

# Irx3 Cas9-CKO Strategy

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Reviewer: Lingyan Wu

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## **Project Overview**



Project Name Irx3

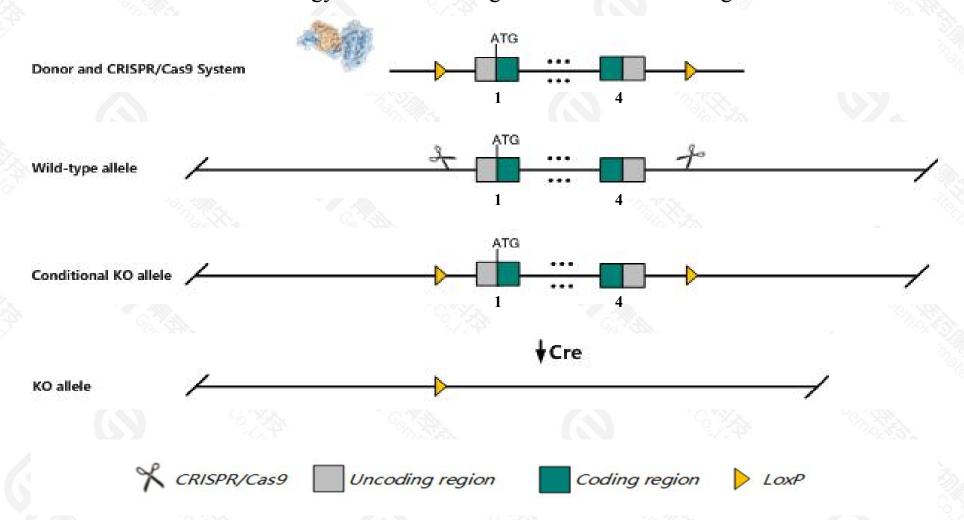
Project type Cas9-CKO

Strain background C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Irx3* gene. The schematic diagram is as follows:



## **Technical routes**



- The *Irx3* gene has 2 transcripts. According to the structure of *Irx3* gene, exon1-exon4 of *Irx3*201(ENSMUST00000093312.5) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock 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: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 null allele display right bundle branch block, decreased body weight, increased energy expenditure, reduced adiposity and decreased susceptibility to diet induced obesity.
- > The KO region contains functional region of the Irx3os gene. Knockout the region may affect the function of Irx3os gene.
- > The *Irx3* gene is located on the Chr8. 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)



#### 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 Al894186

Expression Biased expression in lung adult (RPKM 30.6), CNS E11.5 (RPKM 28.6) and 12 other tissuesSee more

Orthologs human all

## Transcript information (Ensembl)



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

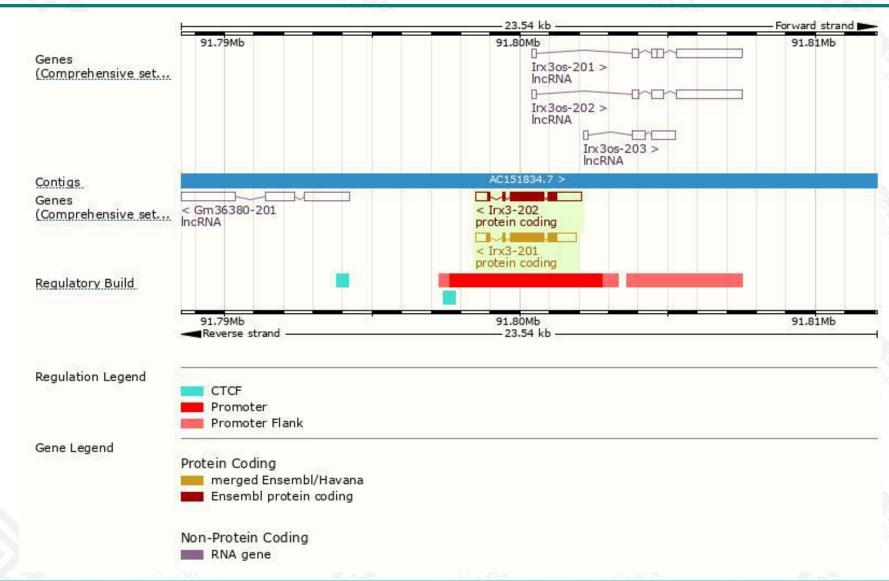
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Irx3-202	ENSMUST00000175795.3	2748	<u>507aa</u>	Protein coding	CCDS57631	P81067	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT:
Irx3-201	ENSMUST00000093312.5	2610	<u>522aa</u>	Protein coding	CCDS57630	P81067	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 P4

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



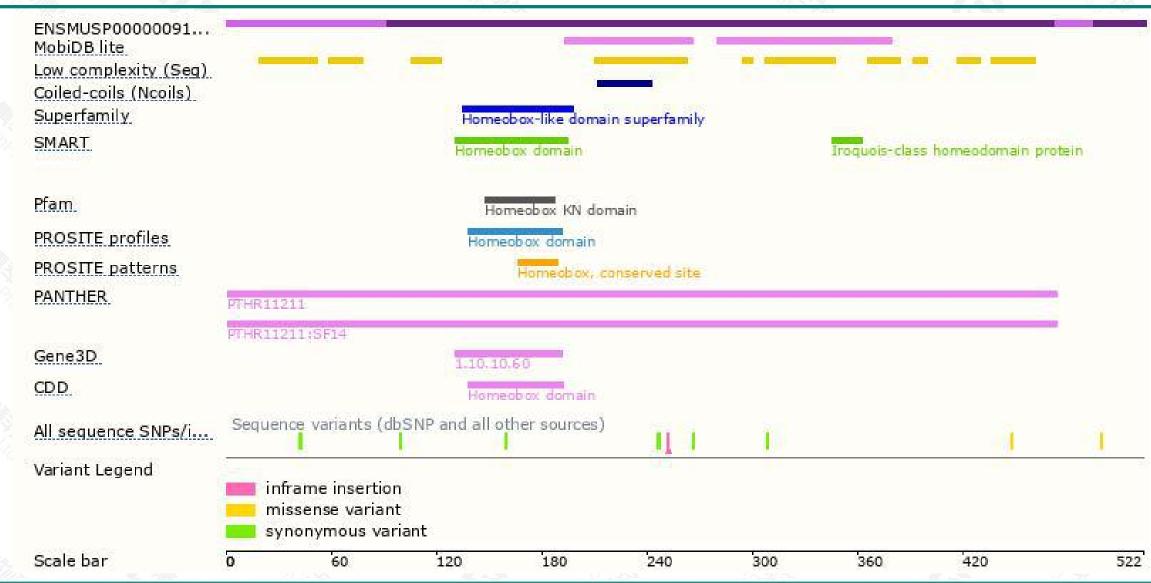
### Genomic location distribution





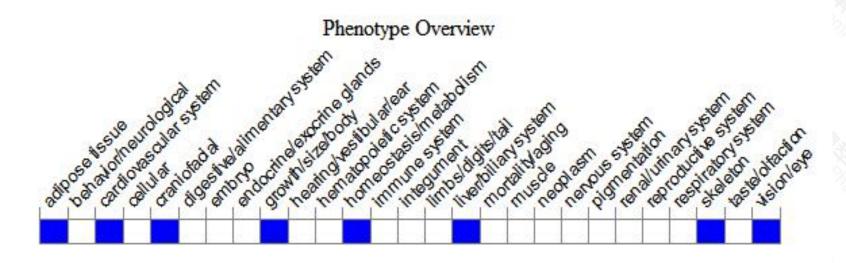
### Protein domain





## 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 right bundle branch block, decreased body weight, increased energy expenditure, reduced adiposity and decreased susceptibility to diet induced obesity.



If you have any questions, you are welcome to inquire.

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