Klf15 Cas9-CKO Strategy

Designer: Daohua Xu

Reviewer: Huimin Su

Design Date: 2019-8-28

Project Overview



Project Name

Klf15

Project type

Cas9-CKO

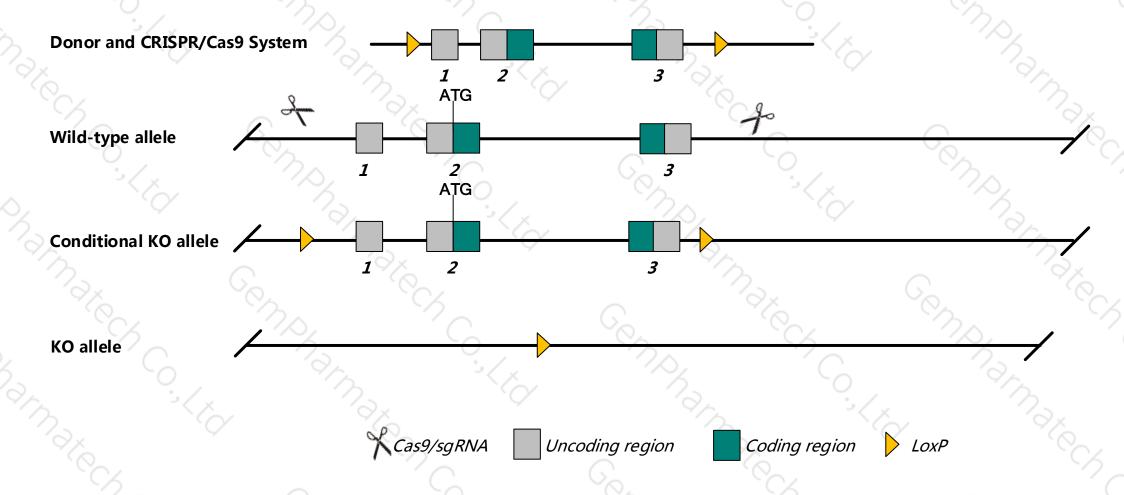
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Klf15* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Klf15* gene has 5 transcripts. According to the structure of *Klf15* gene, exon1-exon3 of *Klf15*-201 (ENSMUST00000203039.2) transcript is recommended as the knockout region. The region contains all coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Klf15* 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 or cell types.

Notice



- According to the existing MGI data, Mice homozygous for a null allele display impaired gluconeogenesis with severe fasting induce hypoglycemia. Homozygotes are also more sensitive to induced cardiac stress and display mild cardiac and aortic abnormalities.
- ➤ The KO region contains functional region of the *Gm44117* gene.Knockout the region may affect the function of *Gm44117* gene.
- ➤ The *Klf15* gene is located on the Chr6. 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)



Klf15 Kruppel-like factor 15 [Mus musculus (house mouse)]

Gene ID: 66277, updated on 8-Dec-2018

Summary

Official Symbol Klf15 provided by MGI

Official Full Name Kruppel-like factor 15 provided by MGI

Primary source MGI:MGI:1929988

See related Ensembl: ENSMUSG00000030087

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 CKLF; KKLF; AV048136; AW494632; 1810013I09Rik

Expression Broad expression in liver adult (RPKM 36.3), kidney adult (RPKM 26.2) and 20 other tissues See more

Orthologs human all

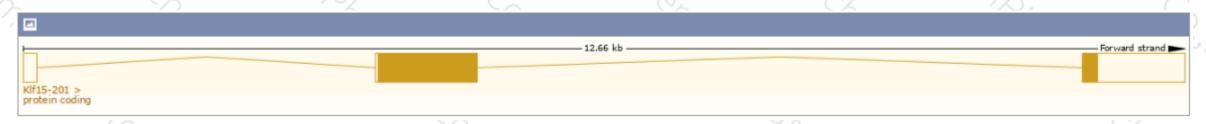
Transcript information (Ensembl)



The gene has 5 transcripts, and all transcripts are shown below:

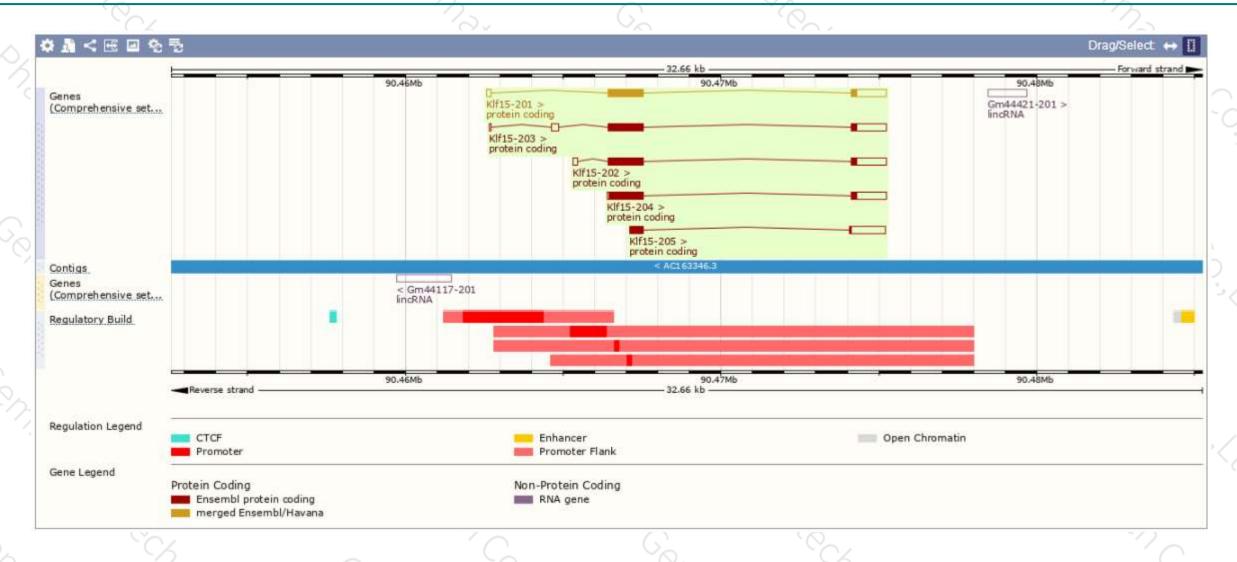
| Show/hide columns (1 hidden) | | | | | | | | | Filter | * |
|------------------------------|-----------------------|------|--------------|----------------|------------|-------------|--------------------------|-------|-------------------|-----------|
| Name 🍦 | Transcript ID 🗼 | bp 🍦 | Protein 🍦 | Biotype | CCDS | UniProt 🍦 | RefSeq | | Flags | \$ |
| Klf15-203 | ENSMUST00000203039.2 | 2482 | <u>415aa</u> | Protein coding | CCDS20360@ | Q9EPW2₽ | - | TSL:5 | GENCODE basic | APPRIS P1 |
| Klf15-202 | ENSMUST00000113530.3 | 2374 | <u>415aa</u> | Protein coding | CCDS20360@ | Q9EPW2₽ | - | TSL:5 | GENCODE basic | APPRIS P1 |
| Klf15-201 | ENSMUST00000032174.11 | 2373 | <u>415aa</u> | Protein coding | CCDS20360₽ | Q9EPW2₽ | NM 023184ଢ NP 075673ଢ | TSL:1 | GENCODE basic | APPRIS P1 |
| Klf15-204 | ENSMUST00000203607.1 | 2240 | <u>415aa</u> | Protein coding | CCDS20360@ | Q9EPW2₽ | - | TSL:1 | GENCODE basic | APPRIS P1 |
| KIf15-205 | ENSMUST00000205136.1 | 1545 | <u>153aa</u> | Protein coding | - | A0A0N4SVC6@ | - | C | CDS 5' incomplete | TSL:1 |

The strategy is based on the design of *Klf15*-201 transcript, The transcription is shown below



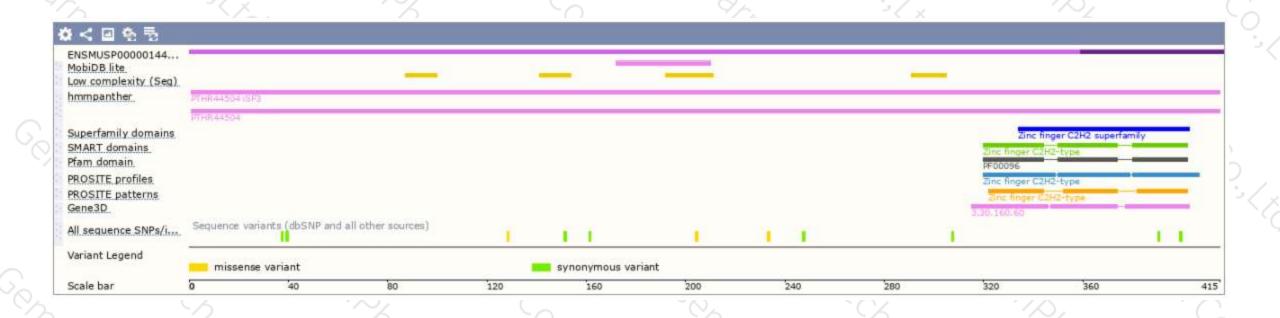
Genomic location distribution





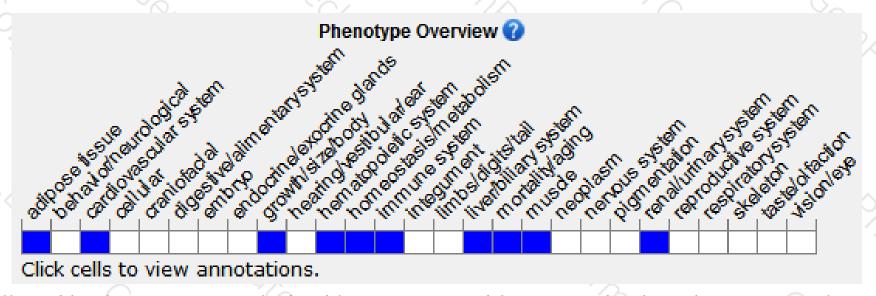
Protein domain





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 null allele display impaired gluconeogenesis with severe fasting induce hypoglycemia. Homozygotes are also more sensitive to induced cardiac stress and display mild cardiac and aortic abnormalities.

If you have any questions, you are welcome to inquire. Tel: 025-5864 1534





