-203 protein coding

Reverse strand

江苏集萃药康生物科技股份有限公司

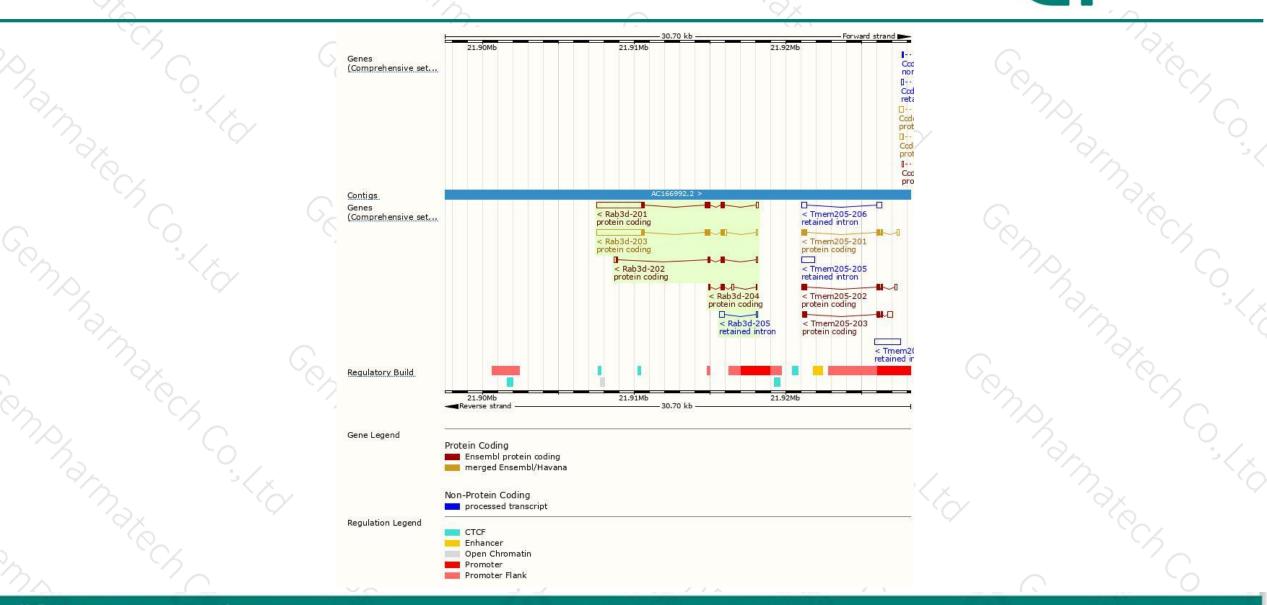
\_\_\_\_^

GemPharmatech Co., Ltd.

10.63 kb

400-9660890

### **Genomic location distribution**



江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

**集萃药康** GemPharmatech

# **Protein domain**

江苏



Scale bar	0	20	40	60	80	100	120	140	160	180	219	
	sy	issense va nonymous	variant	7620	150			100000	70.22	• 10 a 10 a	5	
Variant Legend	<u> </u>							- A - A -				
All sequence SNPs/i	Seque	nce variant:	s (dbSNP	and all oth	er sources)	11	T	TT.			1	
CDD.		Rab3										ý.
	3,40	.50.300										
Gene3D	Second as	R24073:SF3	30									
PANTHER	_	IR24073	88 Q.									
PROSITE profiles		PS51419										
Pfam.		1000	GTPase									
Prints		PR004	A CONTRACTOR OF				-					- C
234252570		SM	00176	-							-	· 2 ~
		SMOO	174									
		SM001	73									
SMART		SM001	75									
Superfamily		P-loop co	ntaining nu	ucleoside tri	phosphate hy	drolase						
TIGRFAM		Small G	TP-binding	protein do	main					_		~?
MobiDB lite Low complexity (Seg)												



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



