

Srsf5 Cas9-CKO Strategy

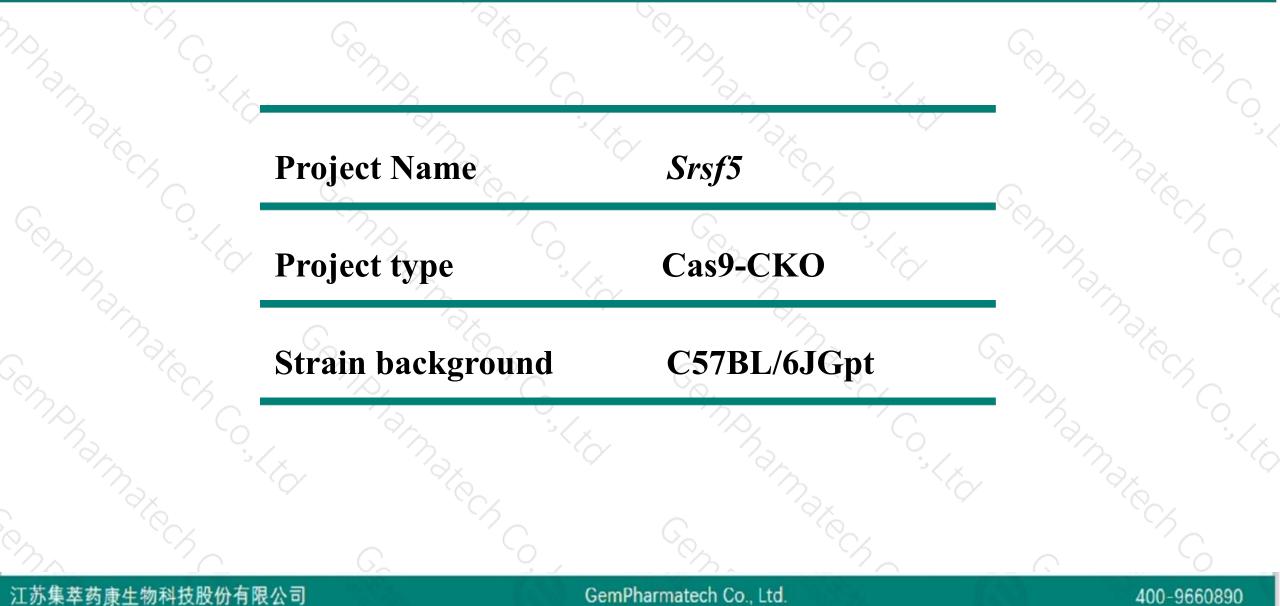
Designer: Xueting Zhang

Reviewer: Daohua Xu

Design Date: 2020-11-10

Project Overview

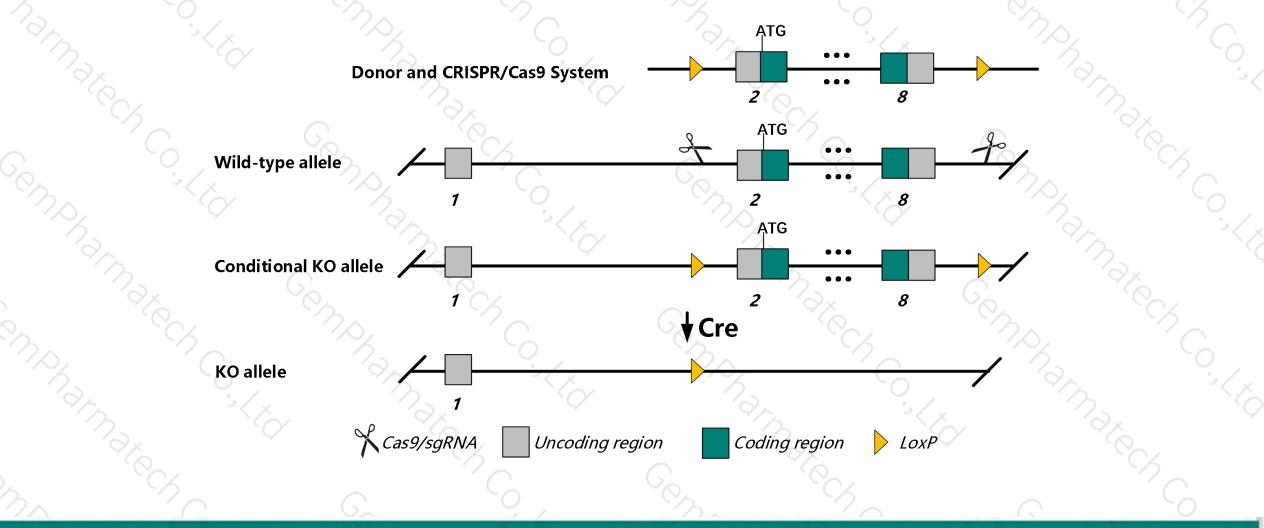




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Srsf5 gene. The schematic diagram is as follows:



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The Srsf5 gene has 12 transcripts. According to the structure of Srsf5 gene, exon2-exon8 of Srsf5-202(ENSMUST00000110351.7) transcript is recommended as the knockout region. The region contains all 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 *Srsf5* 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.



- > The *Srsf5* gene is located on the Chr12. 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.
- > The floxed region is near to the N-terminal of Gm20337 gene, this strategy may influence the regulatory function of the N-terminal of Gm20337 gene.
- > 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.

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Gene information (NCBI)

Srsf5 serine and arginine-rich splicing factor 5 [Mus musculus (house mouse)]

Gene ID: 20384, updated on 13-Mar-2020

- Summary

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Official Symbol	Srsf5 provided by MGI
Official Full Name	serine and arginine-rich splicing factor 5 provided by MGI
Primary source	MGI:MGI:98287
See related	Ensembl:ENSMUSG00000021134
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; Mus; Mus
Also known as	Sfrs5
Summary	The protein encoded by this gene is a member of the serine/arginine (SR)-rich family of pre-mRNA splicing factors, which constitute part of the spliceosome. Each of these factors contains an RNA recognition motif (RRM) for binding RNA and an RS domain for binding other proteins. The RS domain is rich in serine and arginine residues and facilitates interaction between different SR splicing factors. In addition to being critical for mRNA splicing, the SR proteins have also been shown to be involved in mRNA export from the nucleus and in translation. [provided by RefSeq, Nov 2016]
Expression	Ubiquitous expression in CNS E11.5 (RPKM 229.5), bladder adult (RPKM 197.4) and 28 other tissues See more
Orthologs	human all

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Transcript information (Ensembl)



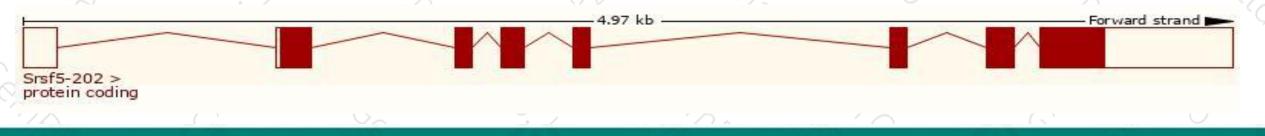
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The gene has 12 transcripts, all transcripts are shown below:

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Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Srsf5-205	ENSMUST00000110356.2	1568	<u>269aa</u>	Protein coding	CCDS36484	<u>035326</u>	TSL:5 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 P3
Srsf5-201	ENSMUST0000094693.10	1527	<u>269aa</u>	Protein coding	CCDS36484	<u>035326</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 P3
Srsf5-202	ENSMUST00000110351.7	1498	<u>270aa</u>	Protein coding	CCDS83974	Q9D8S5	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 ALT
Srsf5-203	ENSMUST00000110352.9	1491	<u>269aa</u>	Protein coding	CCDS36484	<u>035326</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 P3
Srsf5-204	ENSMUST00000110354.7	1489	<u>270aa</u>	Protein coding	CCDS83974	Q9D8S5	TSL:5 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 ALT
Srsf5-208	ENSMUST00000138434.7	547	<u>46aa</u>	Nonsense mediated decay	-	<u>S4R2G3</u>	TSL:3
Srsf5-206	ENSMUST00000125814.8	886	No protein	Processed transcript	-	-	TSL:2
Srsf5-211	ENSMUST00000152662.1	538	No protein	Processed transcript	14 C	2	TSL:1
Srsf5-210	ENSMUST00000151968.1	489	No protein	Processed transcript			TSL:2
Srsf5-209	ENSMUST00000151036.1	3482	No protein	Retained intron		-	TSL:1
Srsf5-207	ENSMUST00000133783.7	2919	No protein	Retained intron	-		TSL:5
Srsf5-212	ENSMUST00000153056.7	2720	No protein	Retained intron	12	2	TSL:5

The strategy is based on the design of *Srsf5-202* transcript, the transcription is shown below:

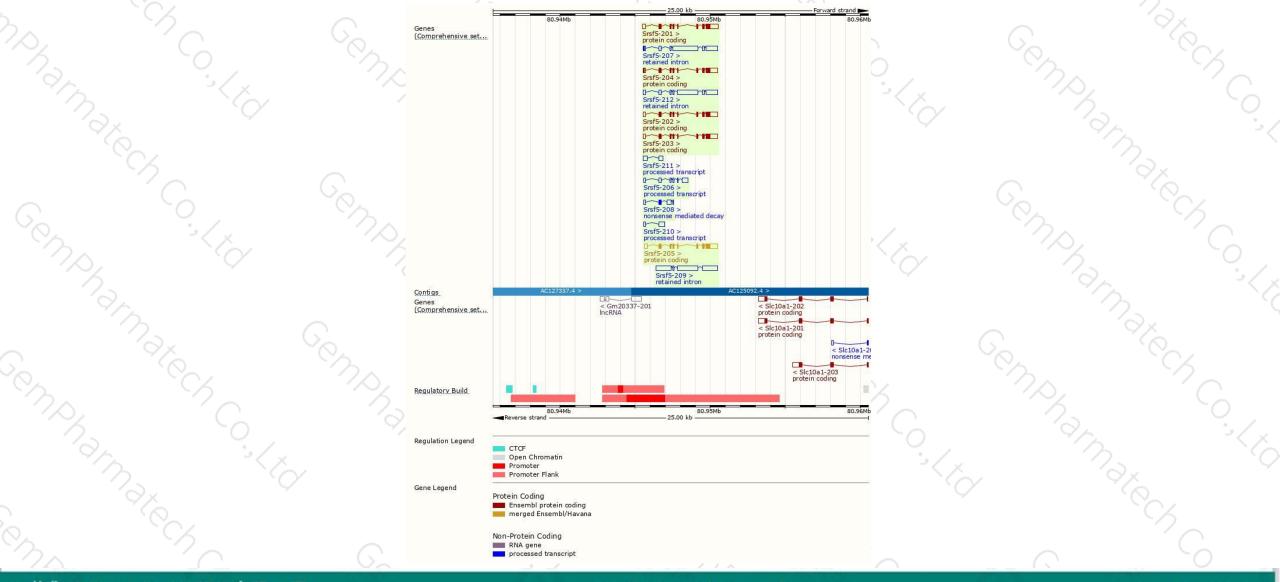


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Genomic location distribution



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Protein domain



10	<u>G</u>					
ENSMUSP00000105 MobiDB lite Low complexity (Seg)					i.s	
Superfamily	RNA-binding domain	n superfamily				
SMART	RNA recognition m	otif domain				
Pfam	RNA recognition m					
PROSITE profiles	RNA recognition mo	otif domain				
PANTHER	PTHR23147:SF68					C
	PTHR23147					
Gene3D	Nucleotide-binding /	alpha-beta plait domain s	superfamily			
CDD	Serine/arginine-ric					
			cd12600			
All sequence SNPs/i	Sequence variants	s (dbSNP and all other	sources)	1	×	
Variant Legend	2 <u></u>					
	📕 stop gained	20. SA				-
	missense var					
	synonymous	variant				
Scale bar	6 4	40 80	120	160	200	270
			<u>`</u> 25.	10		

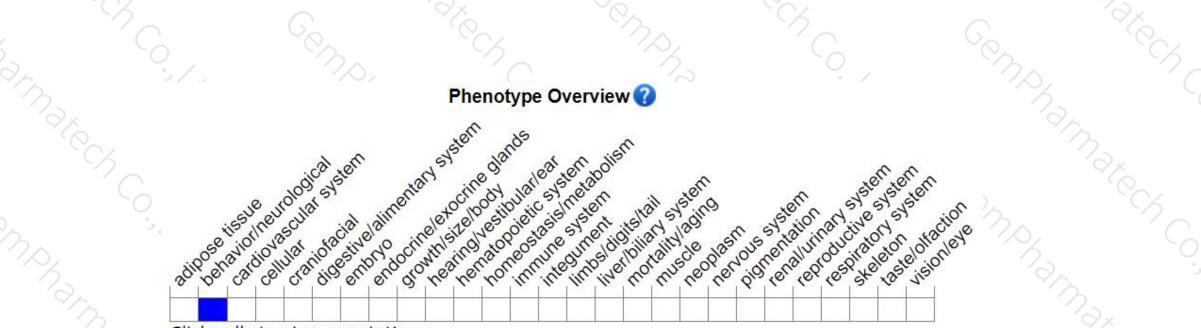
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Mouse phenotype description(MGI)





Click cells to view annotations.

Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).



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



