

Calr Cas9-CKO Strategy

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Project Overview

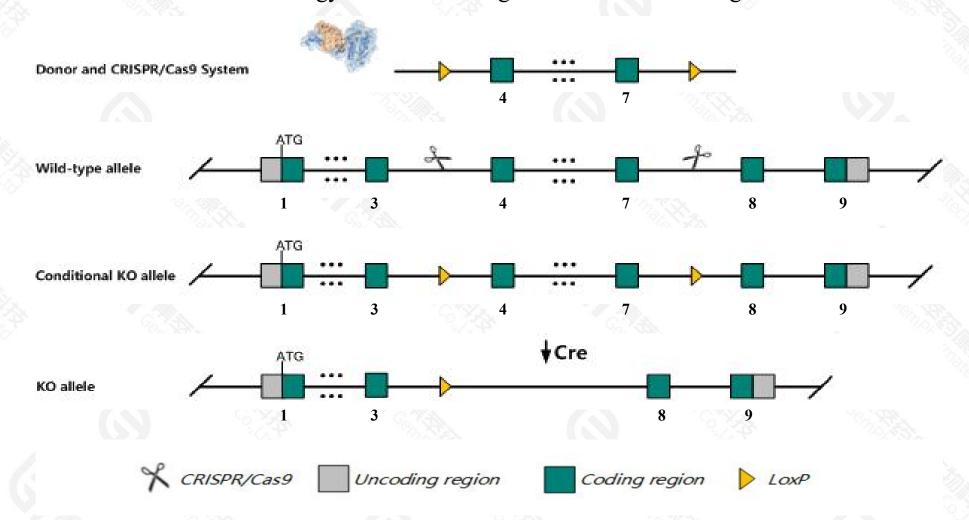


Project Name	Calr	
Project type	Cas9-CKO	
Strain background	C57BL/6JGpt	

Conditional Knockout strategy



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



Technical routes



- ➤ The *Calr* gene has 4 transcripts. According to the structure of *Calr* gene, exon4-exon7 of *Calr*201(ENSMUST0000003912.7) transcript is recommended as the knockout region. The region contains 563bp coding sequence.

 Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Calr* 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, homozygotes for targeted null mutations exhibit decreased cardiac cell mass, increased apoptosis of cardiac myocytes, neural tube defects (sometimes associated with exencephaly), omphalocele, and mid- to late-gestational lethality.
- > The *Calr* gene is located on the Chr8. 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)



Calr calreticulin [Mus musculus (house mouse)]

Gene ID: 12317, updated on 13-Mar-2020

Summary

☆ ?

Official Symbol Calr provided by MGI

Official Full Name calreticulin provided by MGI

Primary source MGI:MGI:88252

See related Ensembl: ENSMUSG00000003814

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 CRT, Calregulin

Expression Ubiquitous expression in placenta adult (RPKM 574.7), genital fat pad adult (RPKM 279.2) and 28 other tissuesSee more

Orthologs <u>human all</u>

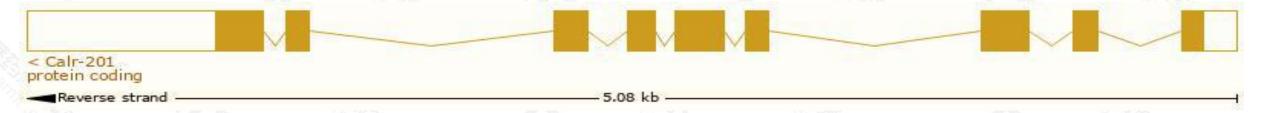
Transcript information (Ensembl)



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

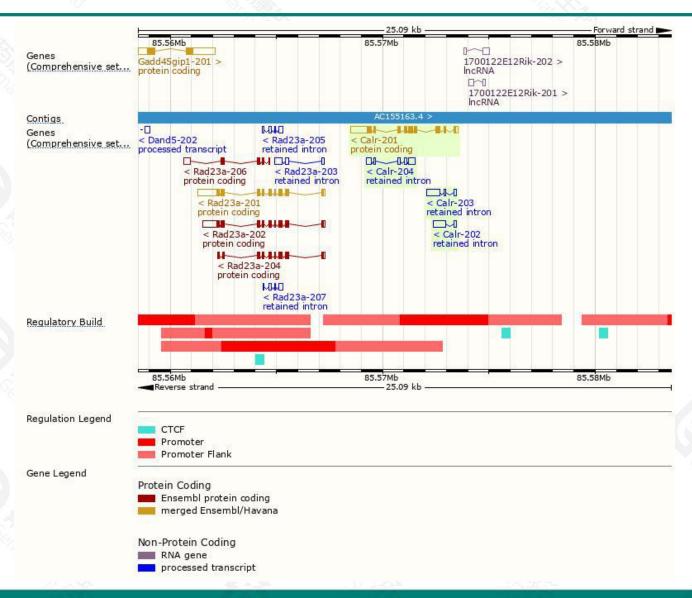
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Calr-201	ENSMUST00000003912.6	2184	416aa	Protein coding	CCDS22479	B2MWM9 P14211	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Calr-204	ENSMUST00000154774.1	943	No protein	Retained intron	-	-	TSL:2
Calr-203	ENSMUST00000128028.1	854	No protein	Retained intron	=	-	TSL:2
Calr-202	ENSMUST00000125998.1	721	No protein	Retained intron	85	2	TSL:2

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



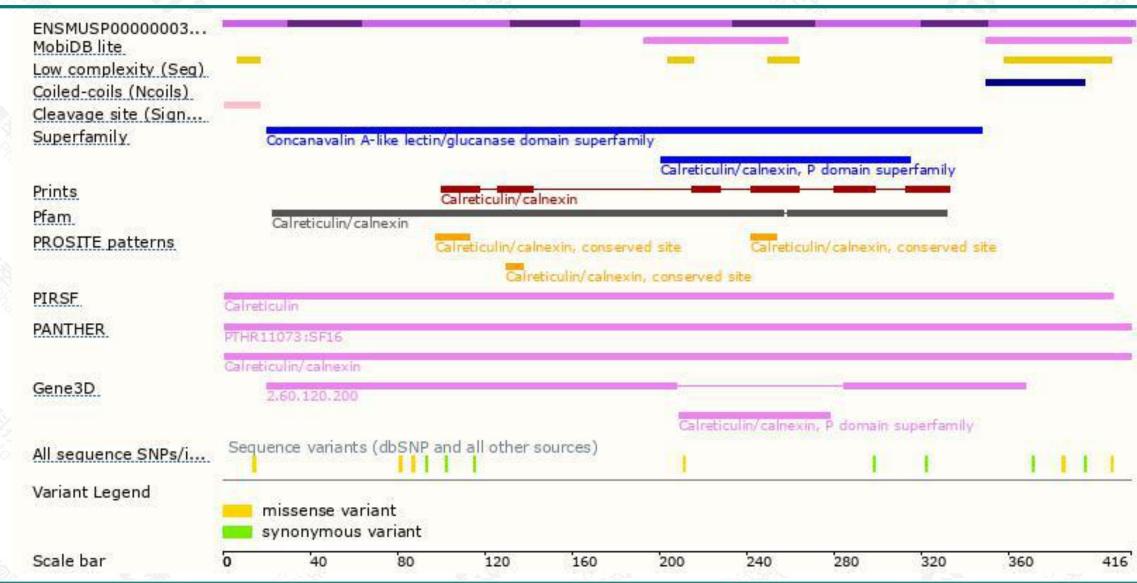
Genomic location distribution





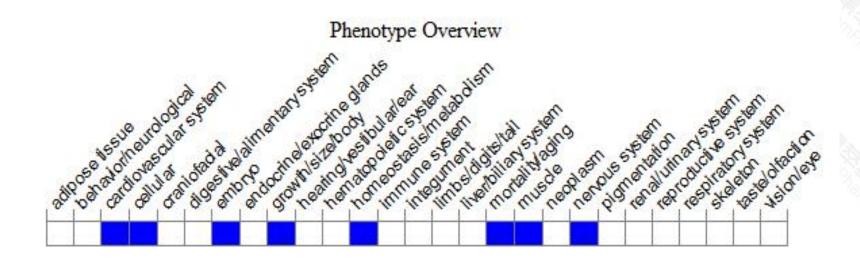
Protein domain





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, homozygotes for targeted null mutations exhibit decreased cardiac cell mass, increased apoptosis of cardiac myocytes, neural tube defects (sometimes associated with exencephaly), omphalocele, and mid- to late-gestational lethality.



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

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