

# Lcn2 Cas9-CKO Strategy

Designer: Huan Wang

**Design Date:** 2019-7-25

## **Project Overview**



**Project Name** 

Lcn2

**Project type** 

Cas9-CKO

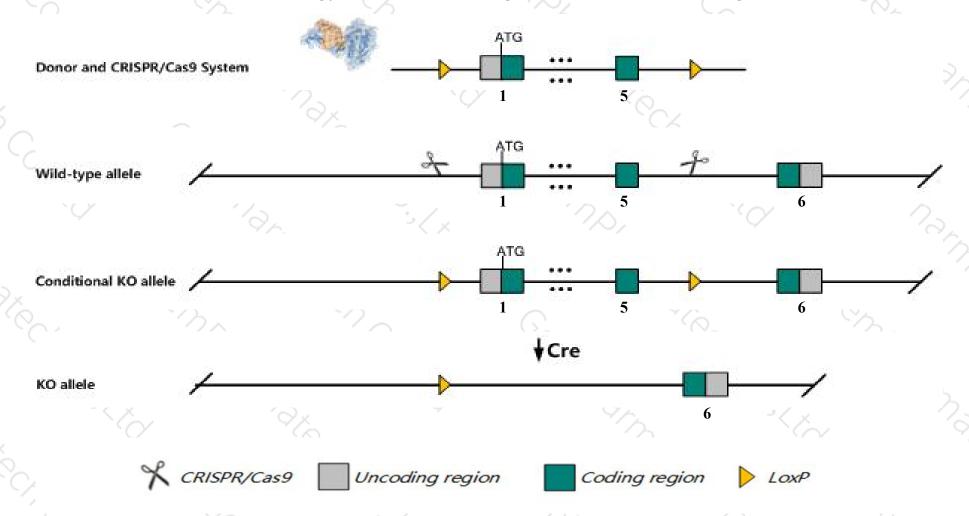
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Lcn2* gene. The schematic diagram is as follows:



### Technical routes



- ➤ The *Lcn2* gene has 6 transcripts. According to the structure of *Lcn2* gene, exon1-exon5 of *Lcn2-201*(ENSMUST0000050785.13) transcript is recommended as the knockout region. The region contains start codon ATG.

  Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Lcn2* 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, Homozygous mutants are more susceptible to infection with bacteria that utilize enterochelin-type siderophores to acquire iron and impaired thermogenesis. Mice homozygous for another knock-out allele exhibit apoptotic defects in hematopoietic cells.
- > The *Lcn2* gene is located on the Chr2. 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)



#### Lcn2 lipocalin 2 [Mus musculus (house mouse)]

Gene ID: 16819, updated on 9-Apr-2019

#### Summary

☆ ?

Official Symbol Lcn2 provided by MGI

Official Full Name lipocalin 2 provided by MGI

Primary source MGI:MGI:96757

See related Ensembl:ENSMUSG00000026822

Gene type protein coding
RefSeq status PROVISIONAL
Organism Mus musculus

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;

Muroidea; Muridae; Murinae; Mus; Mus

Also known as 24p3, AW212229, NRL, Sip24

Expression Biased expression in genital fat pad adult (RPKM 1633.0), liver E18 (RPKM 272.6) and 3 other tissuesSee more

Orthologs <u>human</u> all

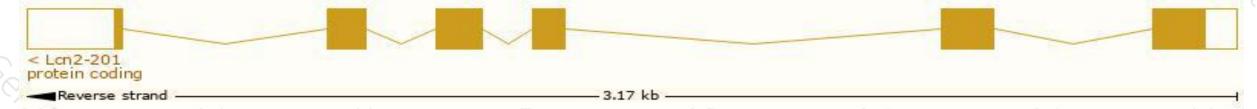
## Transcript information (Ensembl)



The gene has 6 transcripts, all transcripts are shown below:

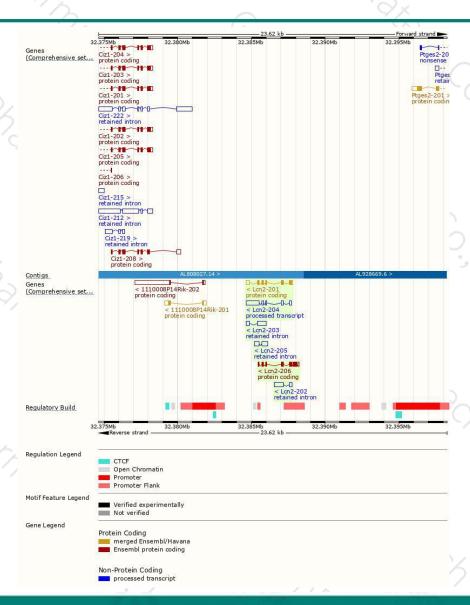
		<u> </u>				
Transcript ID	bp	Protein	Biotype	ccds	UniProt	Flags
ENSMUST00000050785.13	915	200aa	Protein coding	CCDS15913	P11672	TSL:1 GENCODE basic APPRIS P1
ENSMUST00000192241.1	908	<u>271aa</u>	Protein coding	5	A0A0A6YW77	CDS 3' incomplete TSL:5
ENSMUST00000147219.1	1374	No protein	Processed transcript	ų.	-	TSL:2
ENSMUST00000144569.1	827	No protein	Retained intron	2	22	TSL:2
ENSMUST00000136509.1	789	No protein	Retained intron	ā	9	TSL:2
ENSMUST00000155830.1	740	No protein	Retained intron			TSL:2
	ENSMUST00000050785.13  ENSMUST00000192241.1  ENSMUST00000147219.1  ENSMUST00000144569.1  ENSMUST00000136509.1	ENSMUST00000050785.13 915 ENSMUST00000192241.1 908 ENSMUST00000147219.1 1374 ENSMUST00000144569.1 827 ENSMUST00000136509.1 789	ENSMUST00000050785.13         915         200aa           ENSMUST00000192241.1         908         271aa           ENSMUST00000147219.1         1374         No protein           ENSMUST00000144569.1         827         No protein           ENSMUST00000136509.1         789         No protein	ENSMUST00000050785.13         915         200aa         Protein coding           ENSMUST00000192241.1         908         271aa         Protein coding           ENSMUST00000147219.1         1374         No protein         Processed transcript           ENSMUST00000144569.1         827         No protein         Retained intron           ENSMUST00000136509.1         789         No protein         Retained intron	ENSMUST00000050785.13         915         200aa         Protein coding         CCDS15913           ENSMUST00000192241.1         908         271aa         Protein coding         -           ENSMUST00000147219.1         1374         No protein         Processed transcript         -           ENSMUST00000144569.1         827         No protein         Retained intron         -           ENSMUST00000136509.1         789         No protein         Retained intron         -	ENSMUST00000050785.13         915         200aa         Protein coding         CCDS15913         P11672           ENSMUST00000192241.1         908         271aa         Protein coding         -         A0A0A6YW77           ENSMUST00000147219.1         1374         No protein         Processed transcript         -         -           ENSMUST00000144569.1         827         No protein         Retained intron         -         -           ENSMUST00000136509.1         789         No protein         Retained intron         -         -

The strategy is based on the design of *Lcn2-201* transcript, The transcription is shown below



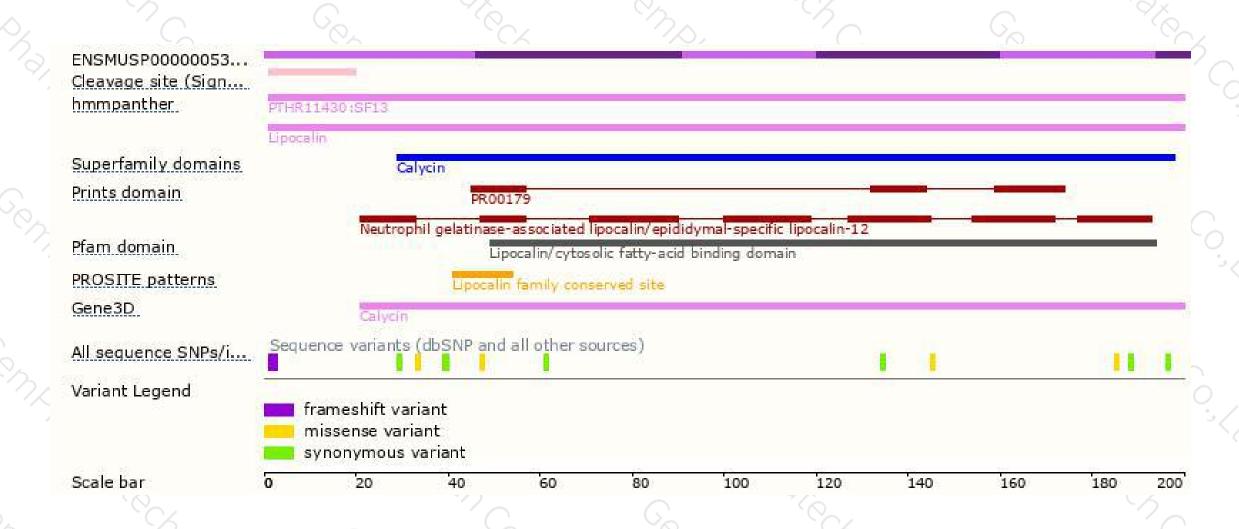
### Genomic location distribution





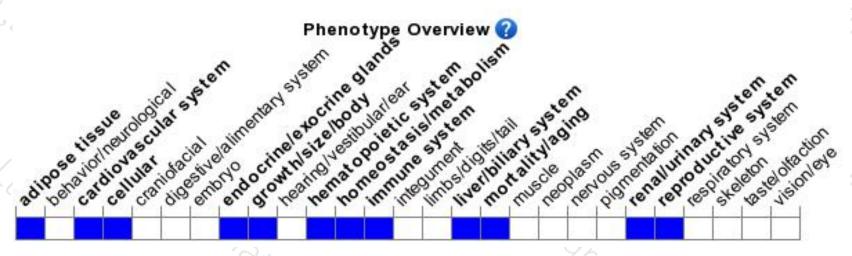
### Protein domain





## 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, Homozygous mutants are more susceptible to infection with bacteria that utilize enterochelin-type siderophores to acquire iron and impaired thermogenesis. Mice homozygous for another knock-out allele exhibit apoptotic defects in hematopoietic cells.



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





