

# **Rbbp4** Cas9-CKO Strategy

Designer: Reviewer:

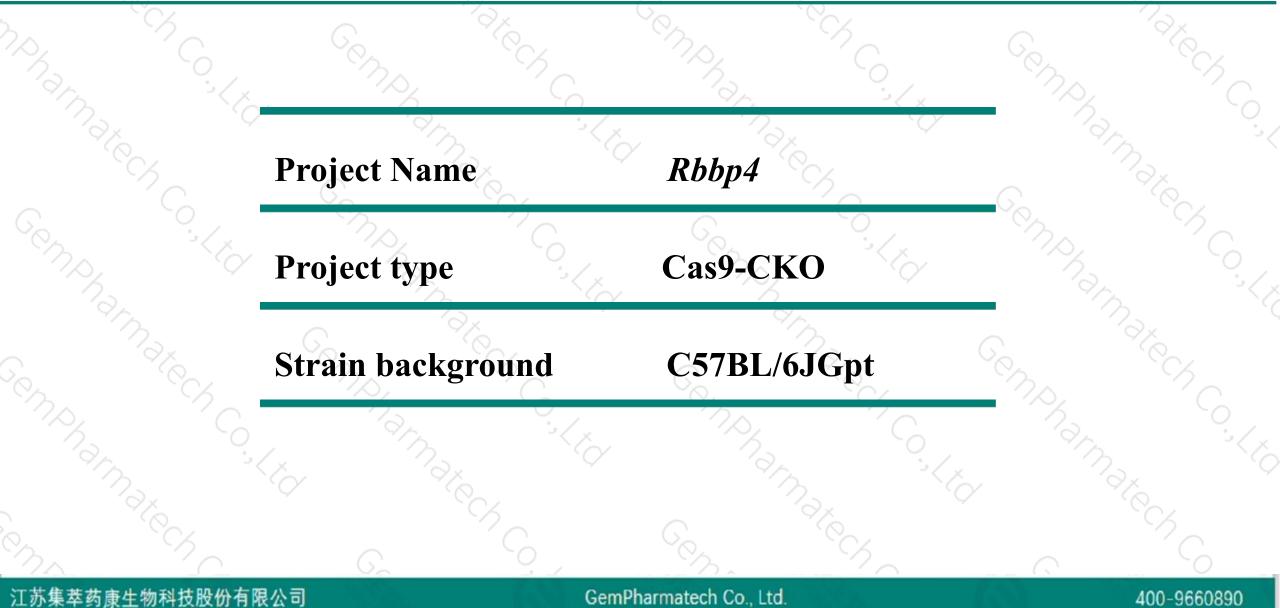
**Design Date:** 

Huan Wang Huan Fan

2020-5-6

# **Project Overview**

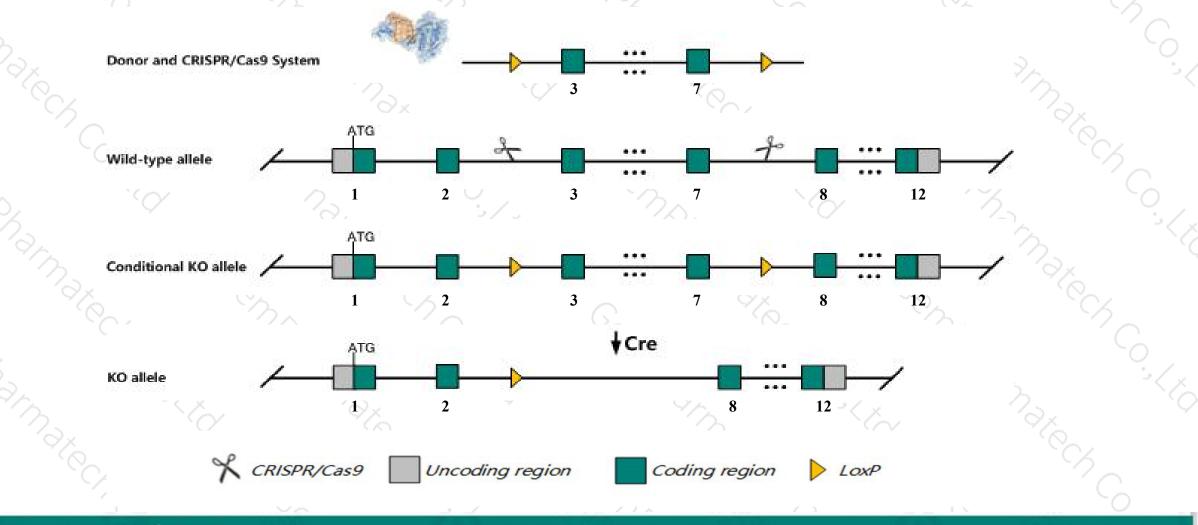




# **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the *Rbbp4* 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 *Rbbp4* gene has 4 transcripts. According to the structure of *Rbbp4* gene, exon3-exon7 of *Rbbp4-201* (ENSMUST00000102598.3) transcript is recommended as the knockout region. The region contains 724bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Rbbp4* 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 knock-out allele show complete embryonic lethality between implantation and somite formation. cultured blastocysts fail to form typical outgrowth colonies.
The *Rbbp4* gene is located on the Chr4. 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)**



☆ ?

Rbbp4 retinoblastoma binding protein 4, chromatin remodeling factor [Mus musculus (house mouse)]

Gene ID: 19646, updated on 13-Mar-2020

#### - Summary

Official SymbolRbbp4 provided by MGIOfficial Full Nameretinoblastoma binding protein 4, chromatin remodeling factor provided byMGIPrimary sourceMGI:MGI:1194912See relatedEnsembl:ENSMUSG0000057236Gene typeprotein codingVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;<br/>Muroidea; Murinae; Mus; MusAlso known asRBAP48, mRbAp48ExpressionUbiquitous expression in CNS E11.5 (RPKM 70.9), liver E14 (RPKM 42.3) and 25 other tissues<br/>See moreOrthologhuman all

#### 江苏集萃药康生物科技股份有限公司

#### GemPharmatech Co., Ltd.

# **Transcript information (Ensembl)**



#### The gene has 4 transcripts, all transcripts are shown below:

// TPQ		1102					
Name	Transcript ID	bp	Protein	Biotype	CCDS	<b>UniProt</b>	Flags
Rbbp4-201	ENSMUST00000102598.3	4407	<u>425aa</u>	Protein coding	CCDS18688	Q60972	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P
Rbbp4-202	ENSMUST00000135585.1	392	<u>48aa</u>	Protein coding		A8Y5E8	CDS 3' incomplete TSL:3
Rbbp4-203	ENSMUST00000140291.1	722	No protein	Processed transcript		1941	TSL:5
Rbbp4-204	ENSMUST00000147183.1	4663	No protein	Retained intron	25	820	TSL:1
	N			5.11	7.)		

The strategy is based on the design of *Rbbp4-201* transcript, the transcription is shown below:

#### < Rbbp4-201 protein coding

Reverse strand

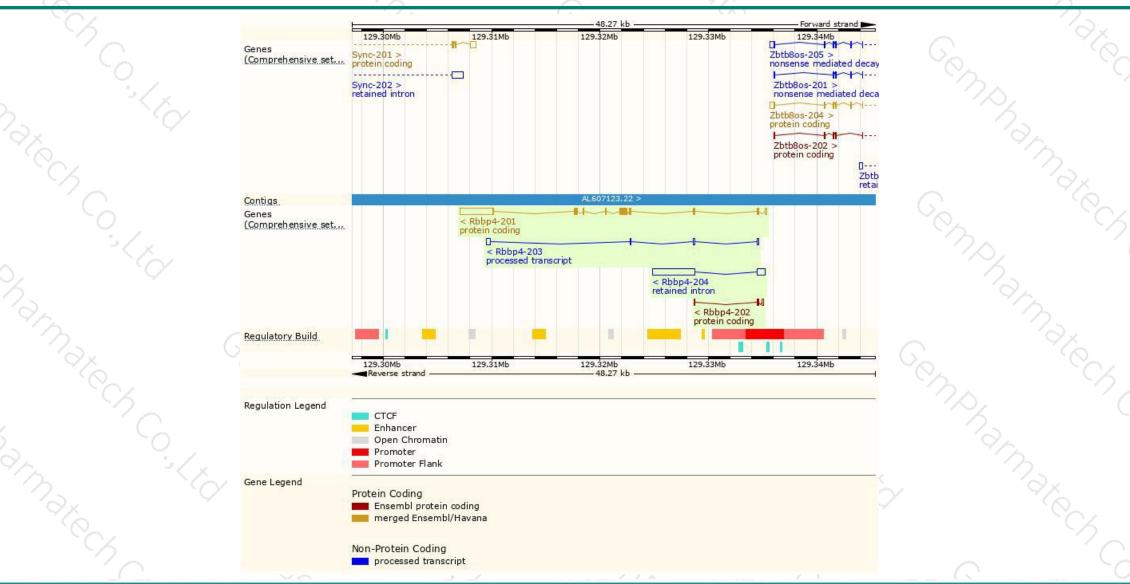
- 28.27 kb --

#### 江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

### **Genomic location distribution**





<u>江苏集萃药康生物科技股份有限公司</u>

GemPharmatech Co., Ltd.

# **Protein domain**



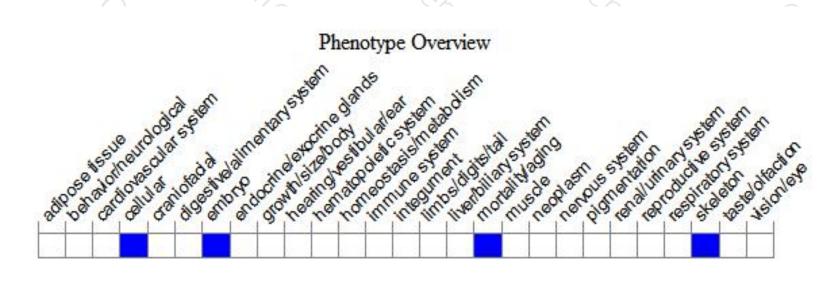
	6	~°?		х. С				5	6	2	°°			
	ENSMUSP00000099 Superfamily	WD40-repeat-containing domain superfamily												
	SMART			WD40 repr			-	-				°*		
	Prints Pfam					and the second s	beta WD-40	repeat						
	PROSITE profiles	Histone-	binding protein	RBBP4, N-term		0 repeat	statek i i				- 1			
	PROSTIL promes				Rear and	0-repeat-co	ntaining don	nain -	-					
	PROSITE patterns				1004	Contraction of the	at, conserv	ed site				-3/		
	PANTHER	PTHR22850:S	F90											
	Cape 2D	PTHR22850												
	Gene3D			aining domain si										
Z	All sequence SNPs/i	Sequence va	anants (dbSNI	P and all other	sources)	1	1	1	11	1		ò		
	Variant Legend	synony	mous variant	5			n Al		10103			-<		
	Scale bar	0 40	0 80	120	160	200	240	280	320	360	425			
											`Ч <sub>С</sub>			
						25.		1						

江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

# 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 a knock-out allele show complete embryonic lethality between implantation and somite formation. Cultured blastocysts fail to form typical outgrowth colonies.



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



