

Sdc4 Cas9-CKO Strategy

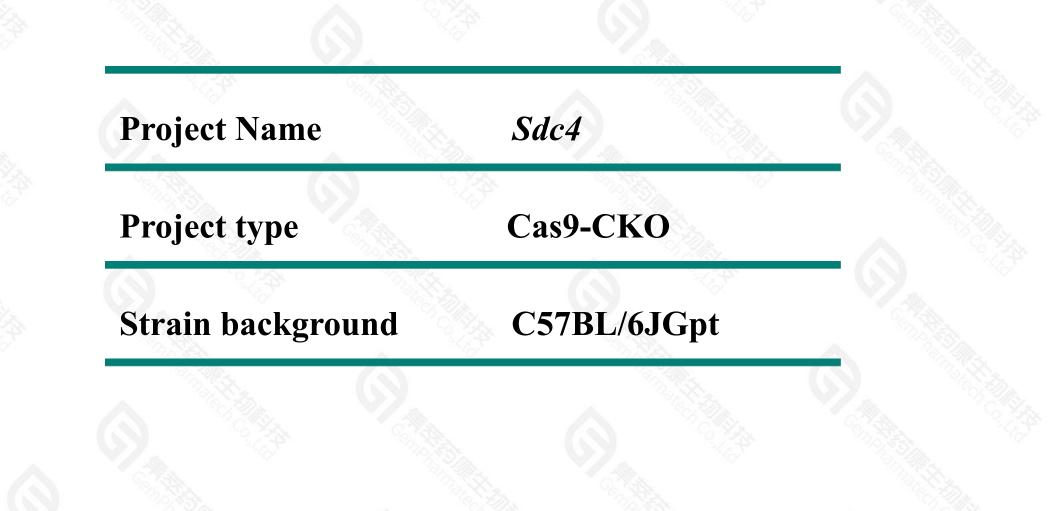
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Reviewer: Xueting Zhang

Design Date: 2021-10-15

Project Overview



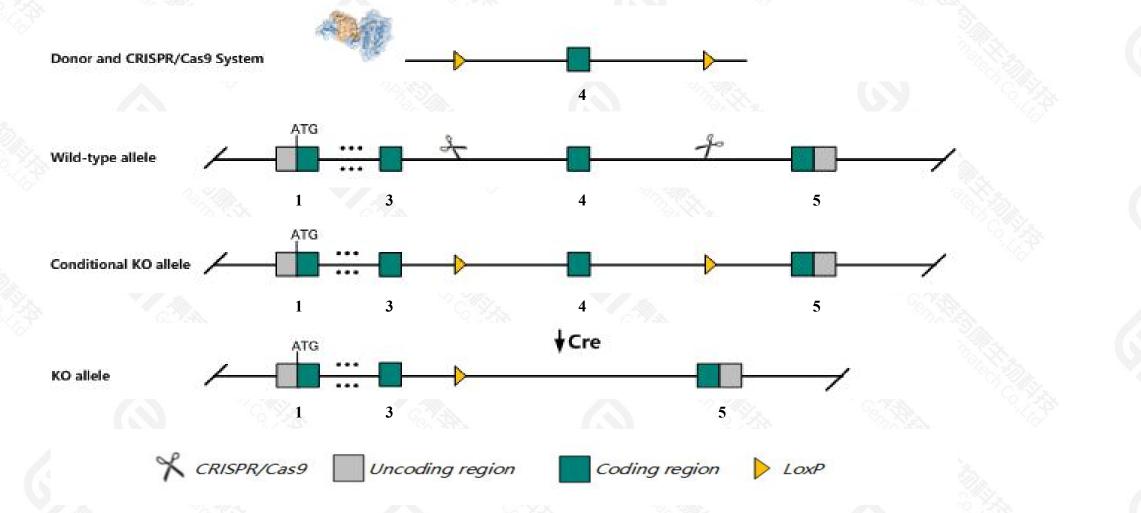


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Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Sdc4* gene. The schematic diagram is as follows:



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Technical routes



> The *Sdc4* gene has 2 transcripts. According to the structure of *Sdc4* gene, exon4 of *Sdc4-201*(ENSMUST00000017153.4) transcript is recommended as the knockout region. The region contains 196bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Sdc4* 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 or heterozygous for a knock-out allele show delayed wound healing and impaired angiogenesis. Homozygotes for a different knock-out allele exhibit degenerated fetal vessels in the placental labyrinth, abnormal cell adhesion, and high susceptibility to induced renal and hepatic injury.
- ➤ The Intron3 and Intron4 are only 591bp and 2655bp,loxp insertion may affect mRNA splicing.
- > The *Sdc4* gene is located on the Chr2. 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)



< ?

Sdc4 syndecan 4 [Mus musculus (house mouse)]

Gene ID: 20971, updated on 2-Feb-2021

Summary

Official SymbolSdc4 provided by MGIOfficial Full Namesyndecan 4 provided byMGIPrimary sourceMGI:MGI:1349164See relatedEnsembl:ENSMUSG0000017009Gene typeprotein codingRefSeq statusPROVISIONALOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Rodentia;
Myomorpha; Muroidea; Murinae; Mus; MusAlso known asAA959608, AW108331, S, S4, Syn, Synd4, ryud, ryudocan, syndec, syndecan-4ExpressionBroad expression in kidney adult (RPKM 79.7), liver adult (RPKM 72.9) and 24 other tissues
See more

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Transcript information (Ensembl)

The gene has 2 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sdc4-201	ENSMUST0000017153.4	2458	<u>198aa</u>	Protein coding	CCD517036		TSL:1 , GENCODE basic , APPRIS P1 ,
Sdc4-202	ENSMUST00000142909.2	797	No protein	Processed transcript	(141)		TSL:3 ,

The strategy is based on the design of *Sdc4-201* transcript, the transcription is shown below:

< Sdc4-201 protein coding

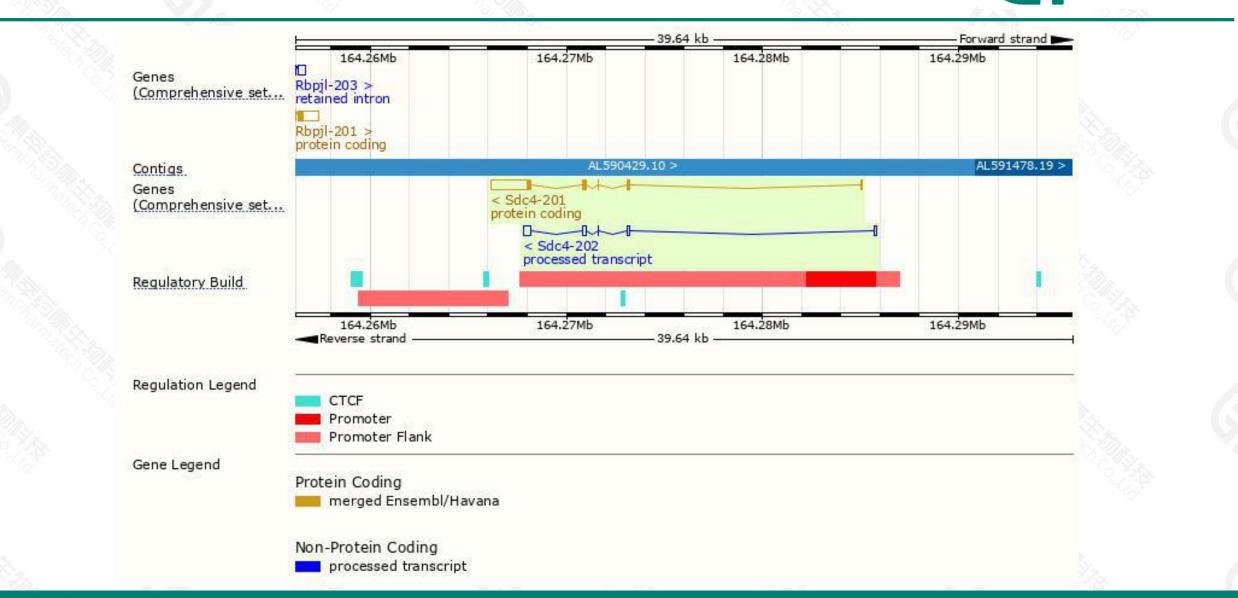
Reverse strand

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18.94 k

Genomic location distribution



集萃

集卒约康 GemPharmatech

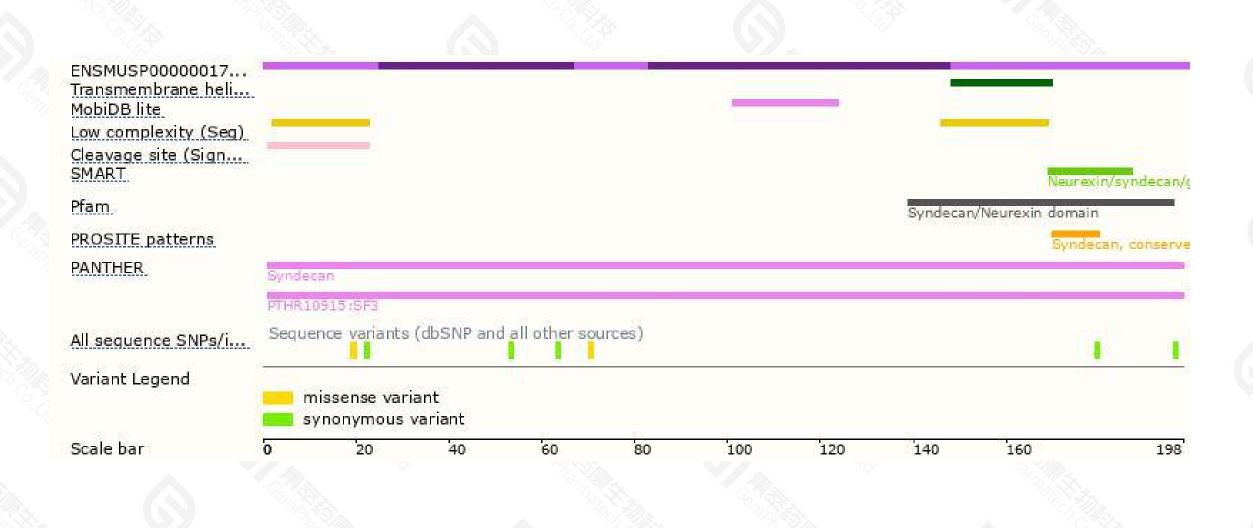
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Protein domain

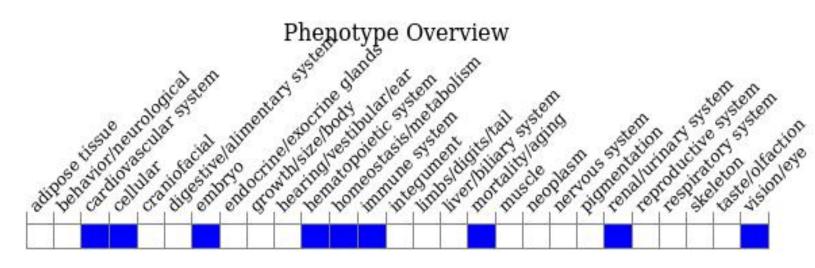




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Mouse phenotype description(MGI)



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 or heterozygous for a knock-out allele show delayed wound healing and impaired angiogenesis. Homozygotes for a different knock-out allele exhibit degenerated fetal vessels in the placental labyrinth, abnormal cell adhesion, and high susceptibility to induced renal and hepatic injury.

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



