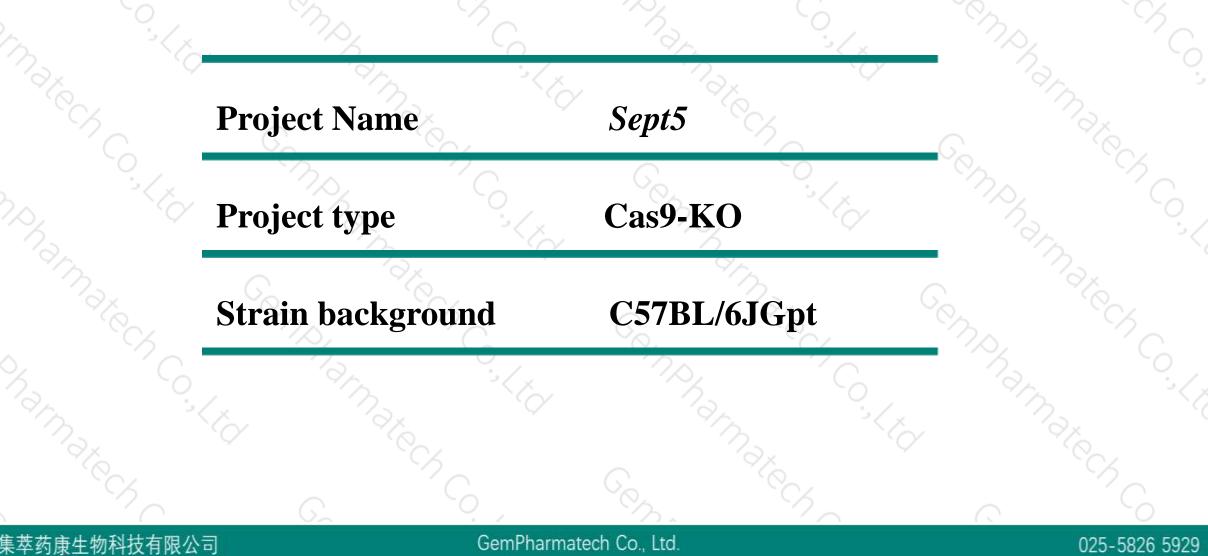


Sept5 Cas9-KO Strategy

Designer:Xueting Zhang Reviewer:Yanhua Shen Design Date:2019-10-14

Project Overview

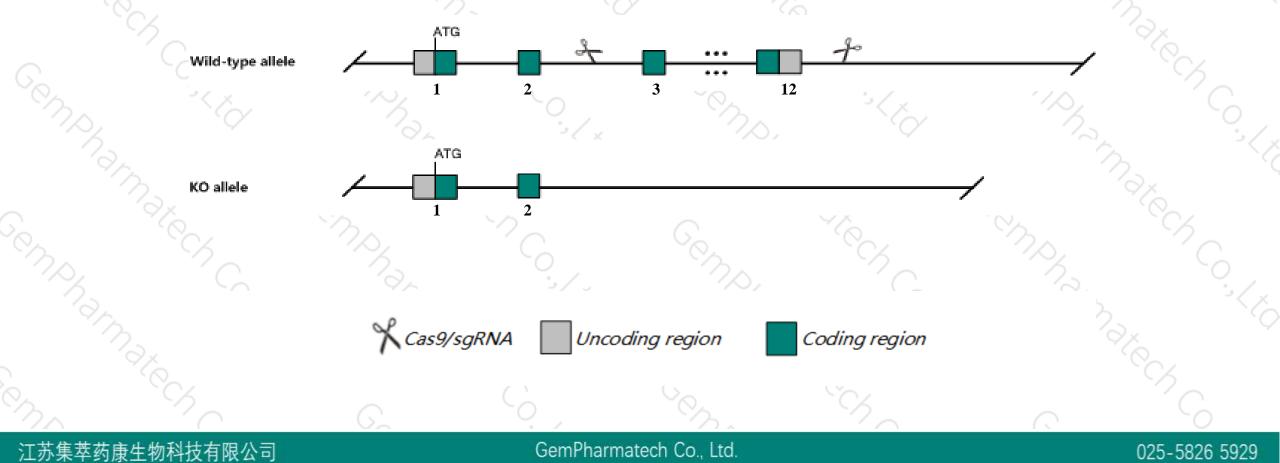




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This model will use CRISPR/Cas9 technology to edit the Sept5 gene. The schematic diagram is as follows:





- The Sept5 gene has 5 transcripts. According to the structure of Sept5 gene, exon3-exon12 of Sept5-201 (ENSMUST00000096987.6) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Sept5* 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.





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- According to the existing MGI data, Mice homozygous for disruptions in this gene show no gross phenotypic changes. Partial defects in synaptic transmission is reported for one allele, and platelet secretion and modest behavioral defects reported for a different allele.
- > Gp1bb and Gm49601 gene will be deleted together in this strategy.
- The Sept5 gene is located on the Chr16. 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.

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Gene information (NCBI)



Sept5 septin 5 [<i>Mus musculus</i> (house mouse)]
Gene ID: 18951, updated	l on 10-Oct-2019
Summary	
Official Sym	Sept5 provided by MGI
Official Full Na	ne septin 5 provided by MGI
Primary sour	ce MGI:MGI:1195461
See relat	ed Ensembl:ENSMUSG00000072214
Gene ty	pe protein coding
RefSeq stat	us VALIDATED
Organi	m <u>Mus musculus</u>
Linea	ge Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia
	Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known	as Pnutl1; Cdcrel1; Septin5; Cdcrel-1
Express	on Broad expression in cortex adult (RPKM 236.3), large intestine adult (RPKM 199.0) and 15 other tissues See more
Ortholo	gs <u>human</u> <u>all</u>

Genomic context

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| ? |

See Sept5 in Genome Data Viewer

2 ?

Location: 16 A3; 16 11.53 cM

Exon count: 12

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 Annotation release
 Status
 Assembly
 Chr
 Location

 108
 current
 GRCm38.p6 (GCF_000001635.26)
 16
 NC_000082.6 (18621811..18629938, complement)

 Build 37.2
 previous assembly
 MGSCv37 (GCF_000001635.18)
 16
 NC_000082.5 (18621904..18630031, complement)



Transcript information (Ensembl)

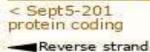


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The gene has 5 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sept5-201	ENSMUST0000096987.6	2143	<u>369aa</u>	Protein coding	CCDS57021	<u>Q9Z2Q6</u>	TSL:1 GENCODE basic APPRIS P2
Sept5-205	ENSMUST00000232653.1	3678	<u>378aa</u>	Protein coding	-	A0A338P769	GENCODE basic APPRIS ALT1
Sept5-204	ENSMUST00000231956.1	1314	<u>381aa</u>	Protein coding	-	B7ZNM7	GENCODE basic
Sept5-203	ENSMUST00000231622.1	521	<u>126aa</u>	Protein coding	-	A0A338P755	CDS 3' incomplete
Sept5-202	ENSMUST00000231244.1	2003	<u>248aa</u>	Nonsense mediated decay	-	A0A338P729	

The strategy is based on the design of Sept5-201 transcript, The transcription is shown below

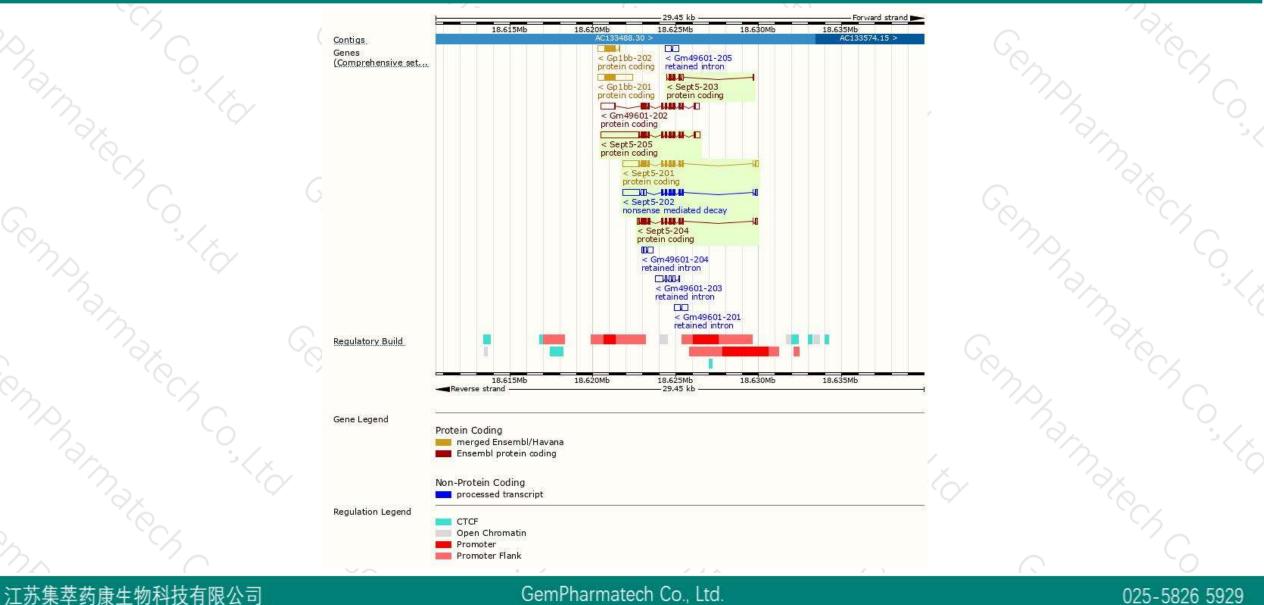


_____ 8.14 kb

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Genomic location distribution





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Protein domain



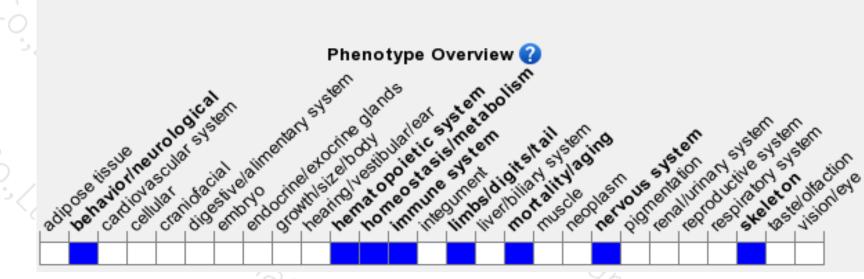
?/.	ENSMUSP00000094 Low complexity (Seg) Coiled-coils (Ncoils) Superfamily	P-loop containi	ng nucleoside triphospha	ite hydrolase			
	Pfam.	Septin-t	ype guanine nucleotide-	binding (G) domain			
	PROSITE profiles	Septin-t	ype guanine nucleotide-	binding (G) domain			
	PIRSF	Septin					
	PANTHER.	Septin S PTHR18884					
	Gene3D	3.40.50.300				15	
	CDD	Septin		- 5000 1000			
K	All sequence SNPs/i	Sequence variants (dbSNP and all other s	ources)	1.1.1	1	0./.
	Variant Legend	synonymous v	ariant				
	Scale bar	o 40	80 120	160	200 240	280 320	369
~	°°G C	G_		Cen .		C	^C C
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Mouse phenotype description(MGI)



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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 disruptions in this gene show no gross phenotypic changes. Partial defects in synaptic transmission is reported for one allele, and platelet secretion and modest behavioral defects reported for a different allele.



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



