

Scrib Cas9-CKO Strategy

Designer: Miaomiao Cui

Reviewer: Shilei Zhu

Design Date: 2020-6-18

Project Overview



Project Name

Scrib

Project type

Cas9-CKO

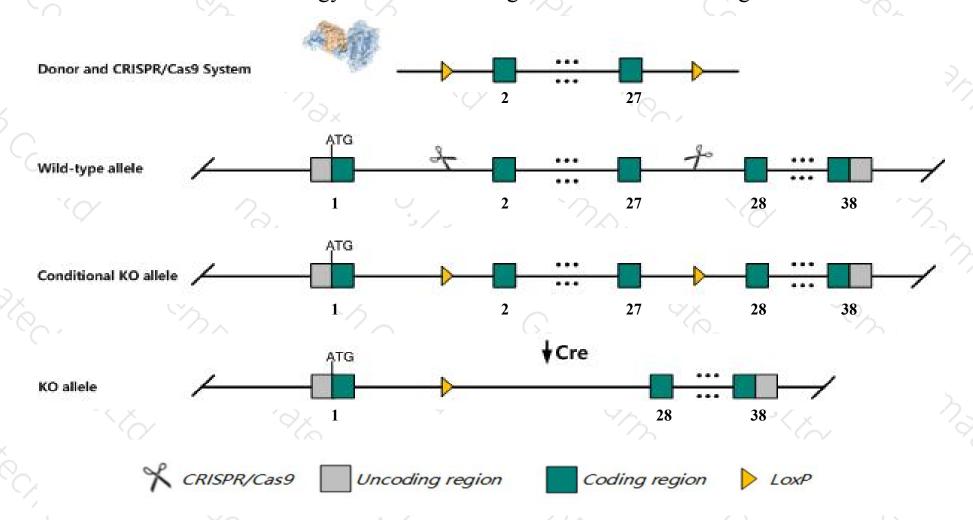
Strain background

C57BL/6JGpt

Conditional Knockout strategy



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



Technical routes



- The *Scrib* gene has 12 transcripts. According to the structure of *Scrib* gene, exon2-exon27 of *Scrib-201* (ENSMUST0000002603.11) transcript is recommended as the knockout region. The region contains most 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 *Scrib* 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 display partial prenatal lethality. mice homozygous for spontaneous or induced alleles exhibit craniofacial or neural tube defects.
- ➤ The KO region deletes most of the coding sequence, but does not result in frameshift.
- ➤ The CKO region contains functional region of the Scrib gene.Conditional Knockout the region may affect the function of Mir6952 gene.
- The *Scrib* gene is located on the Chr15. 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)



Scrib scribbled planar cell polarity [Mus musculus (house mouse)]

Gene ID: 105782, updated on 13-Mar-2020

Summary

☆ ?

Official Symbol Scrib provided by MGI

Official Full Name scribbled planar cell polarity provided by MGI

Primary source MGI:MGI:2145950

See related Ensembl: ENSMUSG00000022568

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 Al118201, CRIB, Crc, SCRIB1, Scrb1, mKIAA0147, vartul

Expression Ubiquitous expression in testis adult (RPKM 45.6), thymus adult (RPKM 36.9) and 28 other tissuesSee more

Orthologs <u>human all</u>

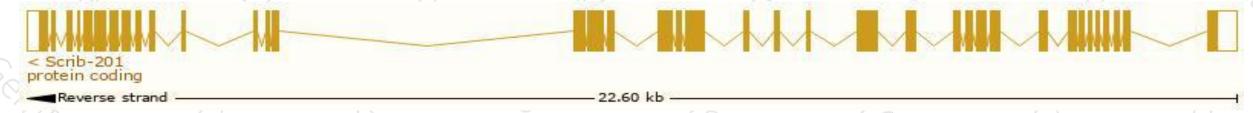
Transcript information (Ensembl)



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

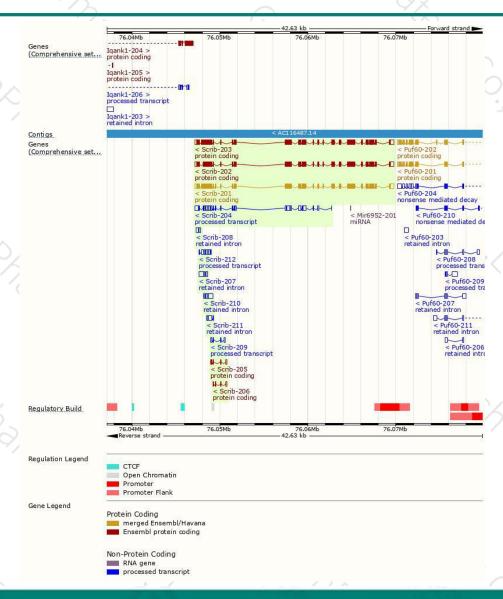
		· /) .					
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Scrib-201	ENSMUST00000002603.11	5599	<u>1665aa</u>	Protein coding	CCDS27560	Q80U72	TSL:1 GENCODE basic APPRIS P3
Scrib-203	ENSMUST00000109946.8	5545	<u>1637aa</u>	Protein coding	CCDS79382	Q80U72	TSL:1 GENCODE basic APPRIS ALT2
Scrib-202	ENSMUST00000063747.11	5440	<u>1612aa</u>	Protein coding	CCDS79381	Q80U72	TSL:1 GENCODE basic APPRIS ALT2
Scrib-205	ENSMUST00000145830.7	423	113aa	Protein coding	-	D3YU97	CDS 3' incomplete TSL:2
Scrib-206	ENSMUST00000148211.2	353	<u>90aa</u>	Protein coding	2	D3YVA3	CDS 3' incomplete TSL:2
Scrib-204	ENSMUST00000136390.7	3071	No protein	Processed transcript	5	-	TSL:1
Scrib-212	ENSMUST00000231218.1	819	No protein	Processed transcript	-	-	
Scrib-209	ENSMUST00000230104.1	454	No protein	Processed transcript	-	2	
Scrib-210	ENSMUST00000230217.1	876	No protein	Retained intron		-	
Scrib-207	ENSMUST00000229896.1	835	No protein	Retained intron	2	-	
Scrib-211	ENSMUST00000230249.1	586	No protein	Retained intron	-	2	
Scrib-208	ENSMUST00000229940.1	439	No protein	Retained intron	,	-	

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



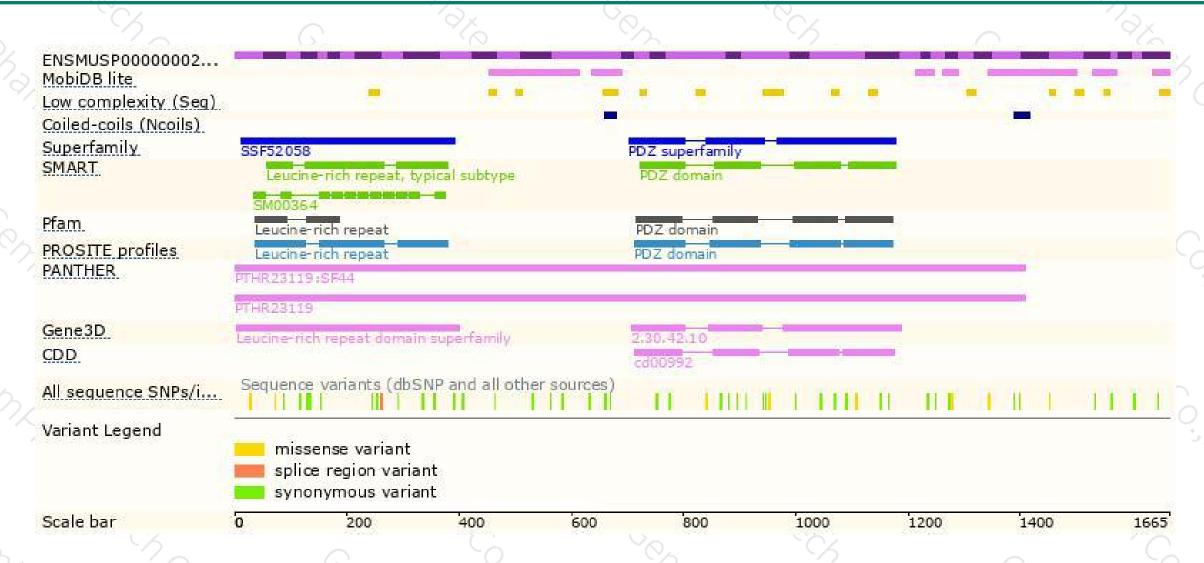
Genomic location distribution





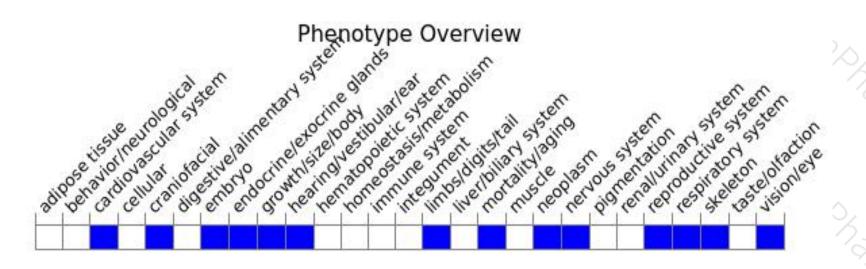
Protein domain





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 display partial prenatal lethality. Mice homozygous for spontaneous or induced alleles exhibit craniofacial or neural tube defects.



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





