

Lcmt1 Cas9-CKO Strategy

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Reviewer: Xiaojing Li

Design Date: 2020-8-3

Project Overview



Project Name

Lcmt1

Project type

Cas9-CKO

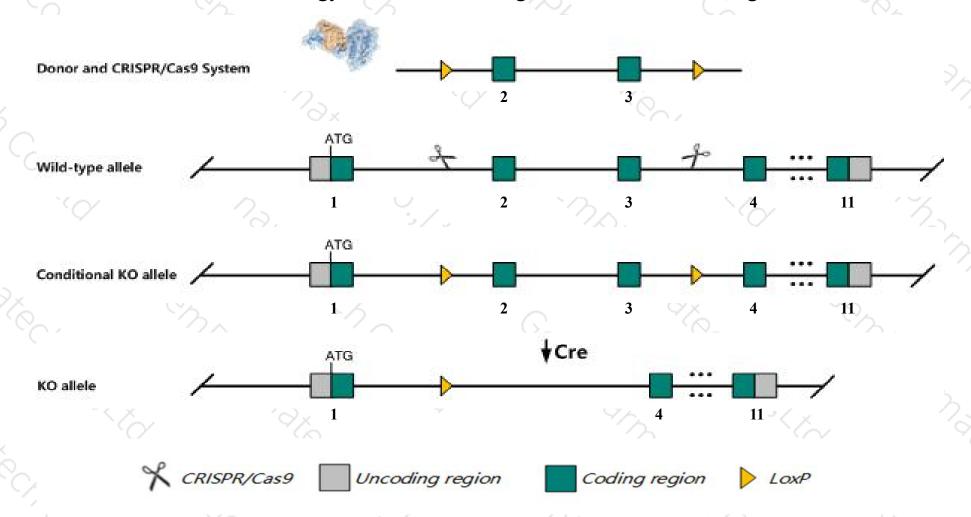
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Lcmt1* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Lcmt1* gene has 6 transcripts. According to the structure of *Lcmt1* gene, exon2-exon3 of *Lcmt1*201(ENSMUST00000033025.6) transcript is recommended as the knockout region. The region contains 214bp coding sequence.

 Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Lcmt1* 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 gene trap allele are embryonic lethal. Mice homozygous for a hypomorphic gene trap allele exhibit partial embryonic lethality, insulin resistance and impaired glucose tolerance. Mice homozygous for a transgenic gene disruption exhibit kidney agenesis.
- Transcript 205 CDS 3' incomplete the influences is unknown.
- > The *Lcmt1* gene is located on the Chr7. 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)



Lcmt1 leucine carboxyl methyltransferase 1 [Mus musculus (house mouse)]

Gene ID: 30949, updated on 13-Mar-2020

Summary

☆ ?

Official Symbol Lcmt1 provided by MGI

Official Full Name leucine carboxyl methyltransferase 1 provided by MGI

Primary source MGI:MGI:1353593

See related Ensembl: ENSMUSG00000030763

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 AL033290, LCMT-1, Lcmt

Expression Ubiquitous expression in CNS E18 (RPKM 17.1), cortex adult (RPKM 13.4) and 28 other tissuesSee more

Orthologs <u>human all</u>

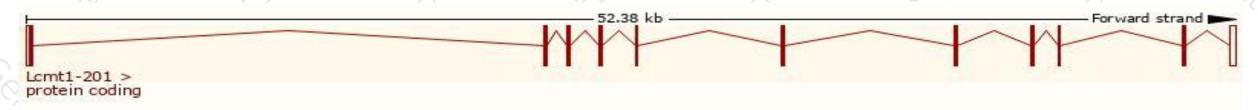
Transcript information (Ensembl)



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

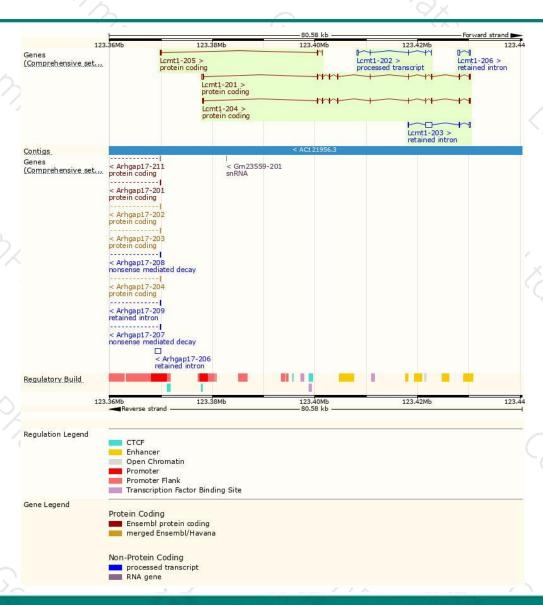
| | Address of the second of the s | 1 3 h | | | <u> </u> | | |
|-----------|--|-------|--------------|----------------------|----------------|------------|---------------------------------|
| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
| Lcmt1-201 | ENSMUST00000033025.6 | 1369 | <u>332aa</u> | Protein coding | CCDS40118 | A2RTH5 | TSL:1 GENCODE basic APPRIS P2 |
| Lcmt1-204 | ENSMUST00000206574.1 | 1286 | <u>332aa</u> | Protein coding | 9 4 | A0A0U1RNF2 | TSL:5 GENCODE basic APPRIS ALT2 |
| Lcmt1-205 | ENSMUST00000206721.1 | 365 | <u>121aa</u> | Protein coding | 625 | A0A0U1RPZ5 | CDS 3' incomplete TSL:3 |
| Lcmt1-202 | ENSMUST00000205880.1 | 632 | No protein | Processed transcript | 0.5 | 18 | TSL:3 |
| Lcmt1-203 | ENSMUST00000206488.1 | 1767 | No protein | Retained intron | - | <u> </u> | TSL:1 |
| Lcmt1-206 | ENSMUST00000206727.1 | 548 | No protein | Retained intron | 1878 | 98 | TSL:2 |

The strategy is based on the design of *Lcmt1-201* transcript, the transcription is shown below:



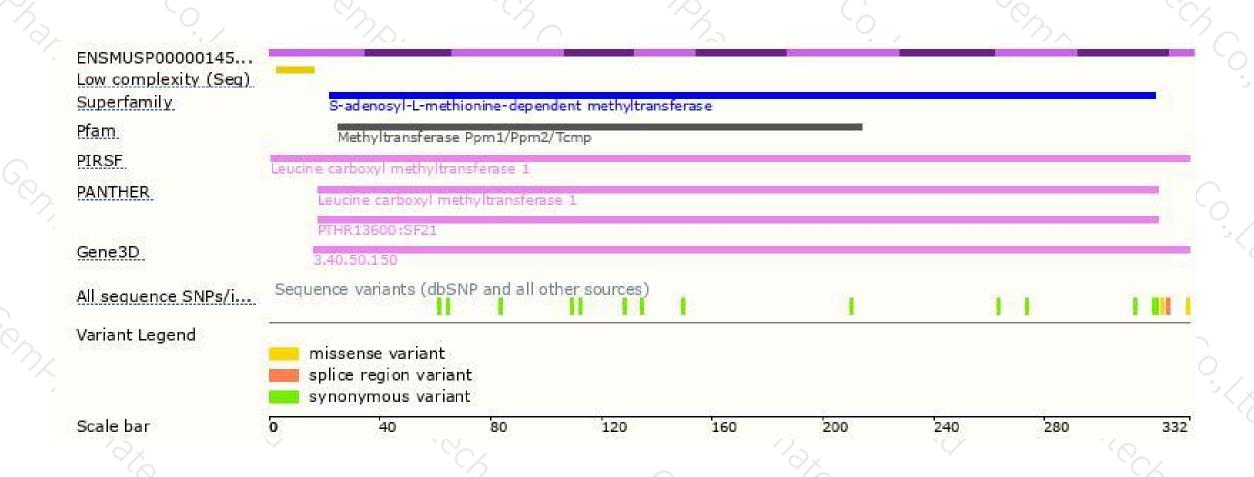
Genomic location distribution





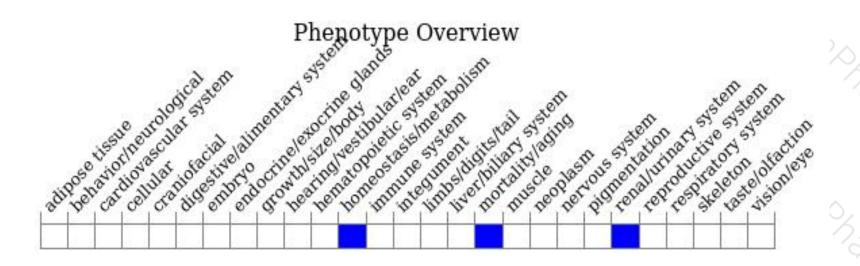
Protein domain





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 gene trap allele are embryonic lethal. Mice homozygous for a hypomorphic gene trap allele exhibit partial embryonic lethality, insulin resistance and impaired glucose tolerance. Mice homozygous for a transgenic gene disruption exhibit kidney agenesis.



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





