Nkx2-5 Cas9-CKO Strategy

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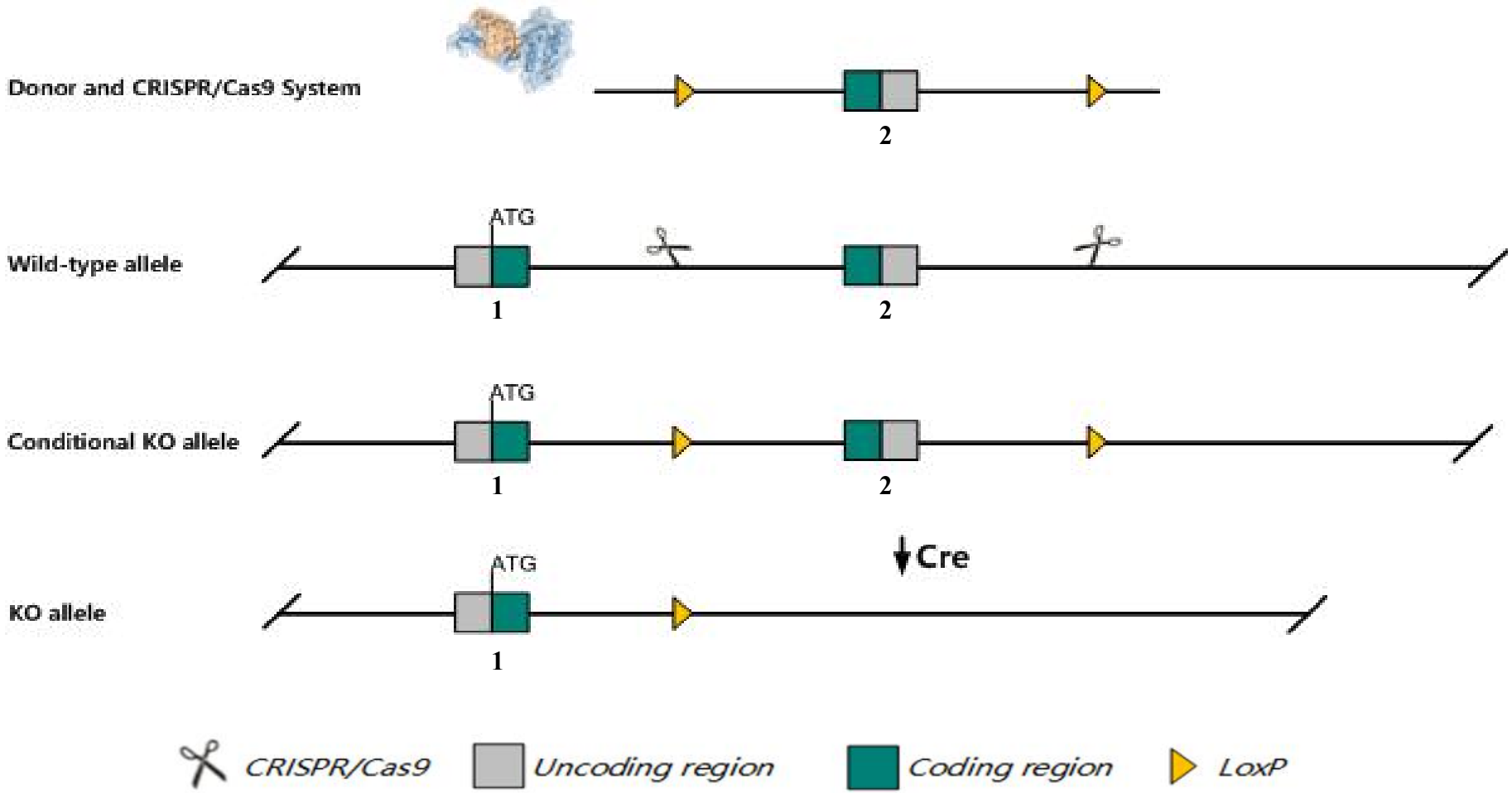
Project Overview

Project Name	***Nkx2-5***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nkx2-5* gene. The schematic diagram is as follows:



Technical routes

The *Nkx2-5* gene has 2 transcripts. According to the structure of *Nkx2-5* gene, exon2 of *Nkx2-5-201* (ENSMUST00000015723.4) transcript is recommended as the knockout region.The region contains 626bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Nkx2-5* 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,Homozygous mutants show cardiac development arrest after looping, growth retardation, hematopoiesis and angiogenesis defects in yolk sac, and die at embryonic day 9-10.
Heterozygotes also show cardiac developmental defects.

The *Nkx2-5* gene is located on the Chr17. 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.

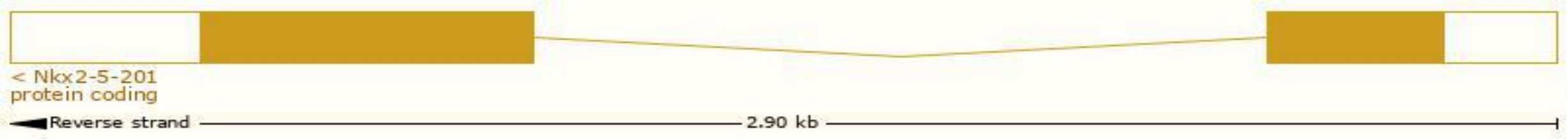
Gene information NCBI

Transcript information Ensembl

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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nkx2-5-201	ENSMUST00000015723.4	1525	318aa	Protein coding	CCDS28556	P42582 Q3UQU2	TSL:1 GENCODE basic APPRIS P1
Nkx2-5-202	ENSMUST00000235454.1	1357	317aa	Protein coding	-	-	CDS 3' incomplete

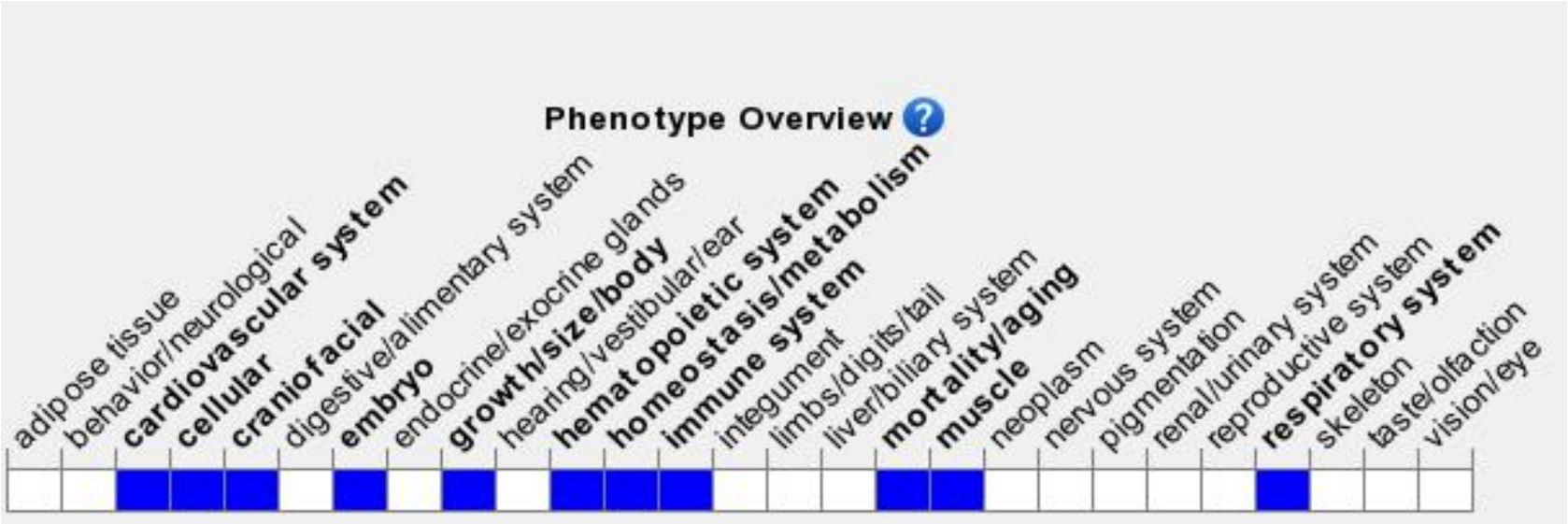
The strategy is based on the design of *Nkx2-5-201* transcript,The transcription is shown below



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/).

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

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