

# Irx5 Cas9-CKO Strategy

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# **Project Overview**



Project Name Irx5

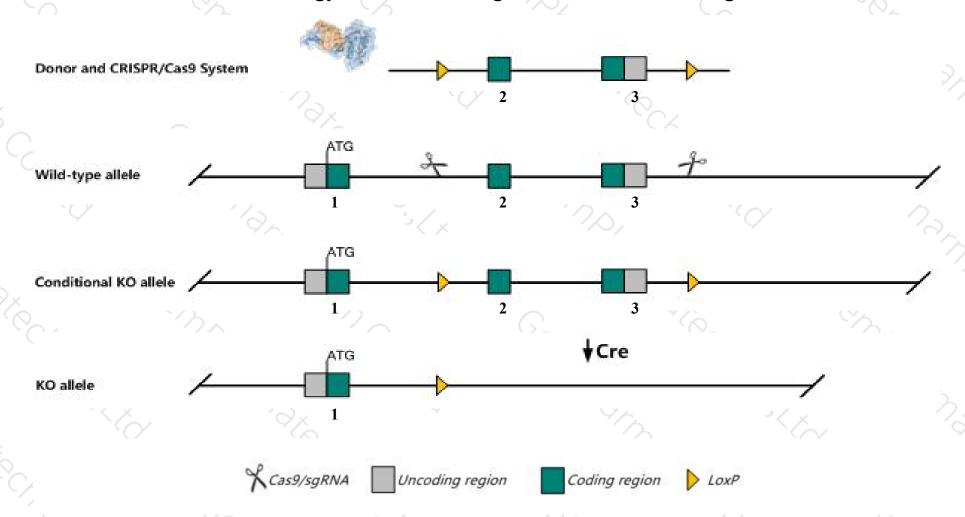
Project type Cas9-CKO

Strain background C57BL/6J

# Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Irx5* gene. The schematic diagram is as follows:



### Technical routes



- ➤ The *Irx5* gene has 2 transcripts. According to the structure of *Irx5* gene, exon2-exon3 of *Irx5-201*(ENSMUST00000034184.11) transcript is recommended as the knockout region. The region contains most 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 *Irx5* 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/6J 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/6J 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 and cell types.

### Notice



- ➤ According to the existing MGI data, Mice homozygous for a knock-out allele exhibit reduced body size, narrow eye opening, and impaired retinal cone bipolar cell development.
- The floxed region is near to the N-terminal of *Crnde* gene, this strategy may influence the regulatory function of the N-terminal of *Crnde* gene.
- The *Irx5* 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)



#### Irx5 Iroquois homeobox 5 [ Mus musculus (house mouse) ]

Gene ID: 54352, updated on 24-Dec-2019

#### Summary

↑ ?

Official Symbol Irx5 provided by MGI

Official Full Name Iroquois homeobox 5 provided by MGI

Primary source MGI:MGI:1859086

See related Ensembl:ENSMUSG00000031737

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

Expression Biased expression in lung adult (RPKM 17.2), limb E14.5 (RPKM 13.4) and 12 other tissues See more

Orthologs <u>human</u> all

#### Genomic context



**Location:** 8 C5; 8 44.93 cM

See Irx5 in Genome Data Viewer

Exon count: 3

Annotation release	Status	Assembly	Chr	Location	
108	current	GRCm38.p6 (GCF_000001635.26)	8	NC_000074.6 (9235779692361456)	
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	8	NC_000074.5 (9488169594885355)	

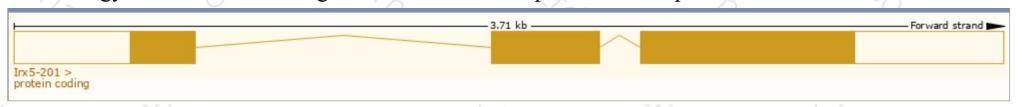
# Transcript information (Ensembl)



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

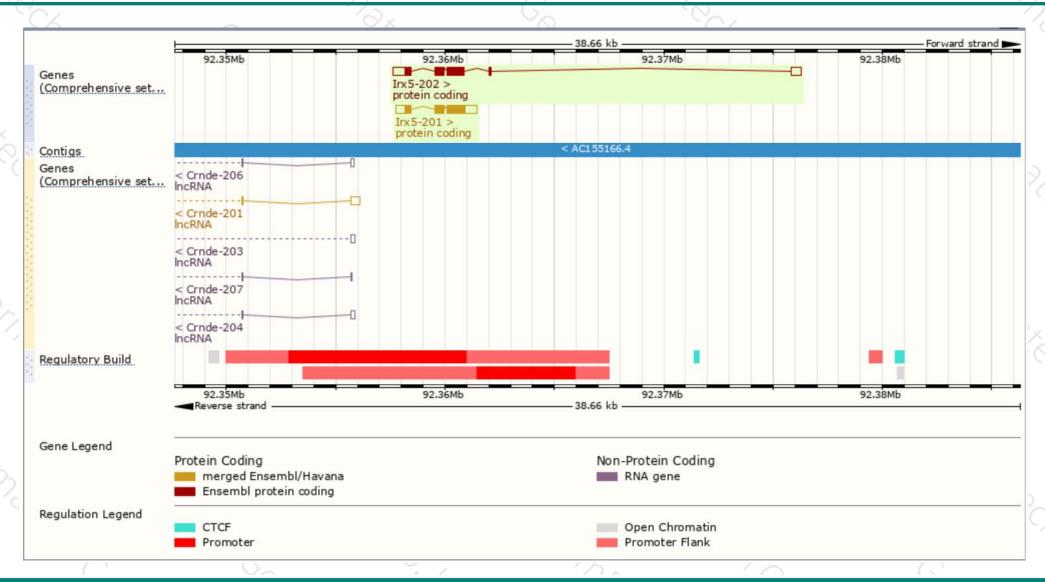
Name 🍦	Transcript ID 🗼	bp 🛊	Protein	Biotype 🍦	CCDS 🍦	UniProt 🝦	Flags			
Irx5-201	ENSMUST00000034184.11	2450	<u>484aa</u>	Protein coding	CCDS22522译	Q9JKQ4&	TSL:1		CODE basic	APPRIS P1
Irx5-202	ENSMUST00000210246.1	2550	<u>516aa</u>	Protein coding	-	A0A1B0GR17&	TSL:5 GENCODE basic			

The strategy is based on the design of *Irx5-201* transcript, The transcription is shown below



### Genomic location distribution





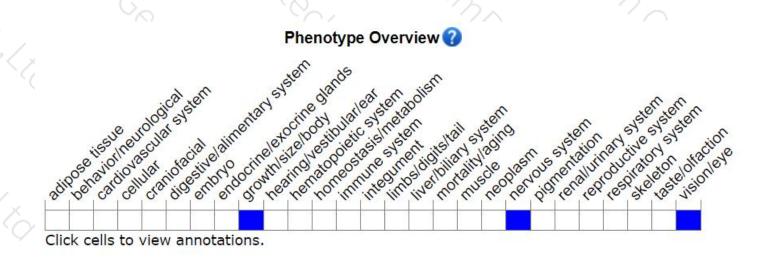
## Protein domain





# 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 knock-out allele exhibit reduced body size, narrow eye opening, and impaired retinal cone bipolar cell development.



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

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