

Spdef Cas9-CKO Strategy

Designer: Daohua Xu

Reviewer: Huimin Su

Design Date: 2020-2-11

Project Overview



Project Name Spdef

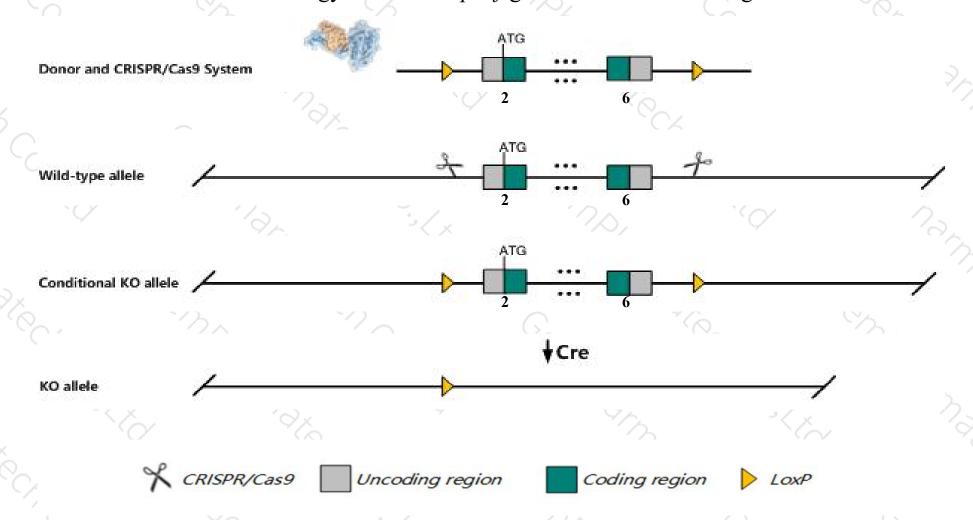
Project type Cas9-CKO

Strain background C57BL/6JGpt

Conditional Knockout strategy



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



Technical routes



- The *Spdef* gene has 6 transcripts. According to the structure of *Spdef* gene, exon2-exon6 of *Spdef-201* (ENSMUST00000025054.9) 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 *Spdef* 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.

Notice



- ➤ According to the existing MGI data, Mice homozygous for a null allele have reduced numbers of intestinal and respiratory mucosa goblet cells. Increased inflammation of the gastric antrum has also been seen.
- The *Spdef* gene is located on the Chr17. 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)



Spdef SAM pointed domain containing ets transcription factor [Mus musculus (house mouse)]

Gene ID: 30051, updated on 5-Mar-2019

Summary

☆ ?

Official Symbol Spdef provided by MGI

Official Full Name SAM pointed domain containing ets transcription factor provided by MGI

Primary source MGI:MGI:1353422

See related Ensembl:ENSMUSG00000024215

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 PDEF, Pse

Expression Biased expression in colon adult (RPKM 65.2), stomach adult (RPKM 24.2) and 4 other tissuesSee more

Orthologs <u>human all</u>

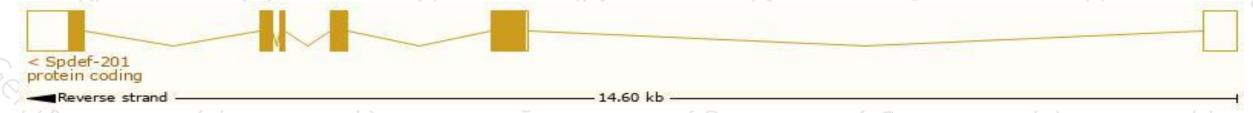
Transcript information (Ensembl)



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

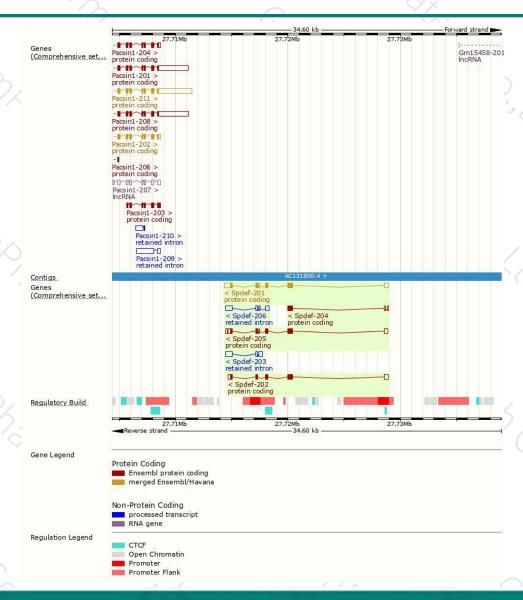
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Spdef-201	ENSMUST00000025054.9	1921	325aa	Protein coding	CCDS28568	Q9WTP3	TSL:1 GENCODE basic APPRIS P2
Spdef-205	ENSMUST00000167489.1	1708	<u>325aa</u>	Protein coding	CCDS28568	Q9WTP3	TSL:5 GENCODE basic APPRIS P2
Spdef-202	ENSMUST00000114870.8	1568	309aa	Protein coding	-	A0A3F2YNL9	TSL:5 GENCODE basic APPRIS ALT2
Spdef-204	ENSMUST00000138970.2	620	<u>126aa</u>	Protein coding	70	B2KF87	CDS 3' incomplete TSL:5
Spdef-206	ENSMUST00000233880.1	1200	No protein	Retained intron	9	65	
Spdef-203	ENSMUST00000127622.2	1045	No protein	Retained intron	le.	87	TSL:2

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



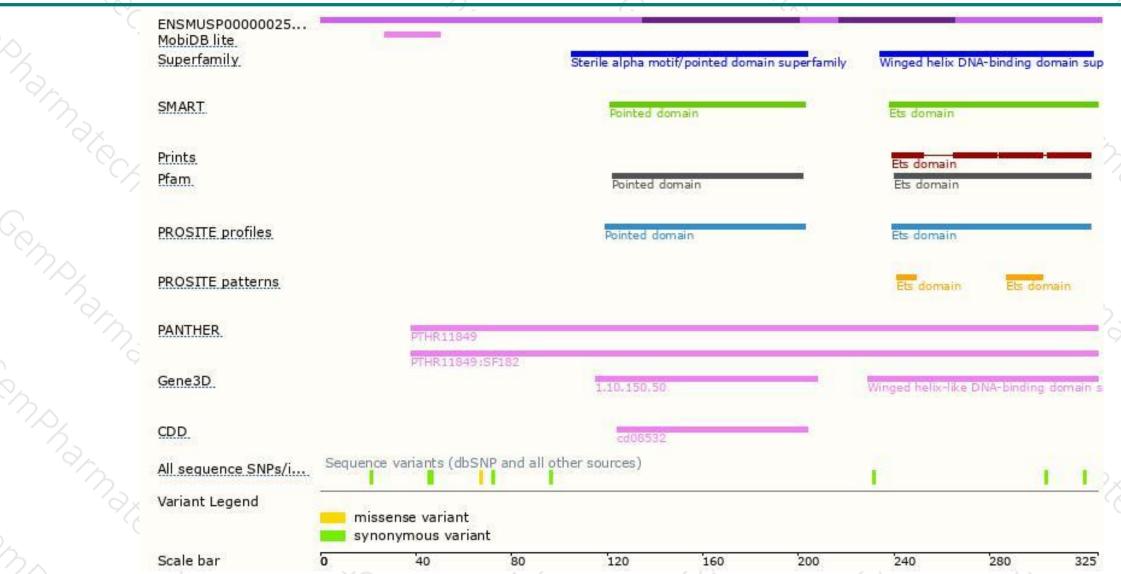
Genomic location distribution





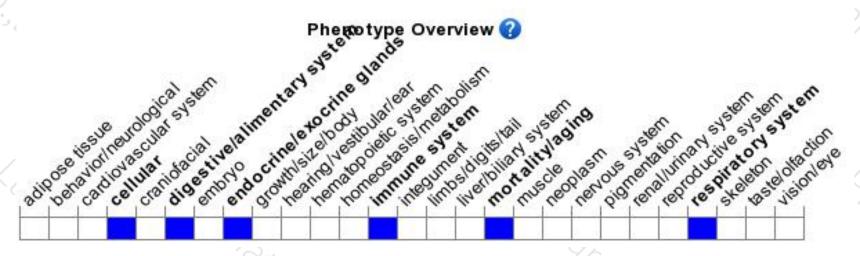
Protein domain





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 null allele have reduced numbers of intestinal and respiratory mucosa goblet cells. Increased inflammation of the gastric antrum has also been seen.



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





