GemPharmatech™

Lfng Cas9-KO Strategy

Designer: Xueting Zhang

Reviewer: Ruirui Zhang

Design Date: 2022-12-12

Overview

Target Gene Name

- Lfng

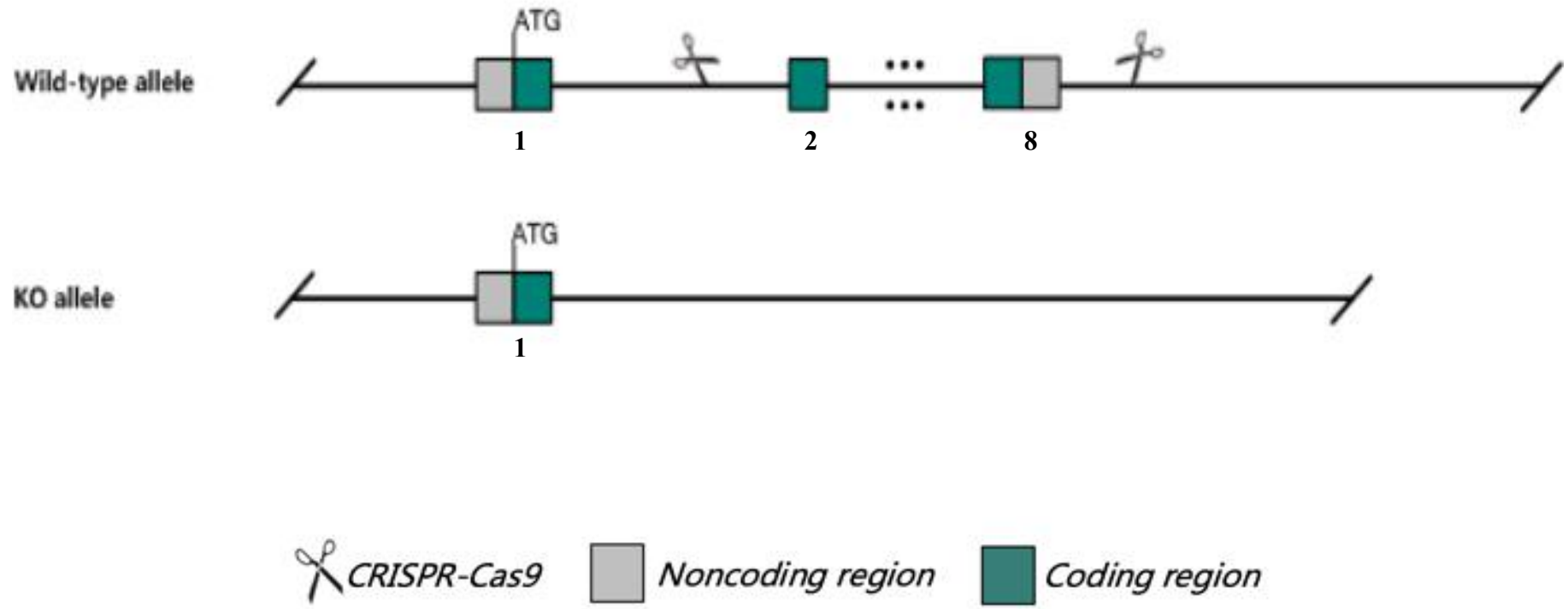
Project Type

- Cas9-KO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Lfng* gene.

Technical Information

- The *Lfng* gene has 3 transcripts. According to the structure of *Lfng* gene, exon2-exon8 of *Lfng-201*(ENSMUST00000031555.3) transcript is recommended as the knockout region.The region contains 708bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Lfng* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.

Gene Information

Lfng LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase [Mus musculus (house mouse)]

Gene ID: 16848, updated on 13-Mar-2020

Summary

Official Symbol	Lfng provided by MGI
Official Full Name	LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase provided by MGI
Primary source	MGI:MGI:1095413
See related	Ensembl:ENSMUSG00000029570
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	AW061165
Expression	Broad expression in spleen adult (RPKM 77.7), mammary gland adult (RPKM 72.5) and 25 other tissues See more
Orthologs	human all

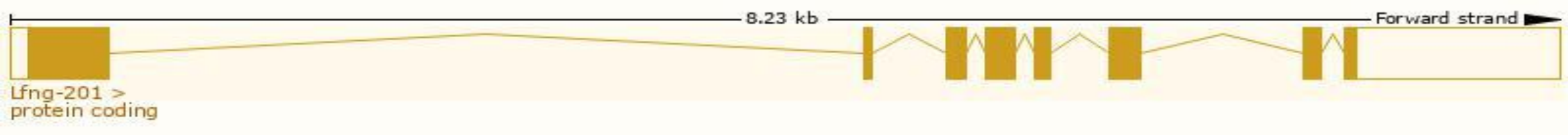
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

The gene has 3 transcripts, all transcripts are shown below:

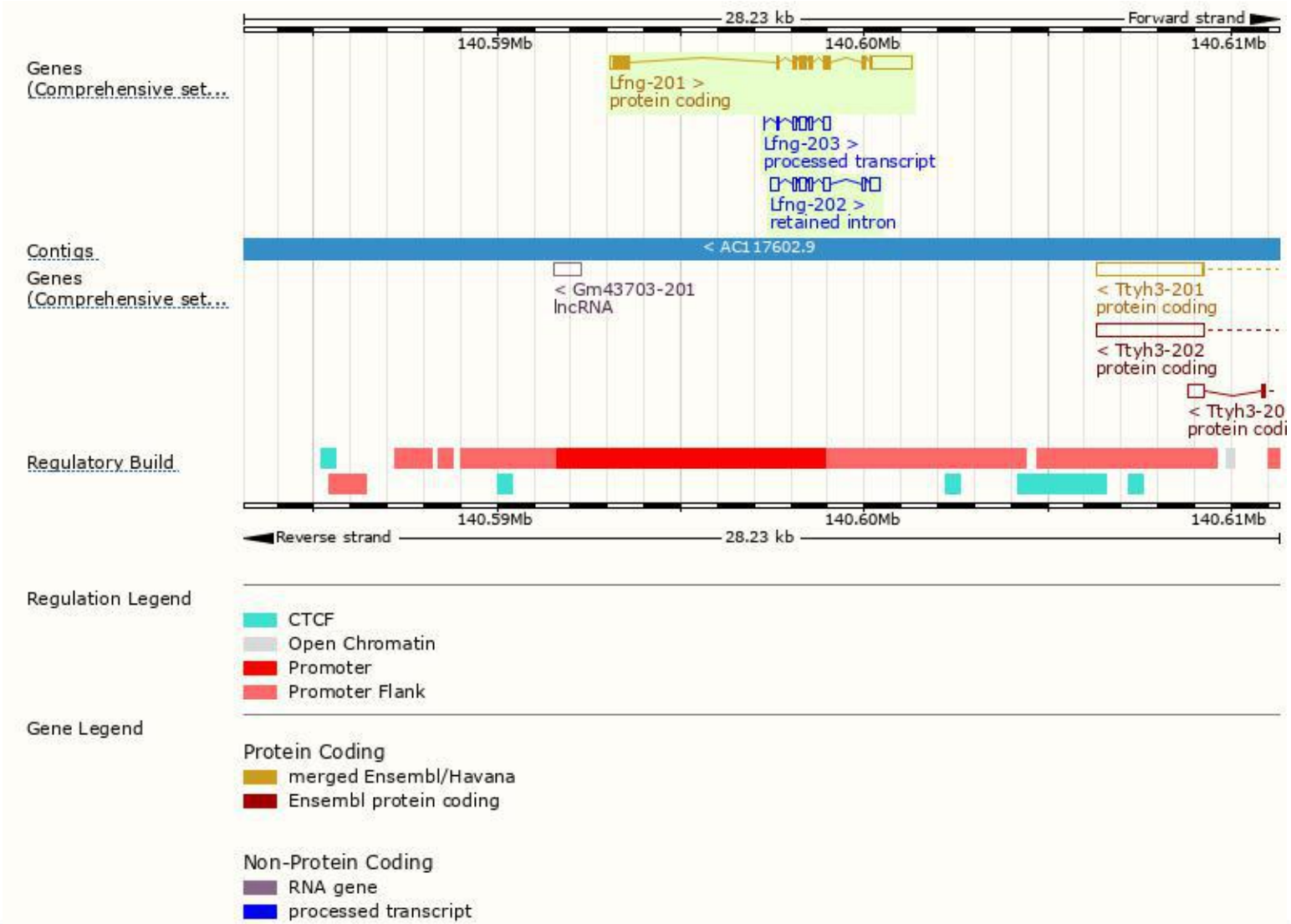
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lfng-201	ENSMUST00000031555.2	2305	378aa	Protein coding	CCDS19821	B2RRW2 O09010	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Lfng-203	ENSMUST00000200626.4	590	No protein	Processed transcript	-	-	TSL:3
Lfng-202	ENSMUST00000199848.1	1074	No protein	Retained intron	-	-	TSL:1

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



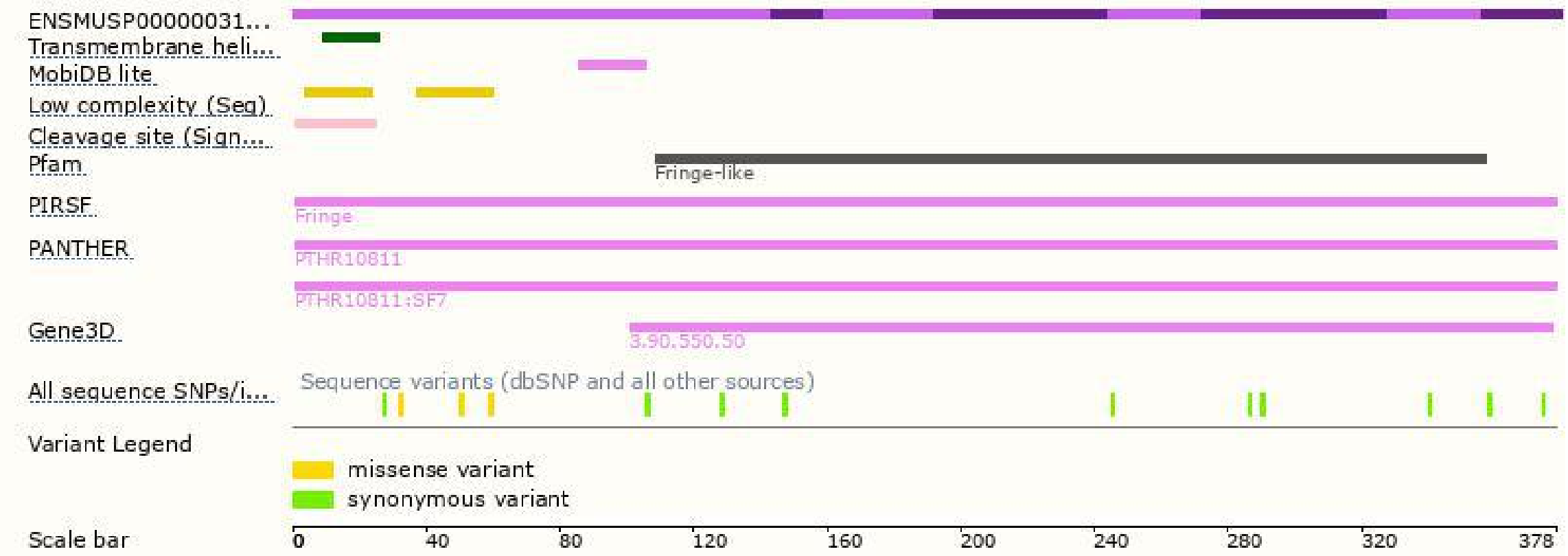
Source: https://www.ensembl.org

Genomic Information



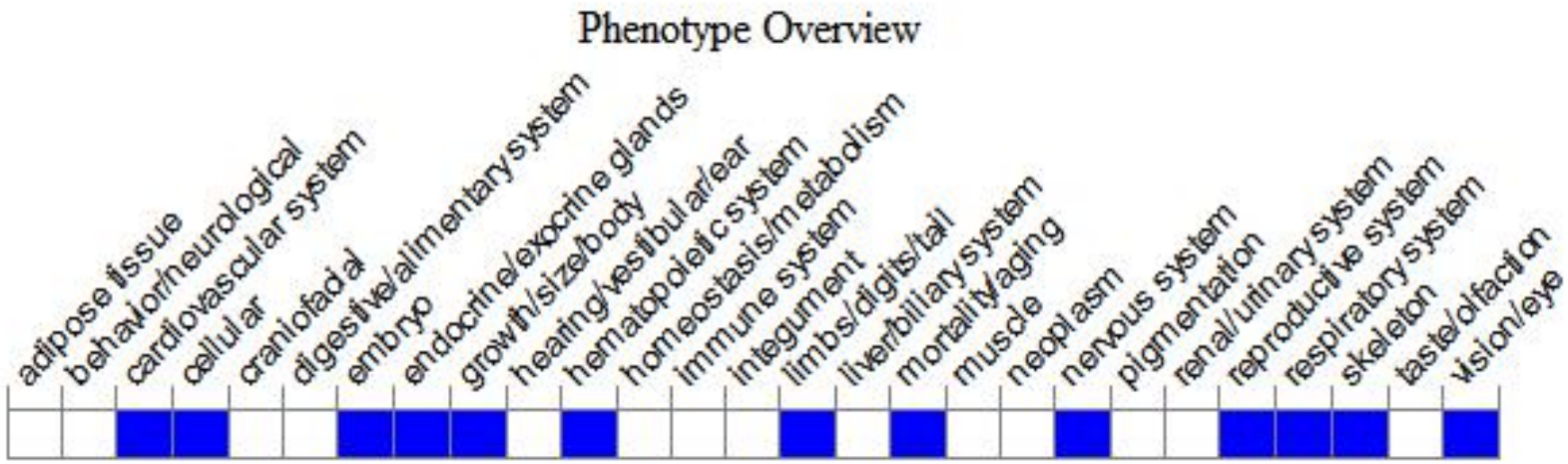
Source: : https://www.ensembl.org

Protein Information



Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



- Mice homozygous for a knock-out allele exhibit a short tail and abnormal rib, somite, and lung development. Mice homozygous mice exhibit reduced female fertility, abnormal hair cells, and abnormal axial skeleton morphology.

Source: https://www.informatics.jax.org

Important Information

- According to the existing MGI data,mice homozygous for a knock-out allele exhibit a short tail and abnormal rib, somite, and lung development. Mice homozygous mice exhibit reduced female fertility, abnormal hair cells, and abnormal axial skeleton morphology.
- According to the breeding data, the gene knockout homozygous mice may die at the embryonic stage.
- The knockout region is near to the N-terminal of *Gm43703* gene,this strategy may influence the regulatory function of the N-terminal of *Gm43703* gene.
- *Lfng* is located on Chr5. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.