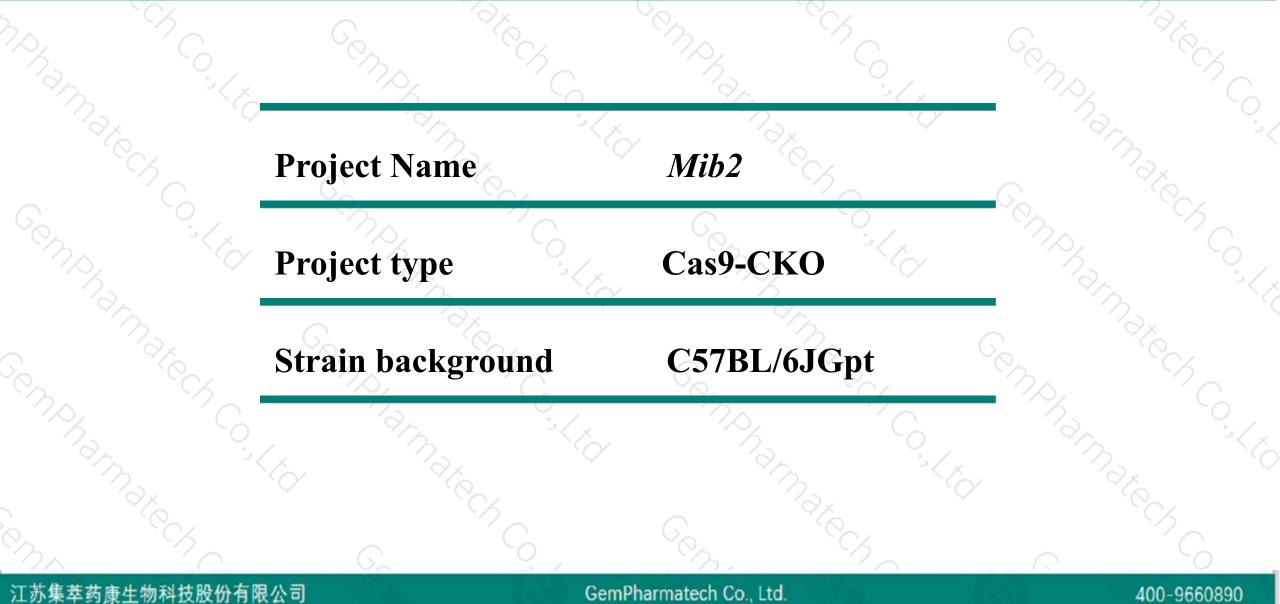


# Mib2 Cas9-CKO Strategy

Designer: Reviewer: Design Date: JiaYu Xiaojing Li 2020-3-4

## **Project Overview**



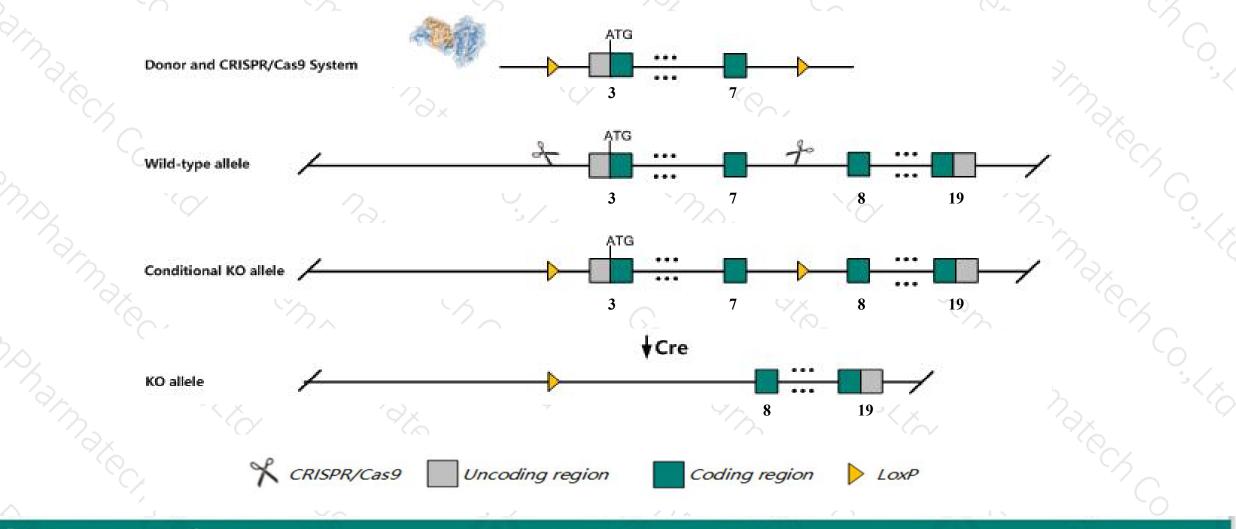


## **Conditional Knockout strategy**



400-9660890

This model will use CRISPR/Cas9 technology to edit the Mib2 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 Mib2 gene has 9 transcripts. According to the structure of Mib2 gene, exon3-exon7 of Mib2-201 (ENSMUST00000103176.9) 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 *Mib2* 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 display exencephaly with a variable penetrance that depends on the genetic background. Mice homozygous for a reporter/null allele are viable, fertile and show normal growth, body weight and brain morphology.

≻Transcript 207 CDS 5' and 3' incomplete the influences is unknown.

- The *Mib2* 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.

#### 江苏集萃药康生物科技股份有限公司

#### GemPharmatech Co., Ltd.

## **Gene information (NCBI)**



\$ ?

#### Mib2 mindbomb E3 ubiquitin protein ligase 2 [Mus musculus (house mouse)]

Gene ID: 76580, updated on 22-Mar-2019

#### Summary

Official Complexit	
Official Symbol	Mib2 provided by MGI
Official Full Name	mindbomb E3 ubiquitin protein ligase 2 provided by MGI
Primary source	MGI:MGI:2679684
See related	Ensembl:ENSMUSG0000029060
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	2210008l11Rik, Zzank1, skd
Expression	Ubiquitous expression in ovary adult (RPKM 36.2), adrenal adult (RPKM 23.1) and 27 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.

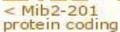


#### The gene has 9 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mib2-201	ENSMUST00000103176.9	3642	<u>921aa</u>	Protein coding	CCDS19035	Q8R516	TSL:1 GENCODE basic APPRIS P1
Mib2-207	ENSMUST00000141108.1	1125	<u>375aa</u>	Protein coding	i.e.	A2A9P8	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Mib2-208	ENSMUST00000151843.7	3282	No protein	IncRNA	4	040	TSL:1
Mib2-202	ENSMUST00000128204.7	2202	No protein	IncRNA	21	1025	TSL:2
Mib2-206	ENSMUST00000139788.7	1558	No protein	IncRNA	17	875	TSL:2
Mib2-209	ENSMUST00000155189.1	751	No protein	IncRNA		-	TSL:3
Mib2-204	ENSMUST00000139134.1	677	No protein	IncRNA	-	0.20	TSL:2
Mib2-203	ENSMUST00000130237.1	622	No protein	IncRNA	12	1028	TSL:3
Mib2-205	ENSMUST00000139289.1	470	No protein	IncRNA	5	1251	TSL:5

14.52 kb

The strategy is based on the design of *Mib2-201* transcript, The transcription is shown below



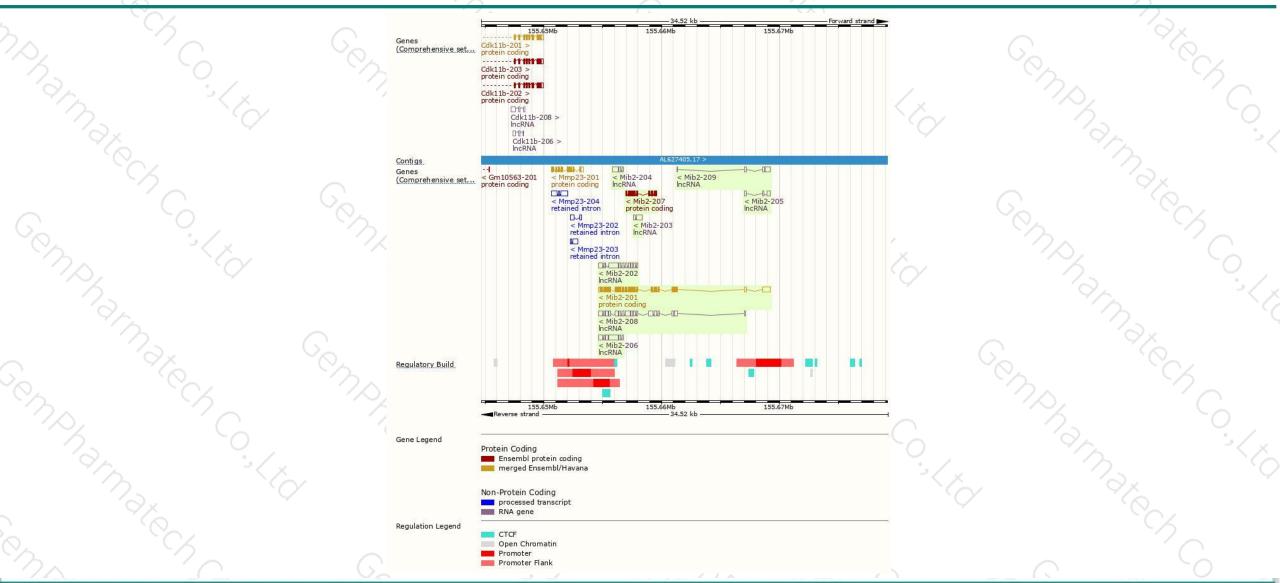
Reverse strand -

汀苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

### **Genomic location distribution**



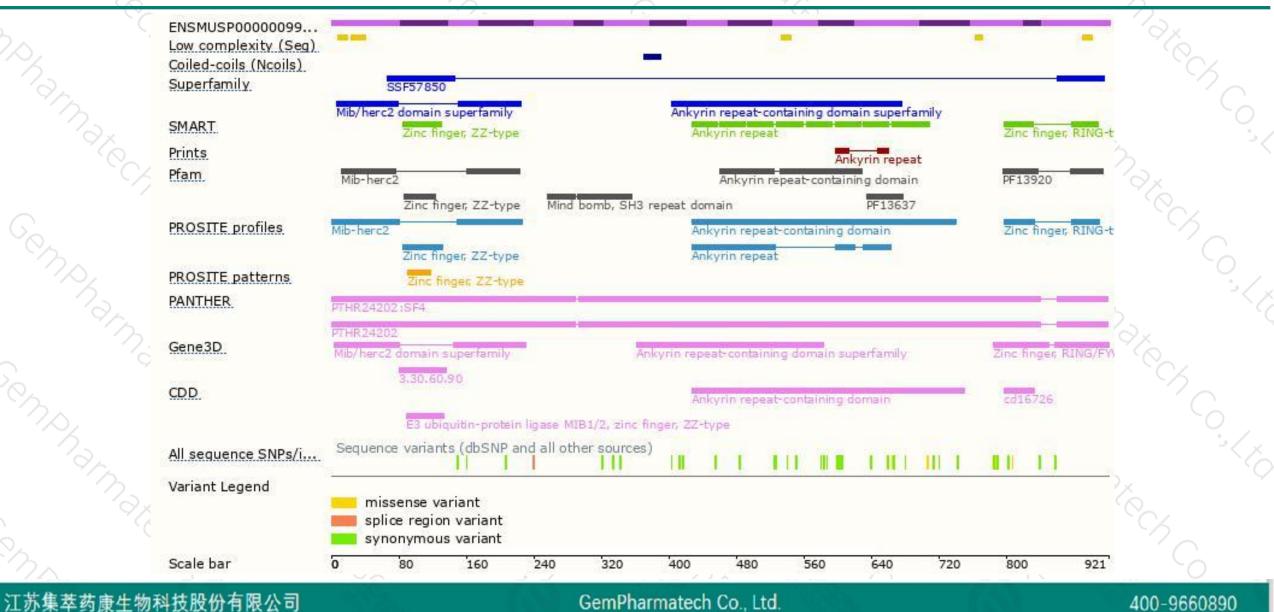


江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

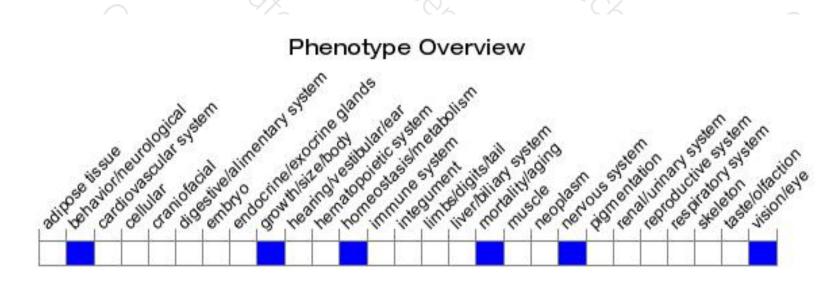
### **Protein domain**





## 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 a knock-out allele display exencephaly with a variable penetrance that depends on the genetic background. Mice homozygous for a reporter/null allele are viable, fertile and show normal growth, body weight and brain morphology.

#### 江苏集萃药康生物科技股份有限公司

#### GemPharmatech Co., Ltd.



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



