

Iscu Cas9-CKO Strategy

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Project Overview



Project Name Iscu

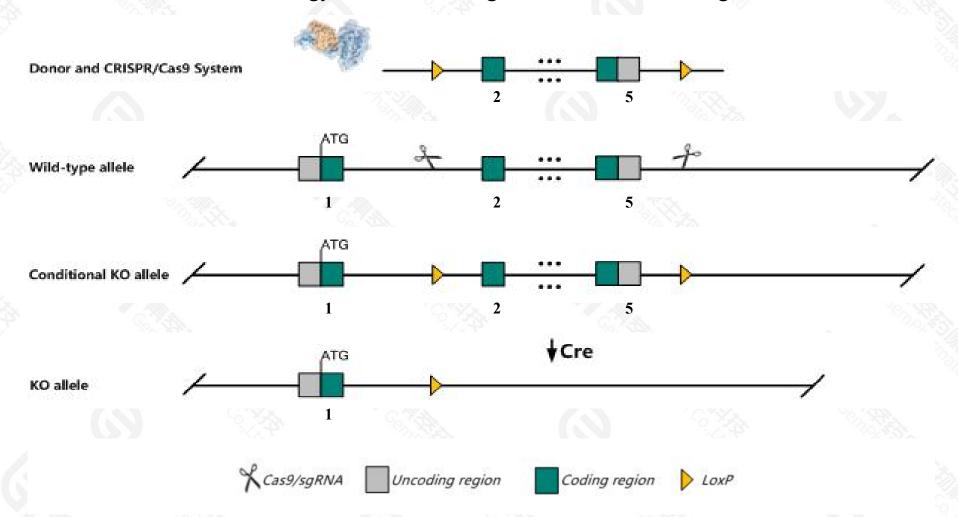
Project type Cas9-CKO

Strain background C57BL/6JGpt

Conditional Knockout strategy



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



Technical routes



- > The *Iscu* gene has 7 transcripts. According to the structure of *Iscu* gene, exon2-exon5 of *Iscu-201*(ENSMUST00000026937.12) 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 *Iscu* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor 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



- > The KO region contains functional region of the Iscu gene. Knockout the region may affect the function of Sart3 gene.
- > The *Iscu* 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)



Iscu iron-sulfur cluster assembly enzyme [Mus musculus (house mouse)]

Gene ID: 66383, updated on 17-Dec-2020

Summary



Official Symbol Iscu provided by MGI

Official Full Name iron-sulfur cluster assembly enzyme provided by MGI

Primary source MGI:MGI:1913633

See related Ensembl: ENSMUSG00000025825

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 2310020H20Rik, AA407971, ISCU2, Ni, Nifu, Nifun

Expression Ubiquitous expression in adrenal adult (RPKM 128.4), kidney adult (RPKM 126.9) and 28 other tissuesSee more

Orthologs <u>human all</u>

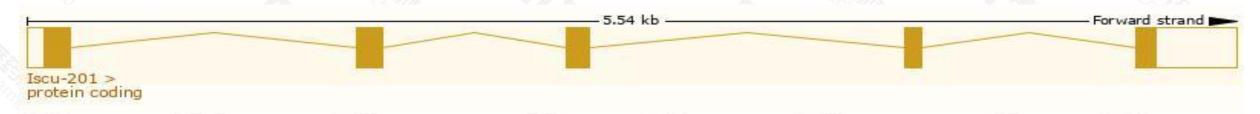
Transcript information (Ensembl)



The gene has 7 transcripts, all transcripts are shown below:

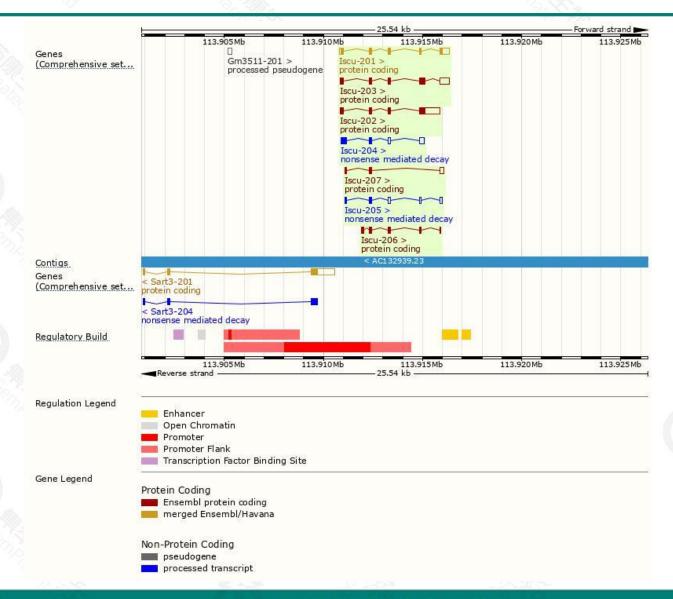
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Iscu-201	ENSMUST00000026937.12	957	<u>168aa</u>	Protein coding	CCDS19553		TSL:1 , GENCODE basic , APPRIS P1
Iscu-202	ENSMUST00000112311.8	1395	209aa	Protein coding	-		TSL:2 , GENCODE basic ,
Iscu-203	ENSMUST00000112312.8	1116	209aa	Protein coding	0		TSL:5 , GENCODE basic ,
Iscu-206	ENSMUST00000145592.2	431	136aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Iscu-207	ENSMUST00000145778.8	388	<u>65aa</u>	Protein coding	2		CDS 5' incomplete , TSL:3 ,
Iscu-204	ENSMUST00000123616.8	773	124aa	Nonsense mediated decay			TSL:3,
Iscu-205	ENSMUST00000134881.2	489	<u>59aa</u>	Nonsense mediated decay	-		CDS 5' incomplete , TSL:3 ,

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



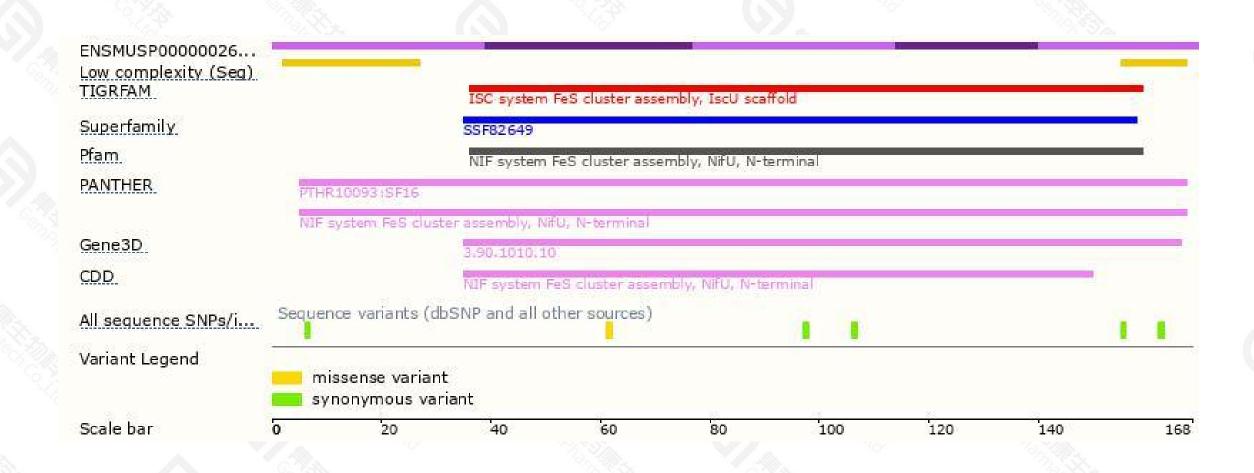
Genomic location distribution





Protein domain







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

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