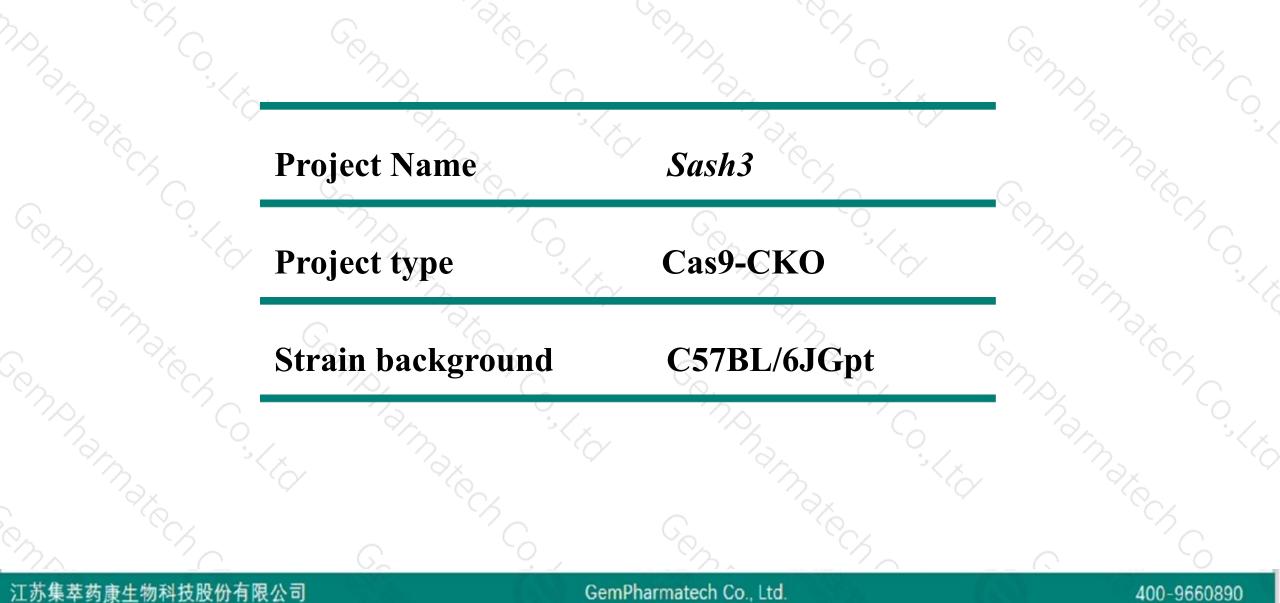


Sash3 Cas9-CKO Strategy

Designer: Reviewer: Design Date: JiaYu Xiaojing Li 2020-2-25

Project Overview

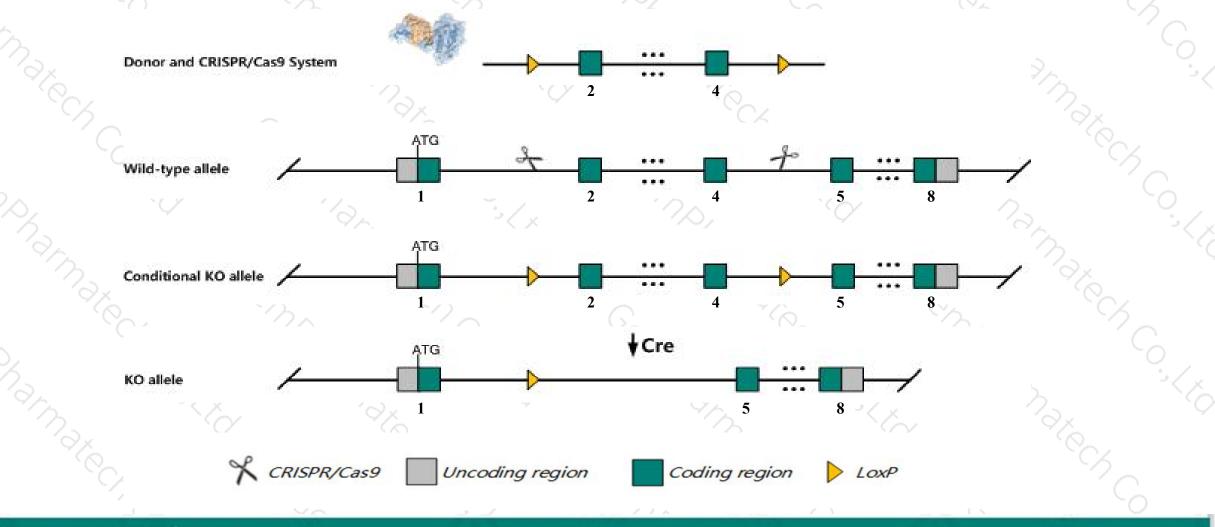




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Sash3 gene. The schematic diagram is as follows:



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The Sash3 gene has 1 transcript. According to the structure of Sash3 gene, exon2-exon4 of Sash3-201 (ENSMUST00000033427.6) transcript is recommended as the knockout region. The region contains 385bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify Sash3 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 truncation allele exhibit decreased T and B cells, altered B cell physiology and increased length of semi-identical allograft survival.
- The Sash3 gene is located on the ChrX. 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)



\$?

Sash3 SAM and SH3 domain containing 3 [Mus musculus (house mouse)]

Gene ID: 74131, updated on 31-Jan-2019

Summary

Official Symbol	Sash3 provided by MGI						
Official Full Name	SAM and SH3 domain containing 3 provided by MGI						
Primary source	MGI:MGI:1921381						
See related	Ensembl:ENSMUSG00000031101						
Gene type	protein coding						
RefSeq status	VALIDATED						
Organism	Mus musculus						
Lineage	ge Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha						
	Muroidea; Muridae; Murinae; Mus; Mus						
Also known as	1200013B08Rik, AW413946, SLY1						
Expression	Biased expression in thymus adult (RPKM 104.2), spleen adult (RPKM 50.8) and 3 other tissues See more						
Orthologs	human all						

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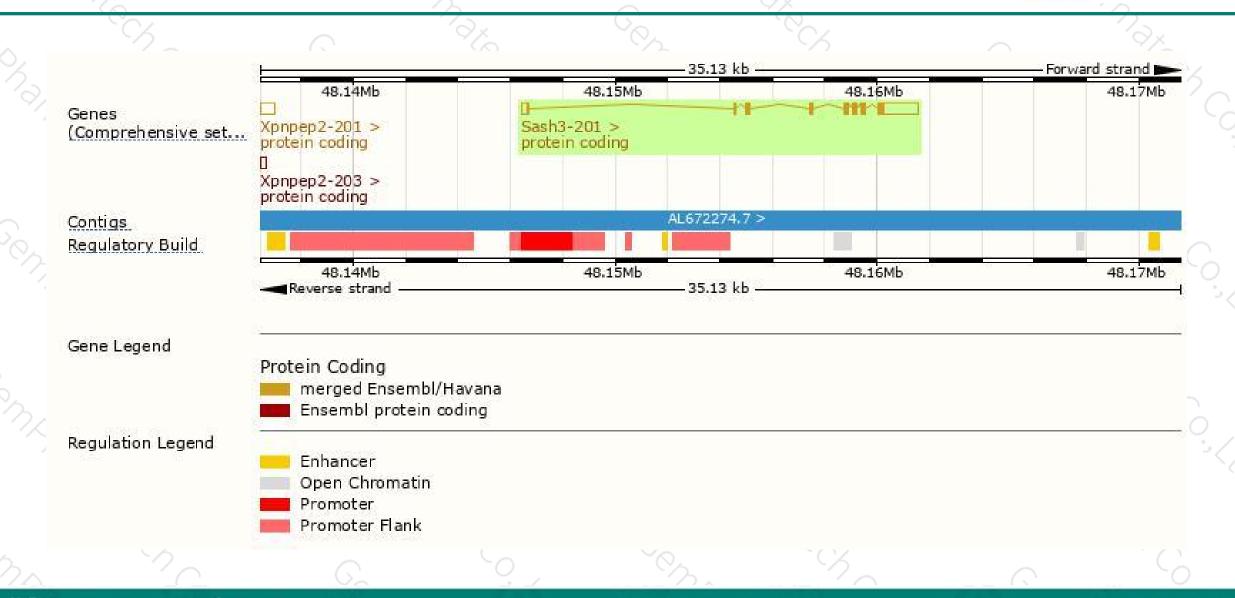
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The gene has 1 transcript, and the transcript is shown below:

Name	Transcript ID	bp	Protein	in Biotype	CCDS	UniProt	Flags		
Sash3-201 ENSMUST00000033427.6		2677 <u>380aa</u>		Protein coding	CCDS40959	<u>Q8K352</u>	TSL:1 GENCODE bas	ENCODE basic APPRIS P1	
	South Cons	2				5 	Cons,	Nate Ch	
Pharm		ann.			(DDJarry	```¢`	George Contractions of the second sec		
e strategy	is based on the design of	Sash3-	-201 trans		cription is sho	own below	Shopping har	C C	
n3-201 > ein coding				15.13 kb			For	ward strand	
萃药康牛物	物科技股份有限公司		10	GemPharmate	ech Co., Ltd.			400-96608	

Genomic location distribution



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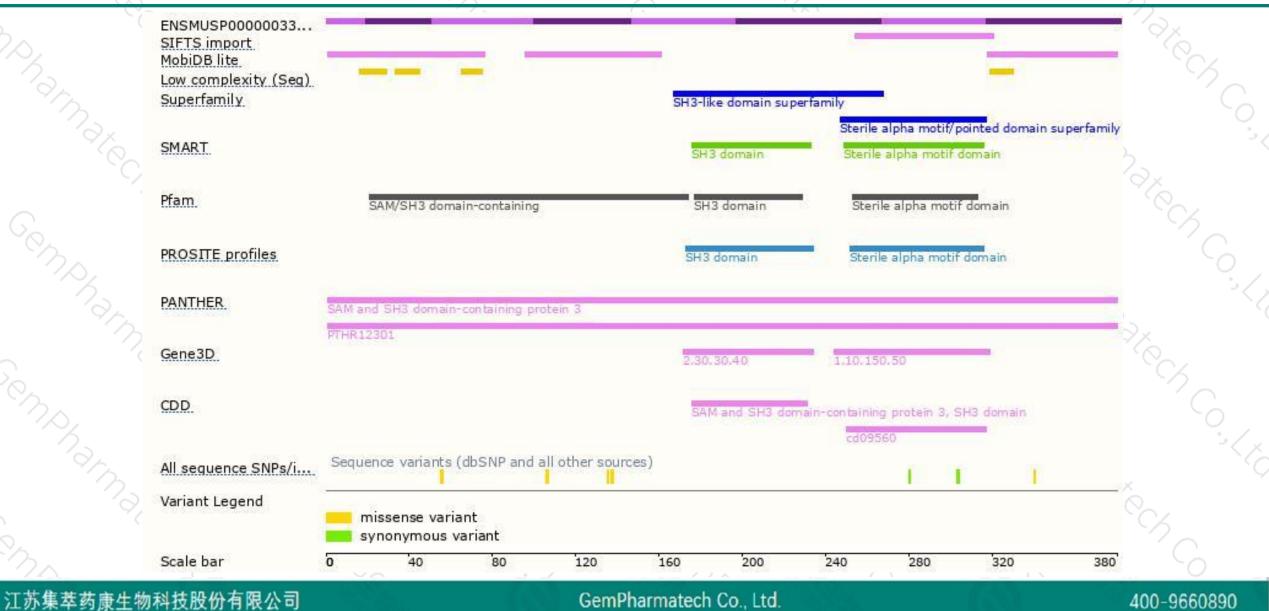
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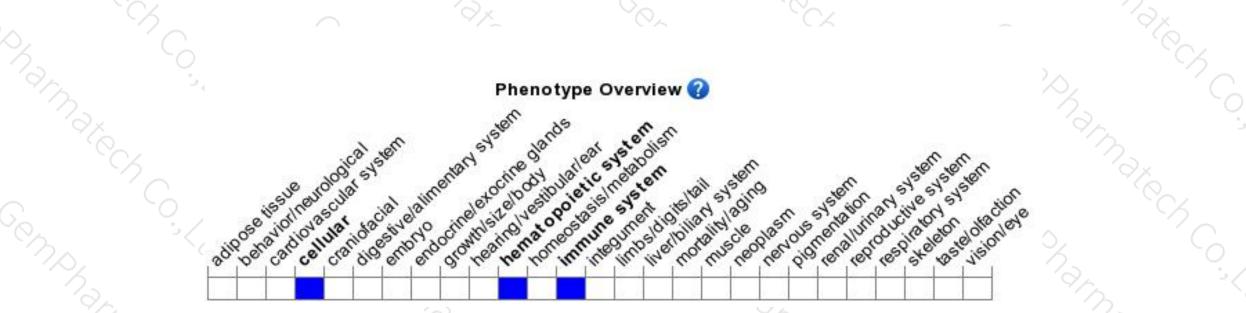
Protein domain





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 truncation allele exhibit decreased T and B cells, altered B cell physiology and increased length of semi-identical allograft survival.



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



