

Sh3glb1 Cas9-CKO Strategy

Designer: Reviewer:

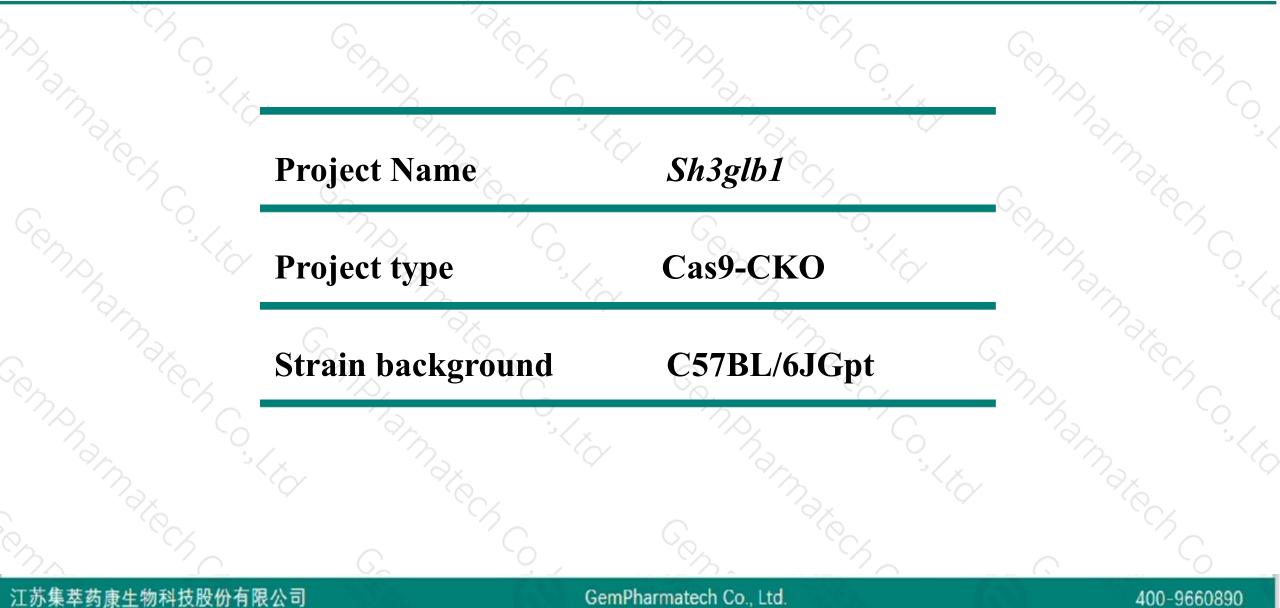
Design Date:

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2020-5-6

Project Overview

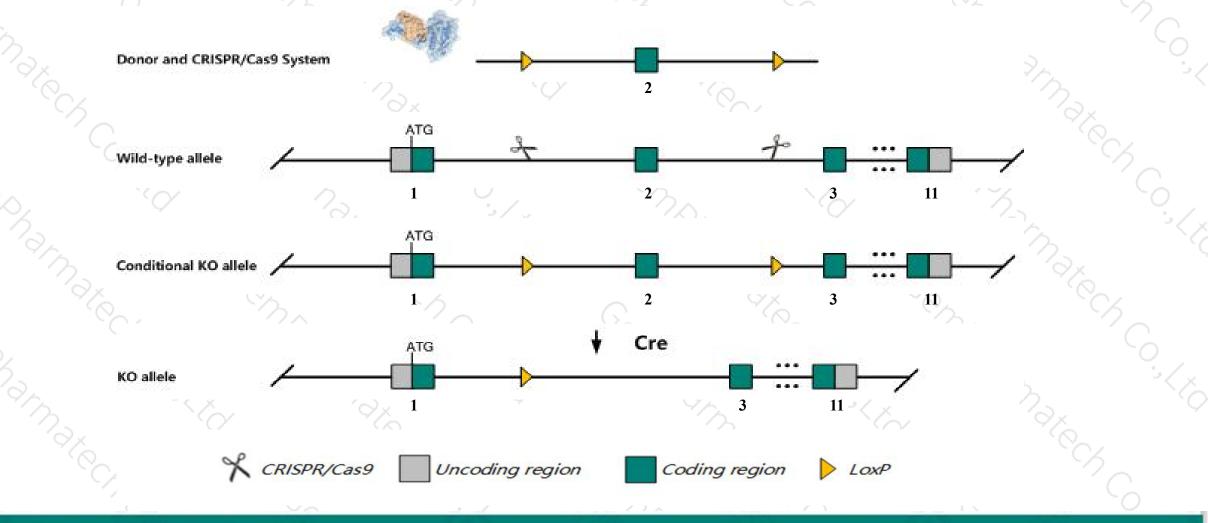




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Sh3glb1* gene. The schematic diagram is as follows:



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400-9660890



 The Sh3glb1 gene has 6 transcripts. According to the structure of Sh3glb1 gene, exon2 of Sh3glb1-202 (ENSMUST00000198254.4) transcript is recommended as the knockout region. The region contains 142bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Sh3glb1* 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, homozygous mutation of this gene results in delayed apoptosis of embryonic fibroblasts in response to serum withdrawal or treatment with a mitochondrial stress inducer.
- ➤ Transcript *Sh3glb1-206* may not be affected.
- The Sh3glb1 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)



< ?

Sh3glb1 SH3-domain GRB2-like B1 (endophilin) [Mus musculus (house mouse)]

Gene ID: 54673, updated on 25-Mar-2020

Summary

Official Symbol	Sh3glb1 provided by MGI										
Official Full Name	SH3-domain GRB2-like B1 (endophilin) provided by MGI										
Primary source	MGI:MGI:1859730										
See related	Ensembl:ENSMUSG0000037062										
Gene type	protein coding										
RefSeq status	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	AA409932, Al314629, AU015566, Bif-1, mKlAA0491										
Expression	Ubiquitous expression in testis adult (RPKM 28.5), CNS E18 (RPKM 9.1) and 27 other tissues See more										
Orthologs	human all										

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Transcript information (Ensembl)



The gene has 6 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Sh3glb1-202	ENSMUST00000198254.4	5945	<u>386aa</u>	Protein coding	CCDS80043	<u>Q9JK48</u>	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT1	
Sh3glb1-201	ENSMUST00000163279.5	3961	<u>365aa</u>	Protein coding	CCDS17885	<u>Q9JK48</u>	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. AF	
Sh3glb1-204	ENSMUST00000199531.4	2584	<u>355aa</u>	Protein coding	CCDS80042	Q9JK48	TSL:1 GENCODE basic	
Sh3glb1-205	ENSMUST00000199854.4	1416	<u>394aa</u>	Protein coding	120	A0A0G2JEC4	TSL:5 GENCODE basic	
Sh3glb1-203	ENSMUST00000199350.4	665	<u>140aa</u>	Protein coding	(5)	A0A0G2JF57	CDS 5' incomplete TSL:3	
Sh3glb1-206	ENSMUST00000200532.1	582	<u>194aa</u>	Protein coding		A0A0G2JE45	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3	

The strategy is based on the design of *Sh3glb1-202* transcript, the transcription is shown below:

< Sh3glb1-202 protein coding

Reverse strand

- 33.41 kb -

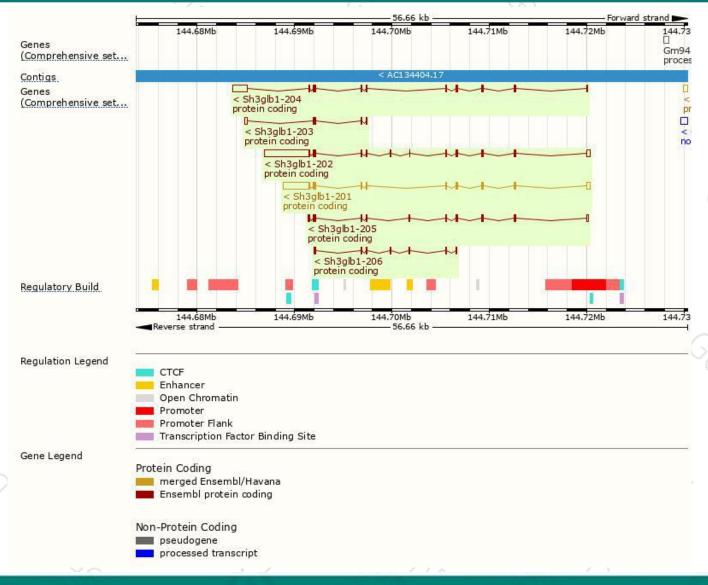
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Genomic location distribution







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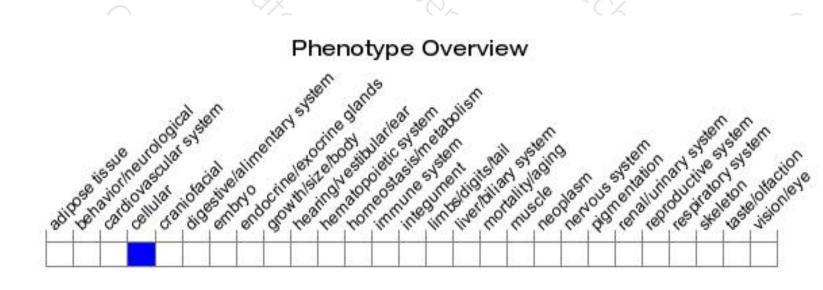
Protein domain



	ENSMUSP00000143 Low complexity (Seg) Coiled-coils (Ncoils) Superfamily	SH3-like domain superfamily	
armar.	SMART	AH/BAR domain superfamily BAR domain SH3 domain	
° C	Pfam.	BAR domain SH3 domain	- ??;
	PROSITE profiles	BAR domain SH3 domain	
n phan	PANTHER	Endophilin-B1	
Str.	Gene3D	PTHR14167 AH/BAR domain superfamily 2.30.30.40	-27
	CDD.	Endophilin-B1, BAR domain	- `X
20 Harry	All sequence SNPs/i	Sequence variants (dbSNP and all other sources)	-0
"Tak	Variant Legend	missense variant synonymous variant	-'* **
	Scale bar		386

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, homozygous mutation of this gene results in delayed apoptosis of embryonic fibroblasts in response to serum withdrawal or treatment with a mitochondrial stress inducer.



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



