

Srd5a3 Cas9-CKO Strategy

Designer: Rui Xiong

Reviewer: Longyun Hu

Design Date: 2021-6-8

Project Overview



Project Name Srd5a3

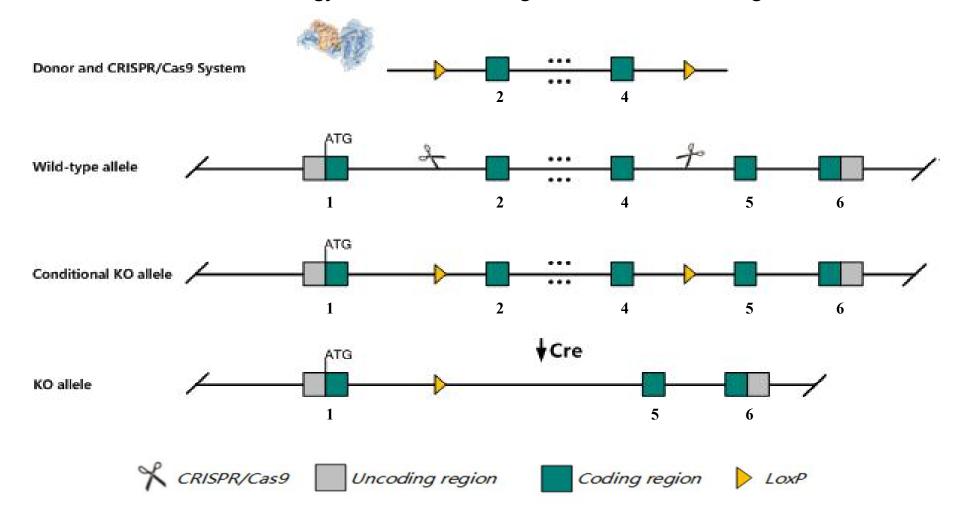
Project type Cas9-CKO

Strain background C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Srd5a3* gene. The schematic diagram is as follows:



Technical routes



The *Srd5a3* gene has 8 transcripts. According to the structure of *Srd5a3* gene, exon2-exon4 of *Srd5a3*-201(ENSMUST00000031143.13) transcript is recommended as the knockout region. The region contains 377bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Srd5a3* 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 gene trapped allele exhibit embryonic lethality between E11.5 and E13.5 with open neural tubes, failure to turn, dilated hearts, and ventral body wall defects.

The *Srd5a3* gene is located on the Chr5. 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



Srd5a3 steroid 5 alpha-reductase 3 [Mus musculus (house mouse)]

Gene ID: 57357, updated on 17-Feb-2021

Summary

☆ ?

Official Symbol Srd5a3 provided by MGI

Official Full Name steroid 5 alpha-reductase 3 provided by MGI

Primary source MGI:MGI:1930252

See related Ensembl:ENSMUSG00000029233

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 1110025P14Rik, A430076C09, AV364670, AW987574, D730040M03Rik, H5ar, S5AR 3, Srd, Srd5a2l

Expression Ubiquitous expression in adrenal adult (RPKM 34.2), ovary adult (RPKM 24.0) and 28 other tissuesSee more

Orthologs <u>human all</u>

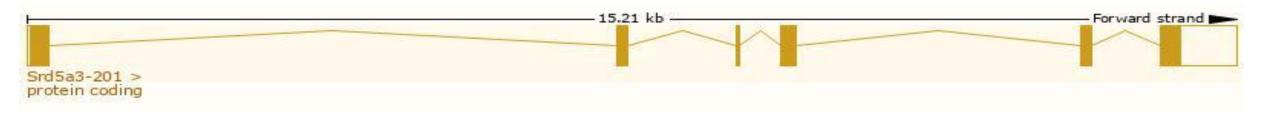
Transcript information Ensembl



The gene has 8 transcripts, all transcripts are shown below:

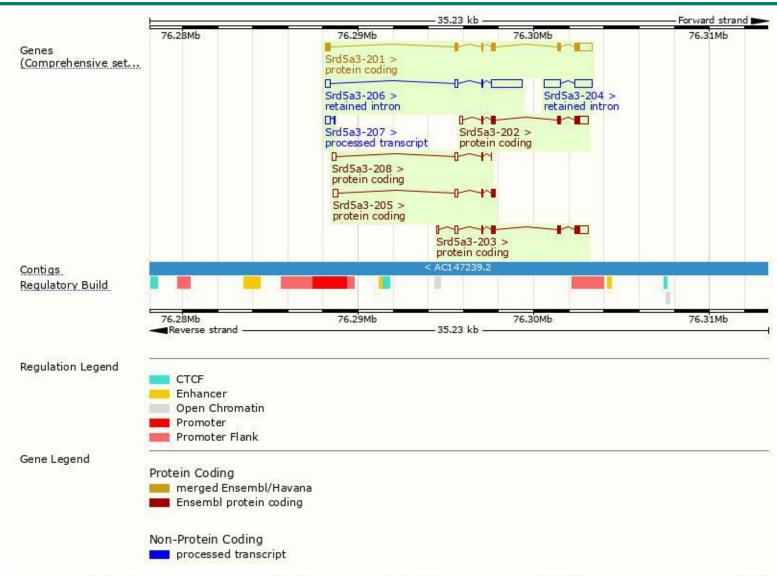
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Srd5a3-201	ENSMUST00000031143.13	1747	330aa	Protein coding	CCDS19358		TSL:1 , GENCODE basic , APPRIS P2 ,
Srd5a3-203	ENSMUST00000113507.8	1358	206aa	Protein coding	=		TSL:1 , GENCODE basic , APPRIS ALT2 ,
Srd5a3-202	ENSMUST00000113506.2	1241	206aa	Protein coding	-		TSL:5 , GENCODE basic , APPRIS ALT2 ,
Srd5a3-205	ENSMUST00000127278.8	628	<u>74aa</u>	Protein coding			CDS 3' incomplete , TSL:3 ,
Srd5a3-208	ENSMUST00000152642.8	351	<u>10aa</u>	Protein coding	2		CDS 3' incomplete , TSL:5 ,
Srd5a3-207	ENSMUST00000149222.2	326	No protein	Processed transcript	-		TSL:3,
Srd5a3-206	ENSMUST00000138699.8	2220	No protein	Retained intron	i -		TSL:1,
Srd5a3-204	ENSMUST00000124217.2	1872	No protein	Retained intron	12		TSL:2,

The strategy is based on the design of *Srd5a3-201* transcript, the transcription is shown below:



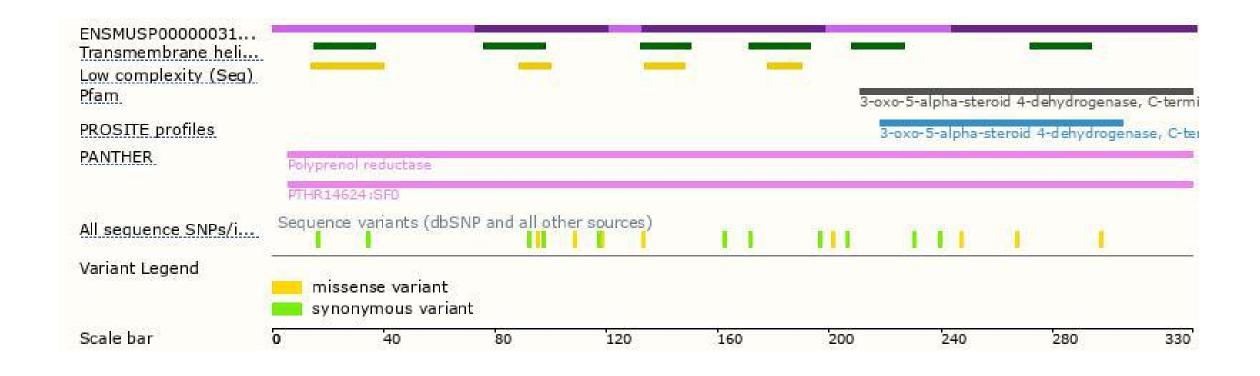
Genomic location distribution





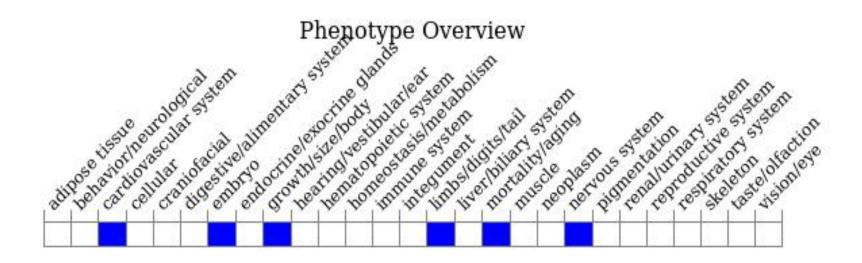
Protein domain





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 gene trapped allele exhibit embryonic lethality between E11.5 and E13.5 with open neural tubes, failure to turn, dilated hearts, and ventral body wall defects.



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





