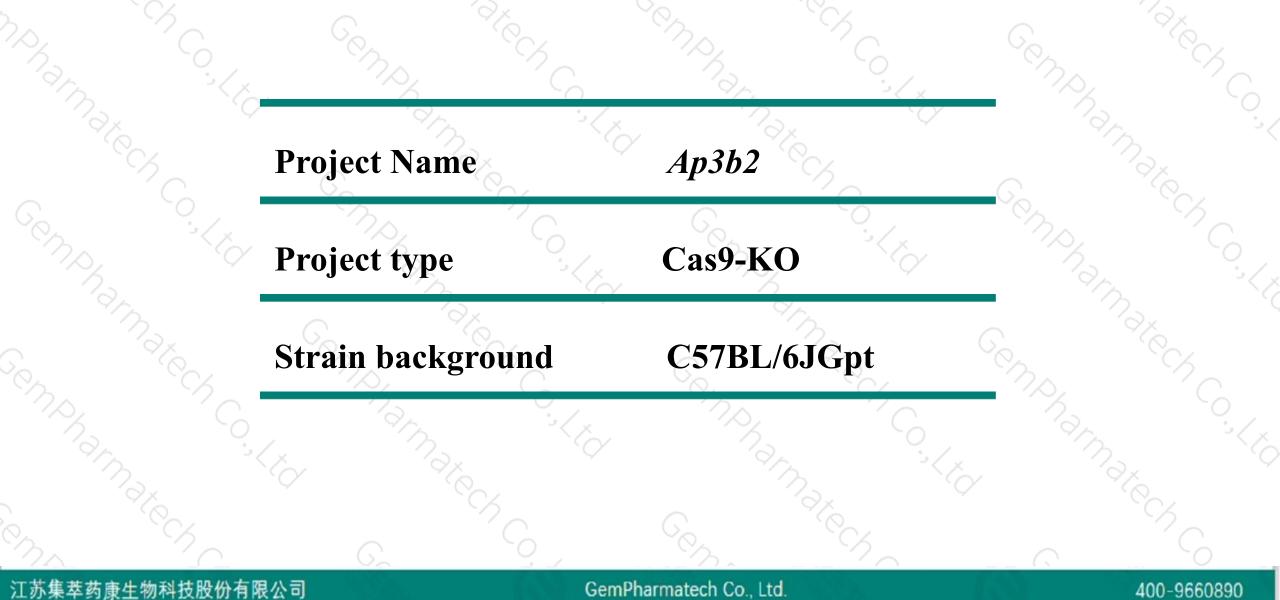


# Ap3b2 Cas9-KO Strategy

Designer: Xueting Zhang Reviewer:Yanhua Shen Date:2020-02-14

## **Project Overview**

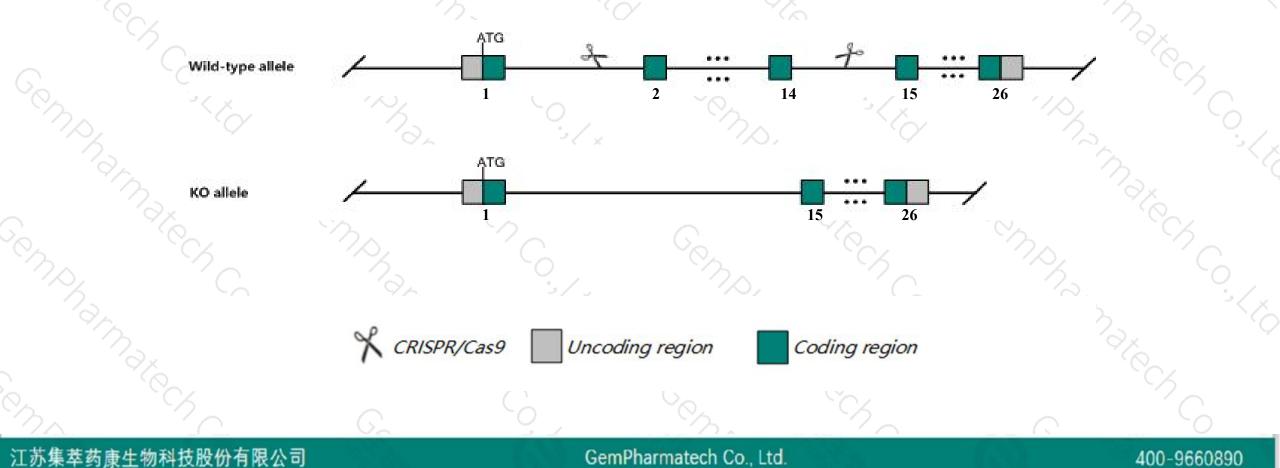




# **Knockout** strategy



This model will use CRISPR/Cas9 technology to edit the *Ap3b2* gene. The schematic diagram is as follows:





- The Ap3b2 gene has 9 transcripts. According to the structure of Ap3b2 gene, exon2-exon14 of Ap3b2-201 (ENSMUST0000082090.14) transcript is recommended as the knockout region. The region contains 1552bp coding sequence Knock out the region will result in disruption of protein function.
- > In this project we use CRISPR/Cas9 technology to modify Ap3b2 gene. The brief process is as follows: CRISPR/Cas9 system



- According to the existing MGI data, Disruption does not alter pigmentation, but causes hyperactivity and tonic-clonic seizures and mice homozygous for a knock-out allele were found to have significantly reduced synaptic zinc levels throughout the brain, with the largest reduction observed in the CA1 stratum oriens.
- ► Transcript *Ap3b2*-208 may not be affected.
- > The effect on transcript Ap3b2-204&206 is unknown.
- The Ap3b2 gene is located on the Chr7. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

# **Gene information (NCBI)**

Build 37.2

江苏集萃药康生物科技股份有限公司





7

NC 000073.5 (88605285..88638811, complement)

### GemPharmatech Co., Ltd.

MGSCv37 (GCF 000001635.18)

previous assembly

## **Transcript information (Ensembl)**



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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ap3b2-201	ENSMUST0000082090.14	3806	<u>1082aa</u>	Protein coding	CCDS21403	Q9JME5	TSL:1 GENCODE basic APPRIS P2
Ap3b2-207	ENSMUST00000238438.1	3383	<u>1101aa</u>	Protein coding	684	-	GENCODE basic APPRIS ALT2
Ap3b2-209	ENSMUST00000238711.1	3230	<u>1050aa</u>	Protein coding	(20)	-	GENCODE basic
Ap3b2-208	ENSMUST00000238692.1	629	<u>199aa</u>	Protein coding	127	-	CDS 3' incomplete
Ap3b2-205	ENSMUST00000152355.8	3161	No protein	Retained intron	(7)	-	TSL:1
Ap3b2-204	ENSMUST00000147624.1	1459	No protein	Retained intron	680	-	TSL:1
Ap3b2-206	ENSMUST00000208911.1	1430	No protein	Retained intron	140	4	TSL:NA
Ap3b2-202	ENSMUST00000119121.1	875	No protein	IncRNA	127	-	TSL:1
Ap3b2-203	ENSMUST00000125634.1	715	No protein	IncRNA	(15)		TSL:3

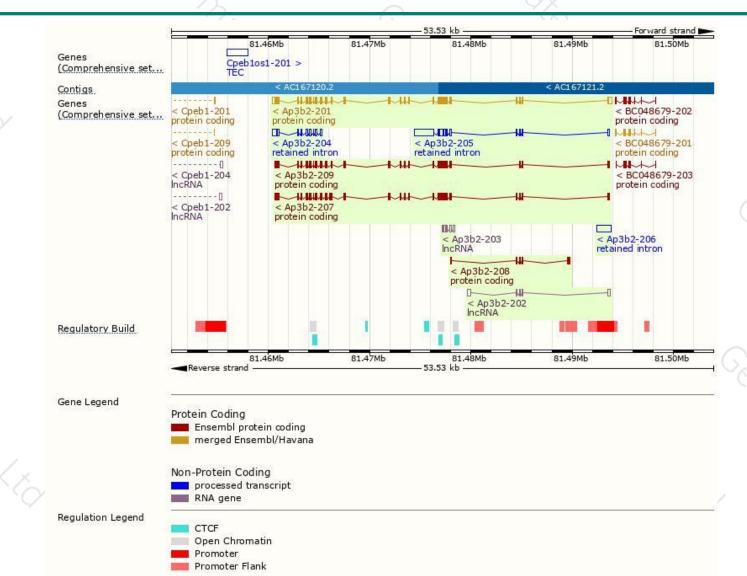
The strategy is based on the design of Ap3b2-201 transcript, The transcription is shown below



- 33.53 kb -

### **Genomic location distribution**





#### 江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

## **Protein domain**

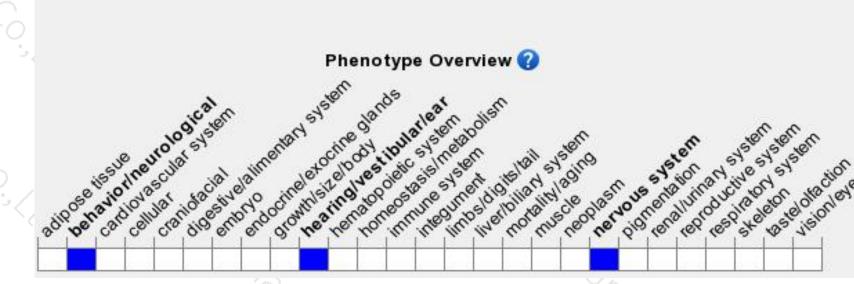
江苏



0,	ENSMUSP00000080											/ X	
23	MobiDB lite Low complexity (Seg)							_				• ?	
	Coiled-coils (Ncoils) Superfamily	Armadillo-typ	e fold							Clathrin adap	tor, app	endage	
	SMART								AP-3 co	mplex subuni	it beta, i	C-term	
ion Co	Pfam.	Clathrin/coate	omer adaptor,	adaptin-like	, N-terminal				AP-3 co	mplex subun	it beta,	C-term	
	PIRSF	AP-3 complex sub	unit beta										~
	PANTHER	PTHR11134:SF11											
	All sequence SNPs/i	AP complex subu Sequence varia		and all othe	r sources)	ų n	$\dot{0}(1)$	ų	1/1	01.0	į		
	Variant Legend	missense synonymo											
	Scale bar	0 100	200	300	400	500	600	700	800	900		1082	
	$^{\prime}$			$\sim 0$		$\Sigma_{\lambda}$		2					
苏集素	<sup>医</sup> 药康生物科技股份有限。	公司		fall 1	GemPharma	atech Co.,	Ltd.		13		400	0-96608	90

### 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, Disruption does not alter pigmentation, but causes hyperactivity and tonic-clonic seizures and mice homozygous for a knock-out allele were found to have significantly reduced synaptic zinc levels throughout the brain, with the largest reduction observed in the CA1 stratum oriens.

### 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



