GemPharmatech™

Irx3 Cas9-KO Strategy

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Reviewer: JiaYu

Design Date: 2023-6-21

Overview

Target Gene Name

- Irx3

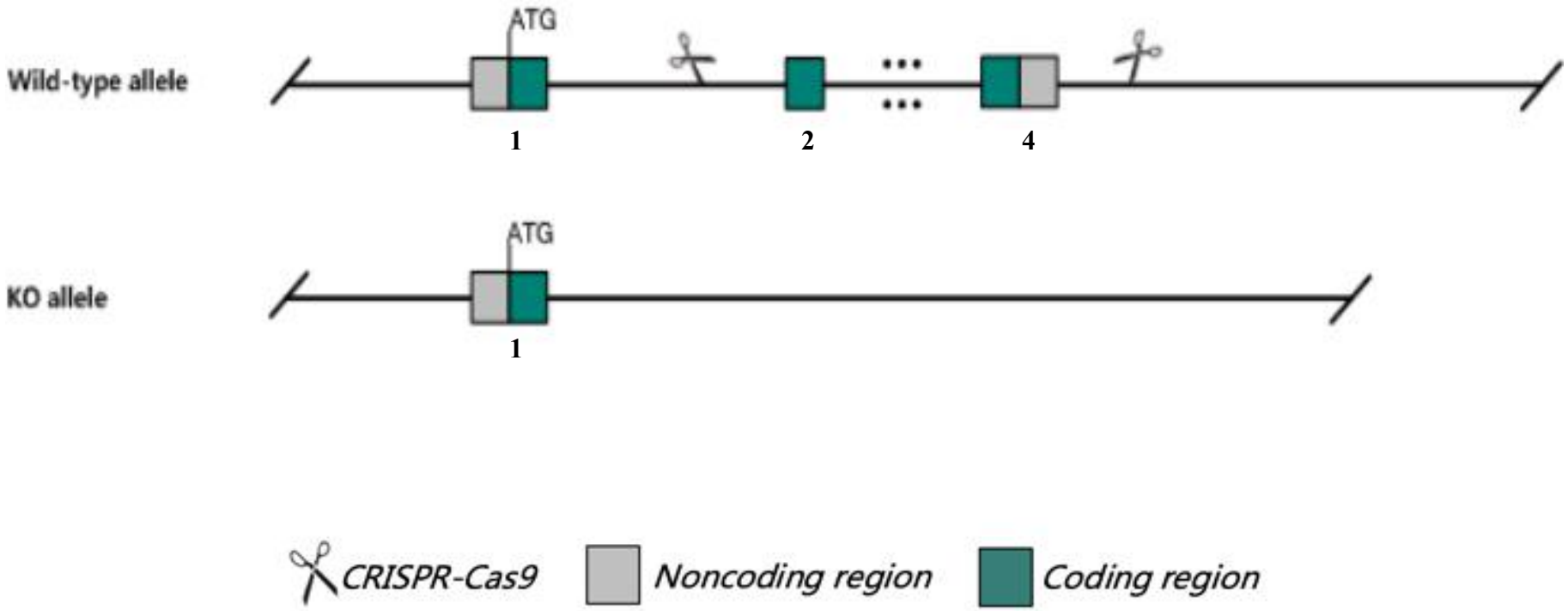
Project Type

- Cas9-KO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Technical Information

- The *Irx3* gene has 2 transcripts. According to the structure of *Irx3* gene, exon2-exon4 of *Irx3*-201 (ENSMUST00000093312.6) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Irx3* 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

Irx3 Iroquois related homeobox 3 [Mus musculus (house mouse)]

Gene ID: 16373, updated on 13-Mar-2020

Summary

Official Symbol	Irx3 provided by MGI
Official Full Name	Iroquois related homeobox 3 provided by MGI
Primary source	MGI:MGI:1197522
See related	Ensembl:ENSMUSG00000031734
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	AI894186
Expression	Biased expression in lung adult (RPKM 30.6), CNS E11.5 (RPKM 28.6) and 12 other tissues See more
Orthologs	human all

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

Transcript Information

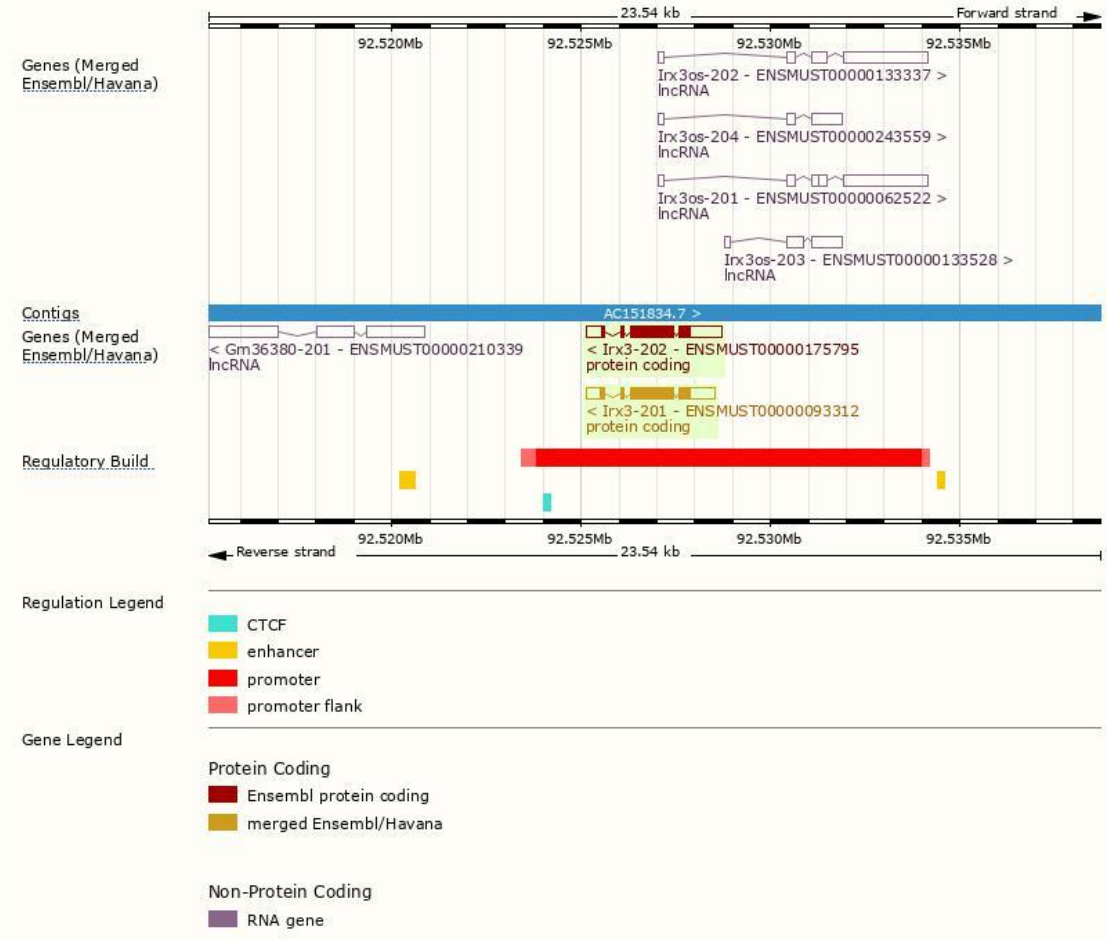
The gene has 2 transcripts,all transcripts are shown below:

Transcript ID	Name ▲	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000093312.6	Irx3-201	2610	522aa	Protein coding	CCDS57630	P81067-2	Ensembl Canonical GENCODE basic TSL:1
ENSMUST00000175795.4	Irx3-202	2748	507aa	Protein coding	CCDS57631	P81067-1	GENCODE basic APPRIS P1 TSL:1

The strategy is based on the design of *Irx3*-201 transcript, the transcription is shown below:

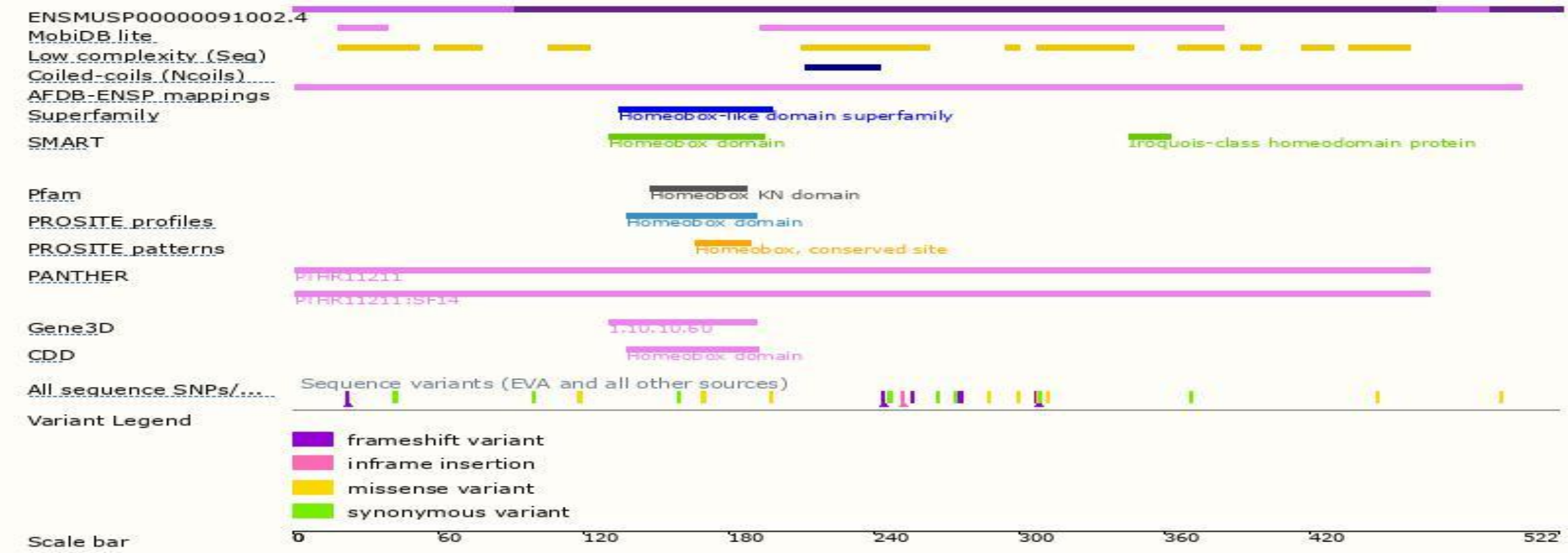
Source: https://www.ensembl.org

Genomic Information



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

Protein Information



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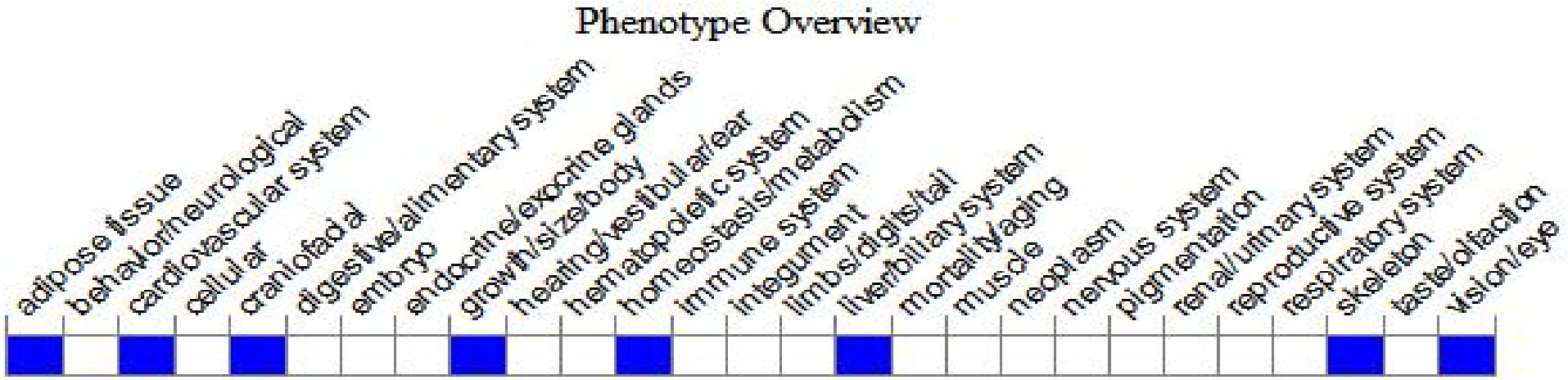
Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



- Mice homozygous for a null allele display right bundle branch block, decreased body weight, increased energy expenditure, reduced adiposity and decreased susceptibility to diet induced obesity.

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

Important Information

- *Irx3* is located on Chr8. 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 knocks out the *Irx3os* lncRNA gene.
- This strategy may affect the 5-terminal regulatory function of the *Gm36380* gene.
- 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.