

# Ankrd50 Cas9-CKO Strategy

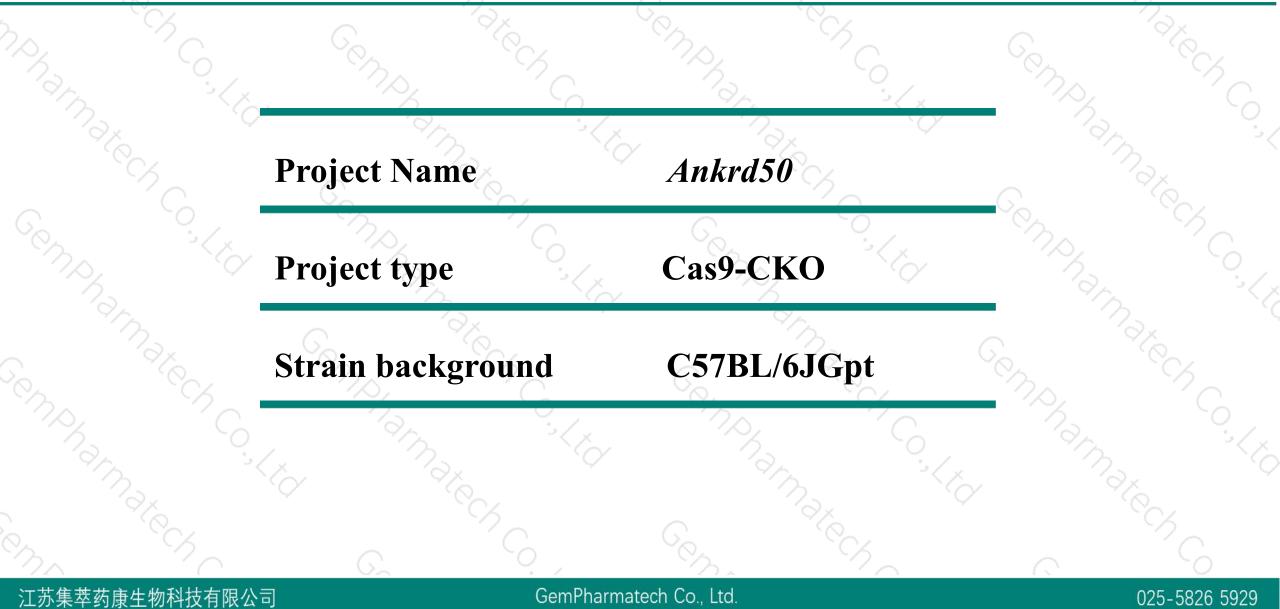
**Designer: Xueting Zhang** 

**Reviewer: Daohua Xu** 

Design Date: 2020-11-3

## **Project Overview**



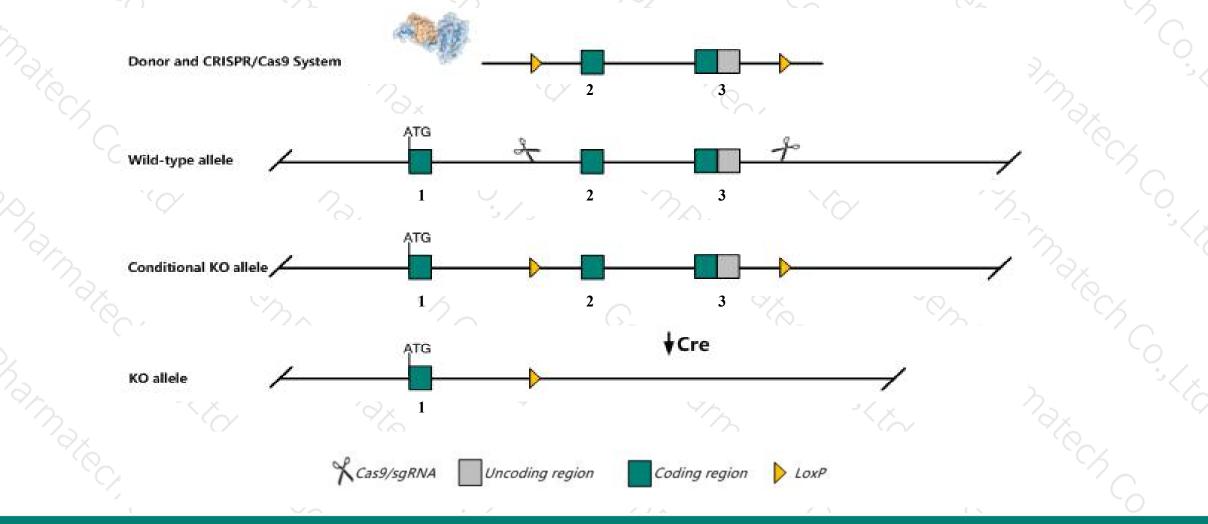


### **Conditional Knockout strategy**



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This model will use CRISPR/Cas9 technology to edit the *Ankrd50* gene. The schematic diagram is as follows:



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The Ankrd50 gene has 4 transcripts. According to the structure of Ankrd50 gene, exon2-exon3 of Ankrd50-204(ENSMUST00000156038.1) 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 *Ankrd50* 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/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 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.



- > The *Ankrd50* gene is located on the Chr3. 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.
- Transcript *Ankrd50*-203 may not be affected.
- ➤ The effect on transcript *Ankrd50*-201 is unknown.
- > 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)**



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Ankrd50 ankyrin repeat domain 50 [Mus musculus (house mouse)]

Gene ID: 99696, updated on 13-Mar-2020

#### Summary

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<b>Official Symbol</b>	Ankrd50 provided by MGI
fficial Full Name	ankyrin repeat domain 50 provided by <u>MGI</u>
<b>Primary source</b>	MGI:MGI:2139777
See related	Ensembl:ENSMUSG00000044864
Gene type	protein coding
<b>RefSeq status</b>	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	Al662170, E430012K20Rik, mKIAA1223, mKIAA3019
Expression	Ubiquitous expression in lung adult (RPKM 12.5), thymus adult (RPKM 11.6) and 28 other tissuesSee more
Orthologs	human all

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# **Transcript information (Ensembl)**

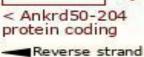


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The gene has 4 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ankrd50-202	ENSMUST00000120875.7	4945	<u>276aa</u>	Protein coding	CCDS50897	<u>D3Z643</u>	TSL:1 GENCODE basic APPRIS P1
Ankrd50-204	ENSMUST00000156038.1	7031	<u>1390aa</u>	Protein coding	-2	F7BE84	CDS 5' incomplete TSL:5
Ankrd50-201	ENSMUST0000094300.3	1944	<u>467aa</u>	Protein coding	-	<u>A6H6J9</u>	TSL:1 GENCODE basic
Ankrd50-203	ENSMUST00000127837.1	462	No protein	Processed transcript	-	-	TSL:5
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The strategy is based on the design of *Ankrd50-204* transcript, the transcription is shown below:



— 33.44 kb —

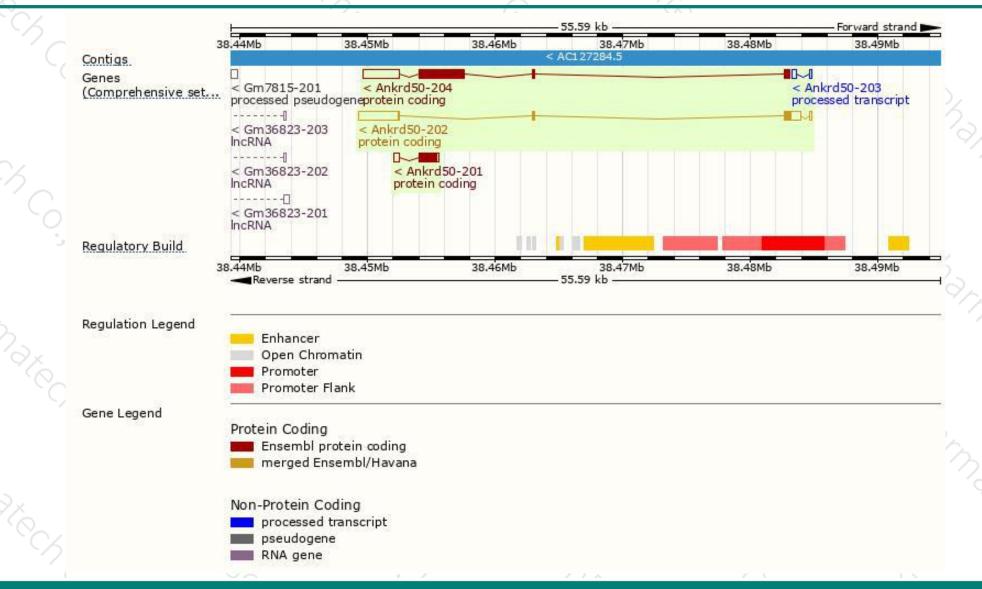
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### **Genomic location distribution**



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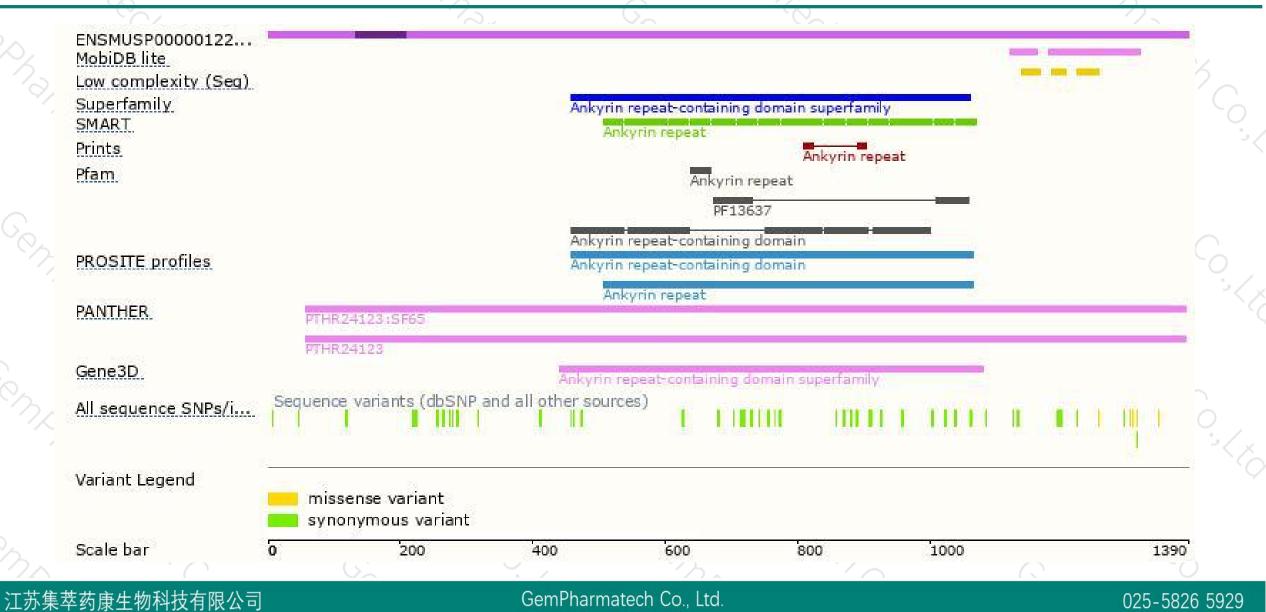


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### **Protein domain**







If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



