

Scn5a Cas9-CKO Strategy

Designer: Xiaojing Li

Reviewer: JiaYu

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Project Overview

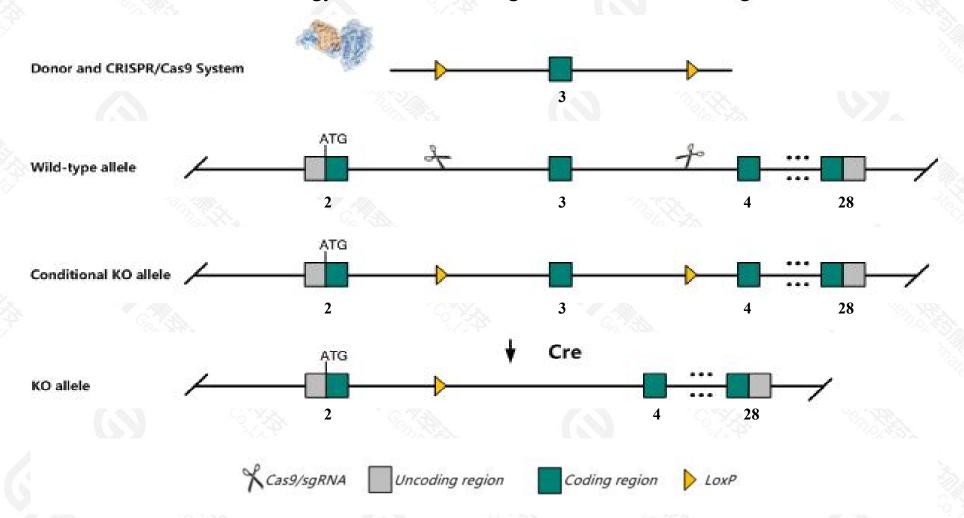


Project Name	Scn5a		
Project type	Cas9-CKO		
Strain background	C57BL/6JGpt		

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Scn5a* gene. The schematic diagram is as follows:



Technical routes



- > The Scn5a gene has 4 transcripts. According to the structure of Scn5a gene, exon3 of Scn5a-202(ENSMUST00000117911.7) transcript is recommended as the knockout region. The region contains 119bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Scn5a* 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 mutations in this gene die prenatally usually during organogenesis and may display decreased embryo size and abnormal cardiovascular system physiology. Heterozygous mice typically display abnormal heartbeats and defects in the function of the impulse conduction system.
- > The Scn5a gene is located on the Chr9. 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)



Scn5a sodium channel, voltage-gated, type V, alpha [Mus musculus (house mouse)]

Gene ID: 20271, updated on 13-Mar-2020

Summary



Official Symbol Scn5a provided by MGI

Official Full Name sodium channel, voltage-gated, type V, alpha provided by MGI

Primary source MGI:MGI:98251

See related Ensembl: ENSMUSG00000032511

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 Nav1.5, Nav1.5c, SkM1, SkM2, mH1

Expression Biased expression in heart adult (RPKM 37.1) and lung adult (RPKM 3.7)See more

Orthologs <u>human all</u>

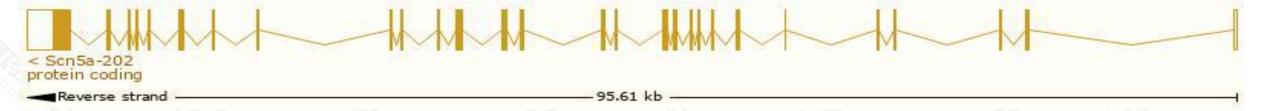
Transcript information (Ensembl)



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

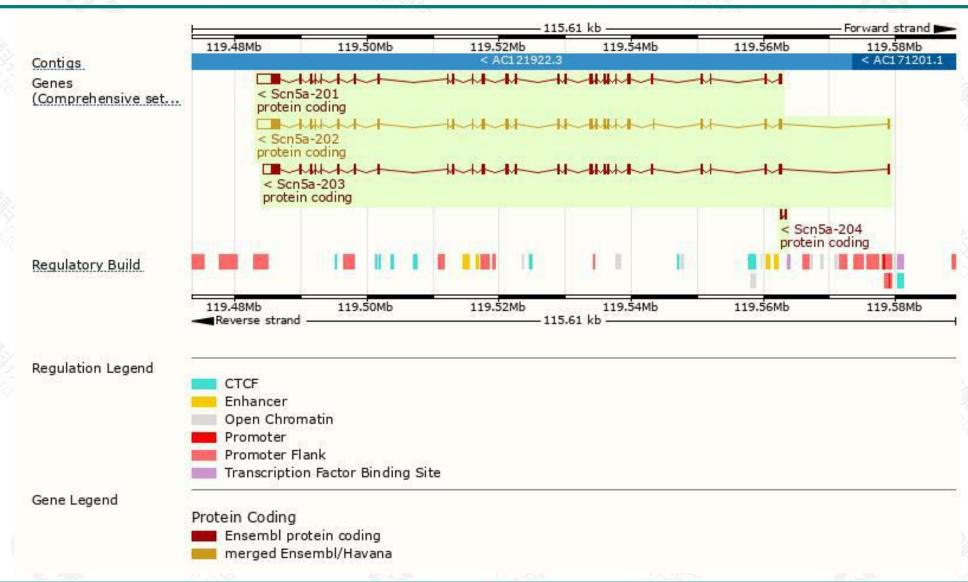
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Scn5a-202	ENSMUST00000117911.7	8453	2020aa	Protein coding	CCDS52965	A0A0R4J1M7	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Scn5a-201	ENSMUST00000065196.12	8287	<u>2020aa</u>	Protein coding	CCDS57715	K3W4N7	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT1
Scn5a-203	ENSMUST00000120420.1	7536	2019aa	Protein coding	12	Q9JJV9	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT1
Scn5a-204	ENSMUST00000138934.1	359	<u>29aa</u>	Protein coding	12	D3YXW2	CDS 3' incomplete TSL:3

The strategy is based on the design of *Scn5a-202* transcript, the transcription is shown below:



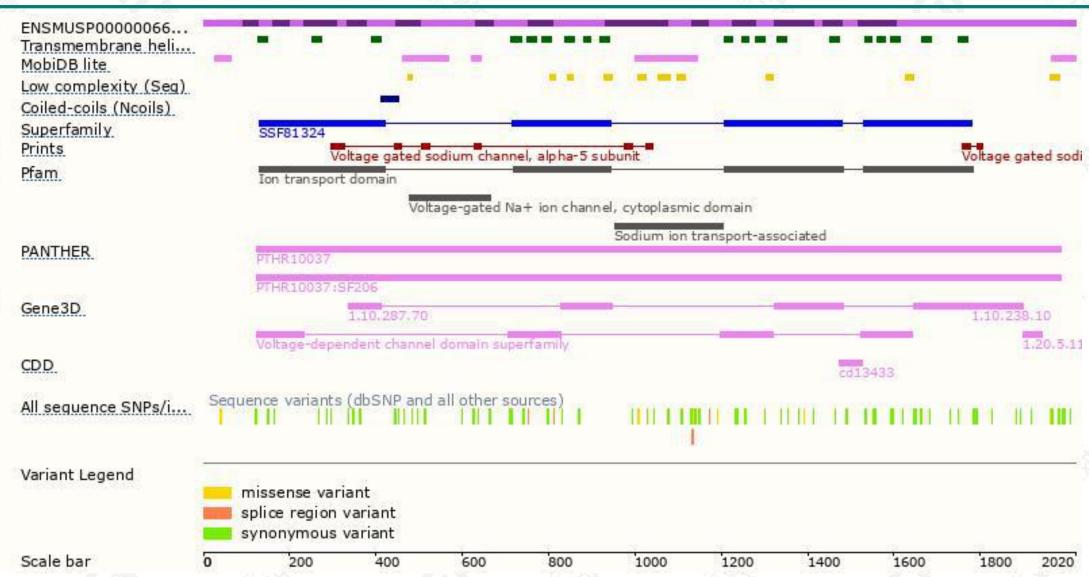
Genomic location distribution





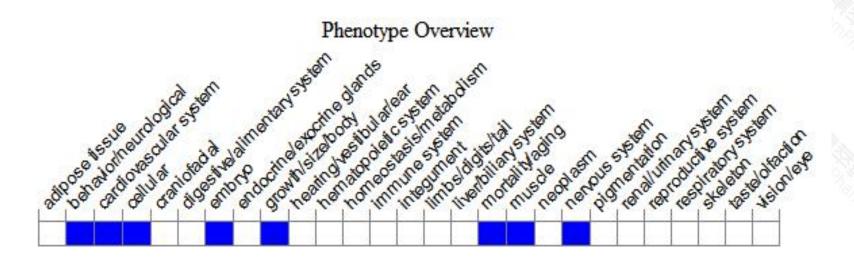
Protein domain





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 mutations in this gene die prenatally usually during organogenesis and may display decreased embryo size and abnormal cardiovascular system physiology. Heterozygous mice typically display abnormal heartbeats and defects in the function of the impulse conduction system.



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

Tel: 025-5864 1534





