

# Actl6a Cas9-CKO Strategy

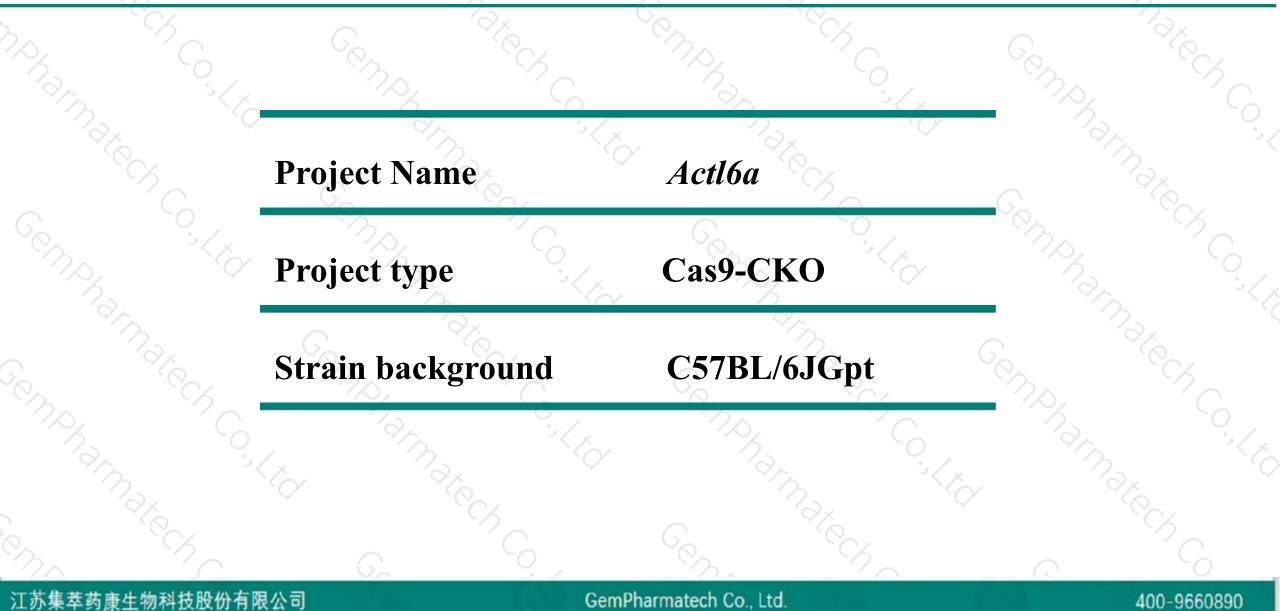
Designer: Reviewer:

**Design Date:** 

Daohua Xu Huimin Su 2019-11-26

# **Project Overview**

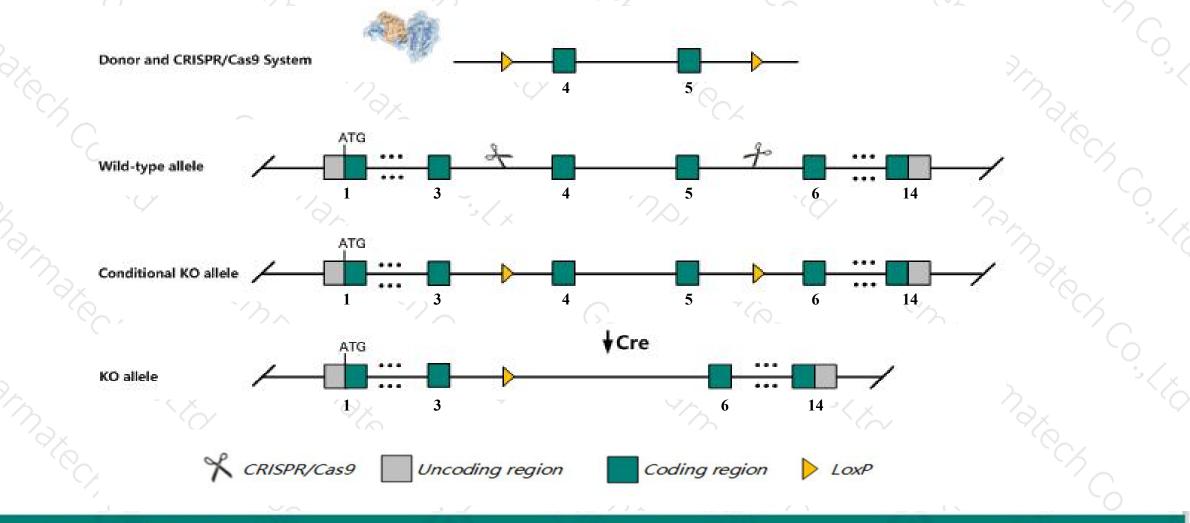




## **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the Actl6a 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.

400-9660890



The Actl6a gene has 8 transcripts. According to the structure of Actl6a gene, exon4-exon5 of Actl6a-201 (ENSMUST00000029214.13) transcript is recommended as the knockout region. The region contains 199bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Actl6a* 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 exhibit embryonic lethality before E6.5. Mice homozygous for a conditional allele activated in hematopoietic cells exhibit bone marrow failure and premature death.
- The Actl6a gene is located on the Chr3. 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)



< ?

### Actl6a actin-like 6A [Mus musculus (house mouse)]

Gene ID: 56456, updated on 31-Jan-2019

#### Summary

Official SymbolActifa provided by MGIOfficial Full Nameactin-like 6A provided by MGIPrimary SoureMGI:MGI:1861453Primary SoureEnsembl:ENSMUSG0000027671Gene typeprotein codingprotein codingvallDATEDOrganismMus musculusLineageEvaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;<br/>Muroidea; Murinae; Mus; MusAlso knowane2810432C06Rik, Al851094, ARP4, Acti6, Baf53a, C79802ExpressionUbiquitous expression in CNS E11.5 (RPKM 32.3), liver E14 (RPKM 17.6) and 27 other tissues

江苏集萃药康生物科技股份有限公司

### GemPharmatech Co., Ltd.

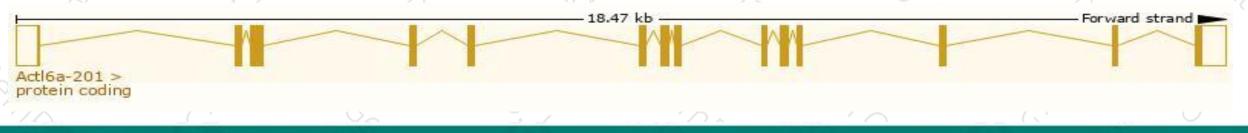
400-9660890



The gene has 8 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Actl6a-201 ENSMUST0000029214.13 2010 429		<u>429aa</u>	Protein coding		Q505L1 Q9Z2N8	TSL:1 GENCODE basic APPRIS P		
Actl6a-202	ENSMUST00000126144.2	922	<u>204aa</u>	Protein coding		D3YVN1	CDS 3' incomplete TSL:5	
Actl6a-208	ENSMUST00000194781.5	831	<u>245aa</u>	Protein coding	20	A0A0A6YWG8	CDS 3' incomplete TSL:5	
Actl6a-206	ENSMUST00000193615.1	635	<u>77aa</u>	Nonsense mediated decay	2	A0A0A6YW15	TSL:3	
Actl6a-205	ENSMUST00000193231.5	539	<u>54aa</u>	Nonsense mediated decay	-	A0A0A6YWR1	CDS 5' incomplete TSL:3	
Actl6a-207	ENSMUST00000194226.1	1457	No protein	Retained intron	-8	•	TSL:NA	
Actl6a-204	ENSMUST00000153779.1	1112	No protein	Retained intron	-	<u>=</u> 2	TSL:3	
Actl6a-203	ENSMUST00000135400.1	632	No protein	Retained intron	2	20	TSL:1	

The strategy is based on the design of Actl6a-201 transcript, The transcription is shown below

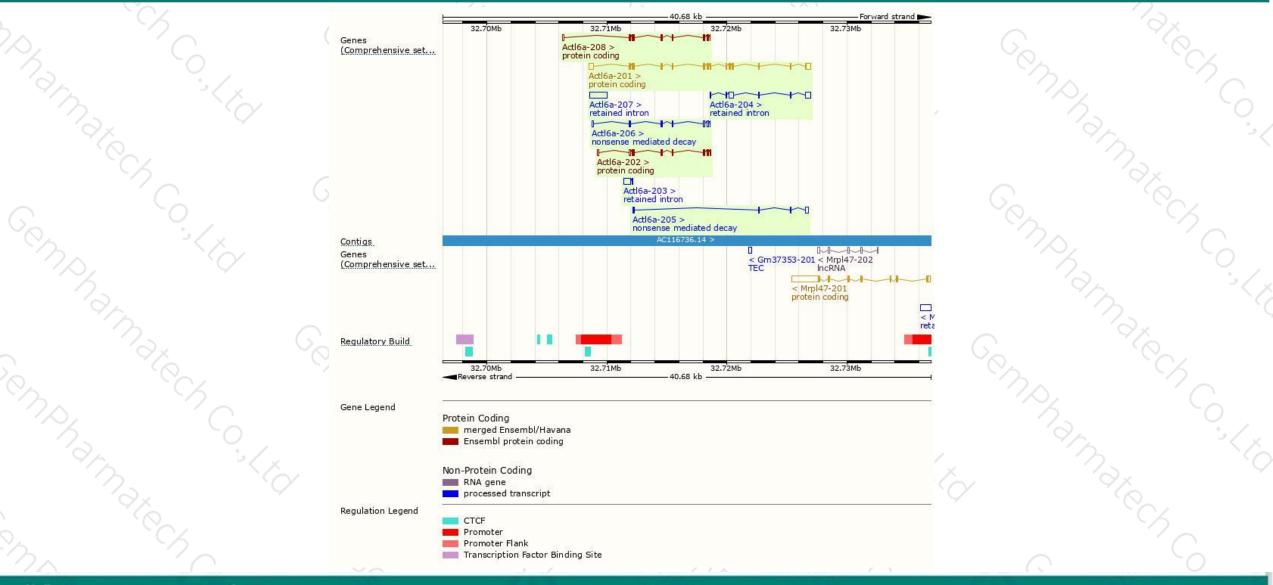


### 江苏集萃药康生物科技股份有限公司

### **Genomic location distribution**



400-9660890



江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

# **Protein domain**



			5	$\sim$			C.	
2	ENSMUSP00000029 Superfamily SMART	SSF53067				R AN		<b>-</b> 6
	Prints Pfam	Actin family Actin family Actin family	_					_
	PROSITE patterns	Accurrentity					A	ctin, «
	PANTHER.	PTHR11937:SF196 Actin family						
	Gene3D	3.30,420,40		3.90.6	10, 10			
	<u>CDD</u>	cd00012			10/10			
K,	All sequence SNPs/i Variant Legend	Sequence variants	(dbSNP and all other	sources)	ET E	<b>1</b> 11 11 11	11	,
	Scale bar	synonymous v	ariant	160 200	240	280 320	360	429
	1 <sup>(2</sup> )		1 C	G <sub>2</sub>	Store Ch		`Y	
	10	G		Š.	-7	<u> </u>	0	0

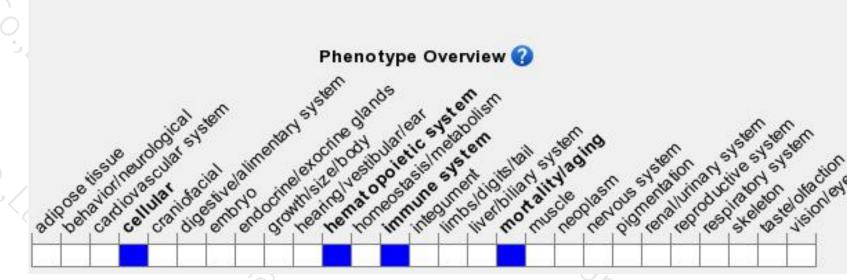
江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

400-9660890

### 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 exhibit embryonic lethality before E6.5. Mice homozygous for a conditional allele activated in hematopoietic cells exhibit bone marrow failure and premature death.



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



