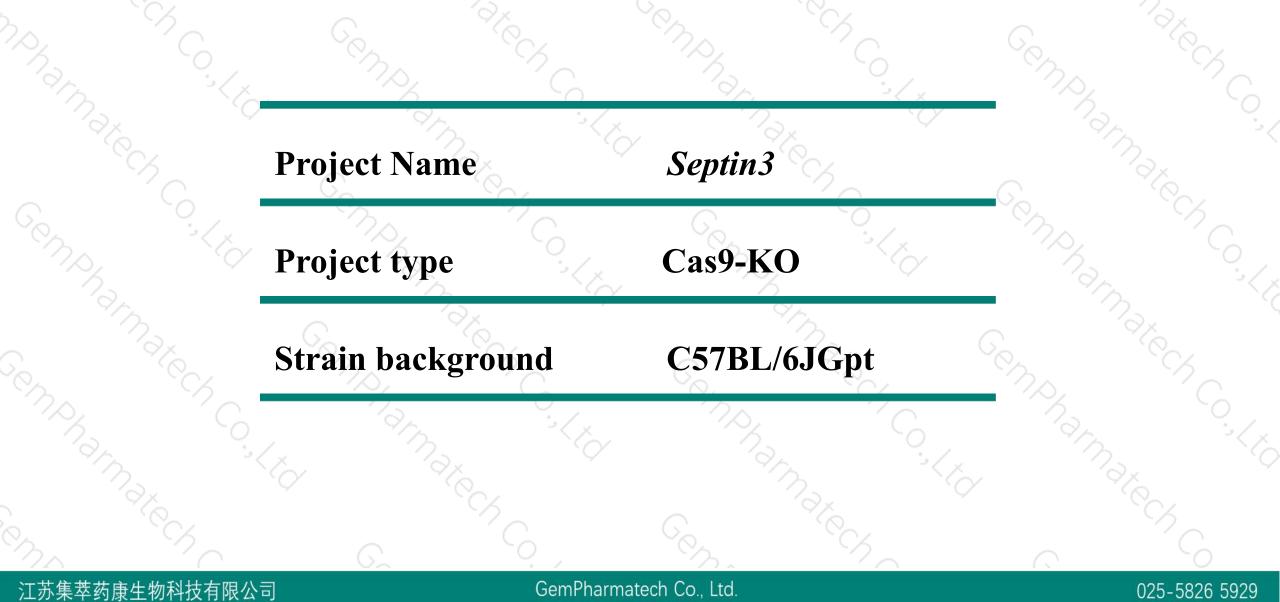


Septin3 Cas9-KO Strategy

Designer:Xueting Zhang Reviwer:Yanhua Shen Date:2020-02-20

Project Overview

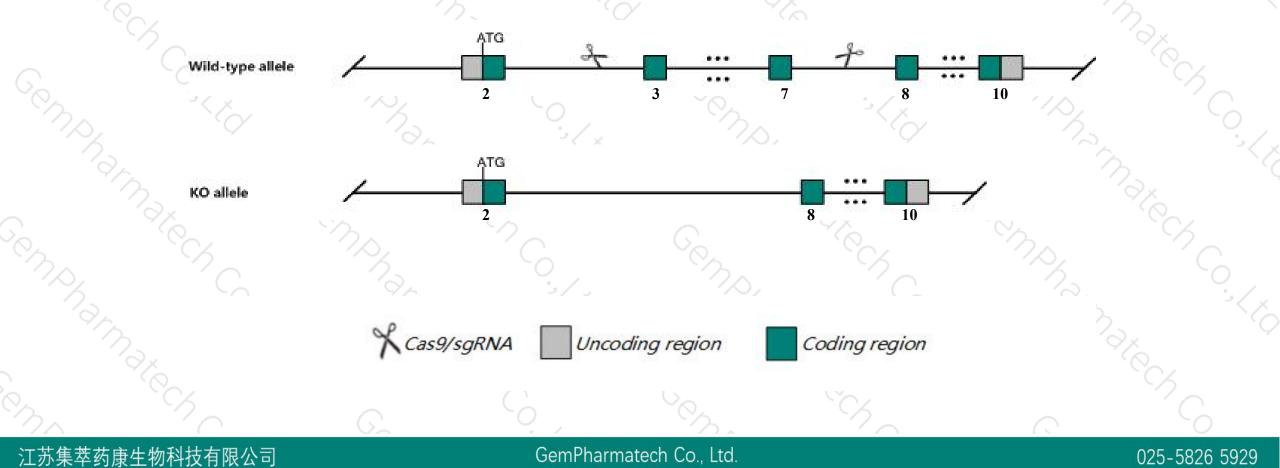




Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Septin3 gene. The schematic diagram is as follows:





- The Septin3 gene has 8 transcripts. According to the structure of Septin3 gene, exon3-exon7 of Septin3-201 (ENSMUST00000023095.13) transcript is recommended as the knockout region. The region contains 563bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Septin3* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

- > According to the existing MGI data, Mice homozygous for a null allele exhibit a normal phenotype.
- ➤ The effect on transcript *Septin3*-205&206 is unknown.
- > The N-terminal of *Septin3* gene will remain some amino acids, it may remain the partial function of *Septin3* gene.
- The Septin3 gene is located on the Chr15. 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.

Notice

Gene information (NCBI)



1	Gene ID: 24050, updated on		s (house mouse)]		
2	Summary				*
Matech (Nohamate	Official Full Name Primary source See related Gene type RefSeq status Organism Lineage Also known as	Ensembl:ENSMUS protein coding VALIDATED <u>Mus musculus</u> Eukaryota; Metazo Myomorpha; Muroi Sep3; Sept3; Gm4	MGI 3G00000022456	us ik; B530002E20Rik	; Eutheria; Euarchontoglires; Glires; Rodentia;
nare.	Expression Orthologs		III CNS E 14 (REKIVI 62.2), WHO	e Dialit E 14.3 (RFRW 01.1)	
hann.	Genomic context Location: 15; 15 E1 Exon count: 15 Annotation release	Status	Assembly	Chr	See Septin3 in <u>Genome Data Vie</u>

江苏集萃药康生物科技有限公司

\$?

\$?

See Septin3 in Genome Data Viewer

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	15	NC_000081.6 (8226880282294574)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	15	NC_000081.5 (8210536582124872)

GemPharmatech Co., Ltd.

025-5826 5929

Transcript information (Ensembl)



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

Sould have							1 P.S. J	
Name 🍦	Transcript ID 💧	bp 🍦	Protein 🖕	Biotype 💧	CCDS	UniProt 🖕	Flags	
Septin3-201	ENSMUST0000023095.13	4992	<u>337aa</u>	Protein coding	<u>CCDS37157</u> &	<u>Q9Z1S5</u> &	TSL:1 GENCODE basic A	PPRIS P2
Septin3-202	ENSMUST00000116423.2	2397	<u>337aa</u>	Protein coding	<u>CCDS37157</u> &	<u>Q9Z1S5</u> &	TSL:1 GENCODE basic A	PPRIS P2
Septin3-209	ENSMUST00000239048.1	6443	<u>818aa</u>	Protein coding	5=8	<u>A0A5F8MPL6</u> &	GENCODE basic APPRI	S ALT2
Septin3-208	ENSMUST00000238416.2	6022	<u>822aa</u>	Protein coding		<u>A0A571BE69</u> &	GENCODE basic APPRI	S ALT2
Septin3-204	ENSMUST00000230365.1	1888	<u>341aa</u>	Protein coding	ς	<u>A0A2R8W6V9</u> &	GENCODE basic APPRI	S ALT2
Septin3-206	ENSMUST00000230507.1	865	<u>201aa</u>	Protein coding	-	<u>A0A2R8VHR7</u> &	CDS 5' incomplete	
Septin3-205	ENSMUST00000230418.1	440	<u>75aa</u>	Protein coding	-	A0A2R8VHB2	CDS 3' incomplete	
Septin3-207	ENSMUST00000230799.1	2130	No protein	Retained intron	-	8-	_	
Septin3-203	ENSMUST00000229067.1	1882	No protein	Retained intron	-		3	

The strategy is based on the design of *Septin3-201* transcript, The transcription is shown below

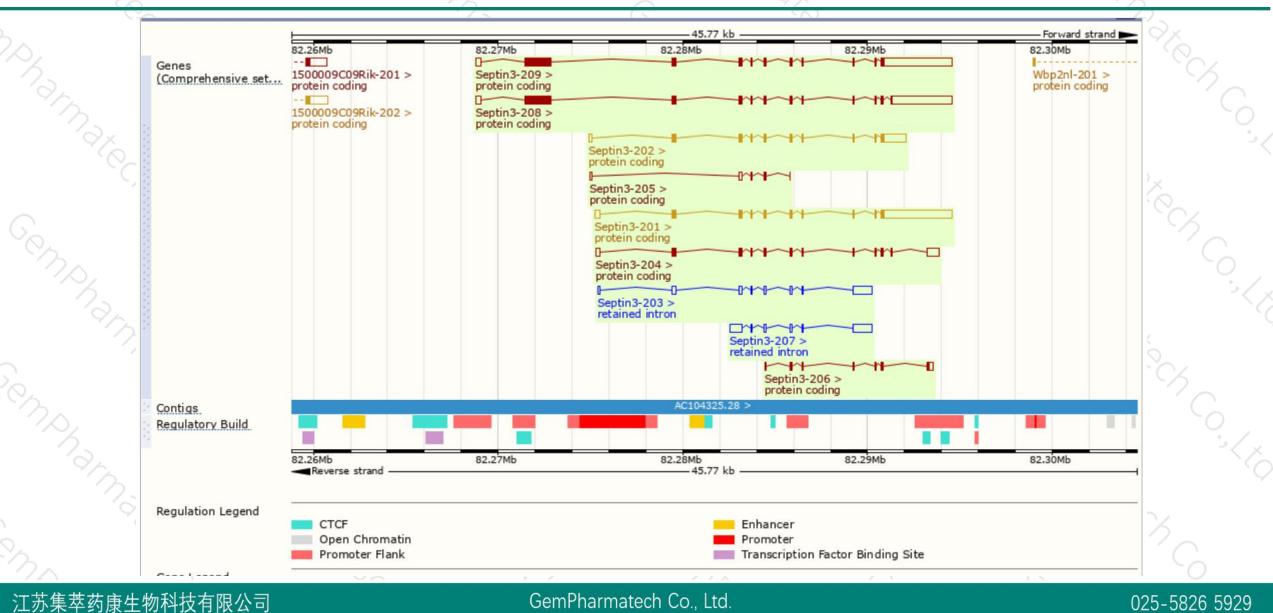
19.34 kb Septin3-201 >						orward strand D
	10	G	~0	×>>	2.	

江苏集萃药康生物科技有限公司

GemPharmatech Co., Ltd.

025-5826 5929

Genomic location distribution



集萃g

集举药康 GemPharmatech

GemPharmatech Co., Ltd.

Protein domain



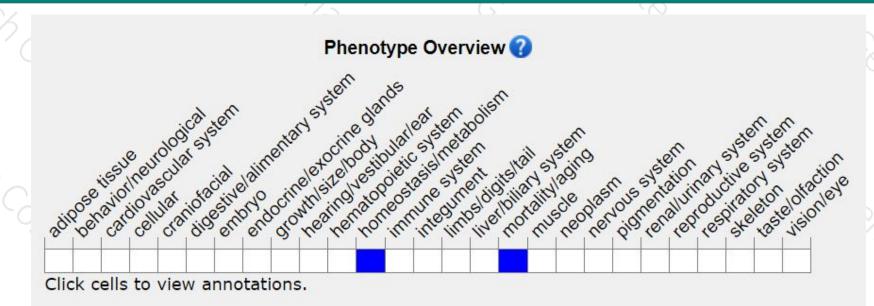
ENSMUSP00000023 MobiDB lite							- ² C
Superfamily	P-loop c	ontaining nucleoside tr	iphosphate hydrolase				
Prints	Septin 3				-		
Pfam.		Septin-type guanine nu	cleatide-binding (G) dor	nain			
PROSITE profiles		Septin-type guanine nu	cleatide-binding (G) don	nain			
PIRSF	Septin						
PANTHER	PTHR18884						
	PTHR18884:SF6.	2					
Gene3D	3.40.50,3	00					
CDD		Septin					
All sequence SNPs/i	Sequence variar	nts (dbSNP and all ot	her sources)		19 M 1	1.0	
							<u> </u>
Variant Legend	splice regio	on variant					
	🗾 synonymou	ıs variant					
Scale bar	o 40	80	120 16	0 200	240	280	337
							~?~
10		\sim	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	$\sim \sim $			

江苏集萃药康生物科技有限公司

GemPharmatech Co., Ltd.

025-5826 5929

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 null allele exhibit a normal phenotype.



If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



