

Sf3a1 Cas9-CKO Strategy

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Reviewer: Xiaojing Li

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Project Overview



Project Name Sf3a1

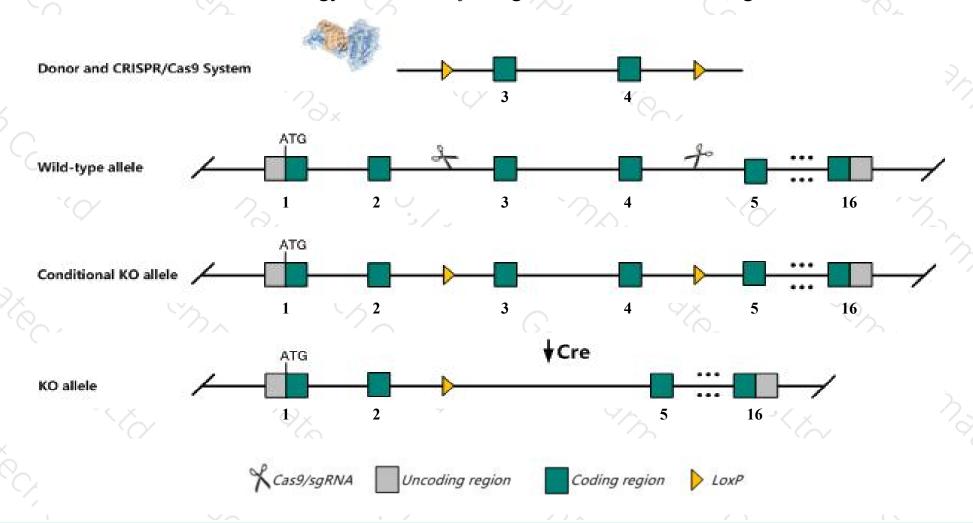
Project type Cas9-CKO

Strain background C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Sf3a1* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Sf3a1* gene has 4 transcripts. According to the structure of *Sf3a1* gene, exon3-exon4 of *Sf3a1*201(ENSMUST00000002198.3) transcript is recommended as the knockout region. The region contains 466bp coding sequence.

 Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Sf3a1* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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.

Notice



- ➤ According to the existing MGI data, mice homozygous for a transgenic gene disruption may exhibit preimplantation lethality.
- The *Sf3a1* gene is located on the Chr11. 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)



Sf3a1 splicing factor 3a, subunit 1 [Mus musculus (house mouse)]

Gene ID: 67465, updated on 13-Mar-2020

Summary

☆ ?

Official Symbol Sf3a1 provided by MGI

Official Full Name splicing factor 3a, subunit 1 provided by MGI

Primary source MGI:MGI:1914715

See related Ensembl:ENSMUSG00000002129

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 1200014H24Rik, 5930416L09Rik, Al159724

Expression Ubiquitous expression in CNS E11.5 (RPKM 27.8), thymus adult (RPKM 22.6) and 28 other tissuesSee more

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Orthologs <u>human all</u>

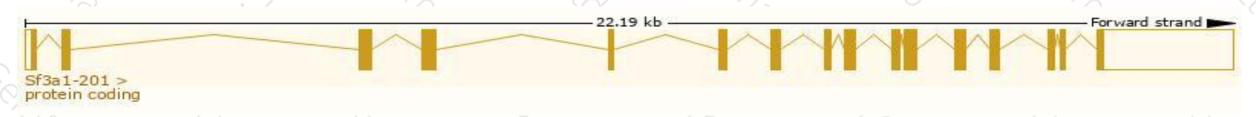
Transcript information (Ensembl)



The gene has 4 transcripts, all transcripts are shown below:

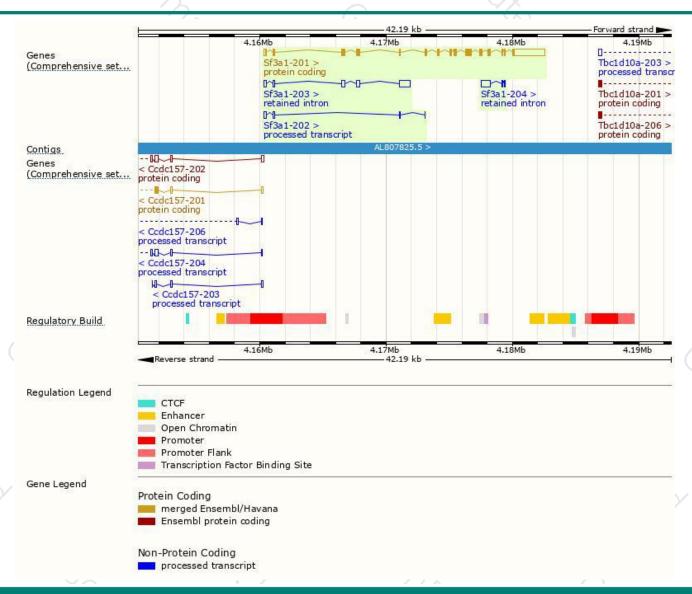
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sf3a1-201	ENSMUST00000002198.3	4920	<u>791aa</u>	Protein coding	CCDS24380	Q8K4Z5	TSL:1 GENCODE basic APPRIS P1
Sf3a1-202	ENSMUST00000129583.7	424	No protein	Processed transcript	-	-	TSL:3
Sf3a1-203	ENSMUST00000132666.1	1636	No protein	Retained intron	2	1 12	TSL:1
Sf3a1-204	ENSMUST00000133095.1	846	No protein	Retained intron		-	TSL:5

The strategy is based on the design of *Sf3a1-201* transcript, the transcription is shown below:



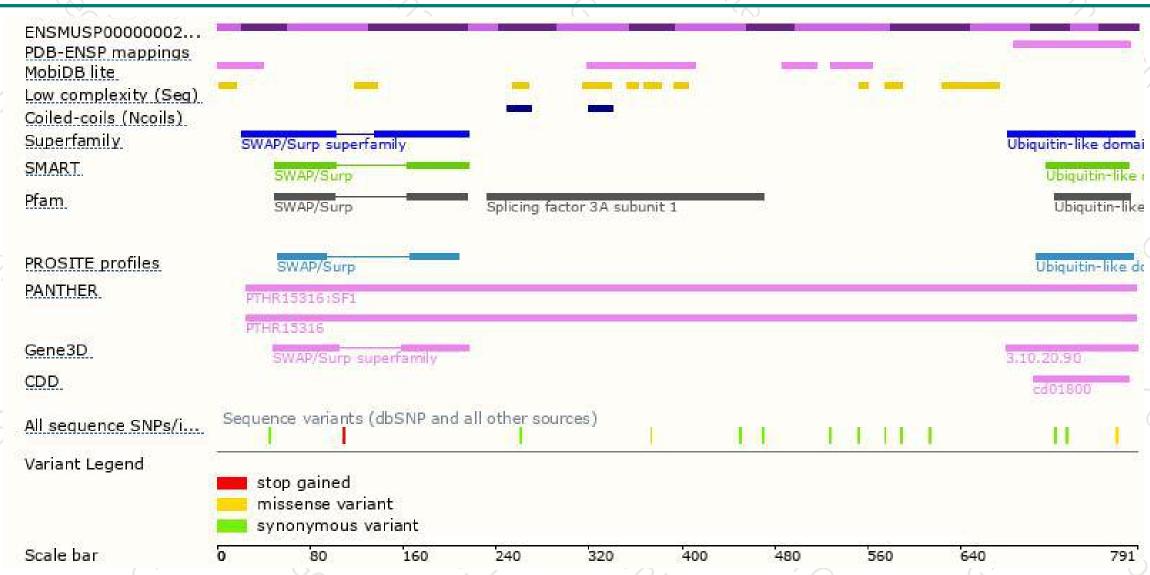
Genomic location distribution





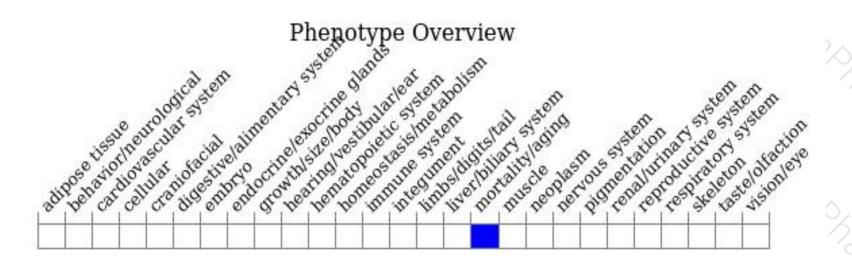
Protein domain





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 transgenic gene disruption may exhibit preimplantation lethality.



If you have any questions, you are welcome to inquire.

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