

# Ankfy1 Cas9-CKO Strategy

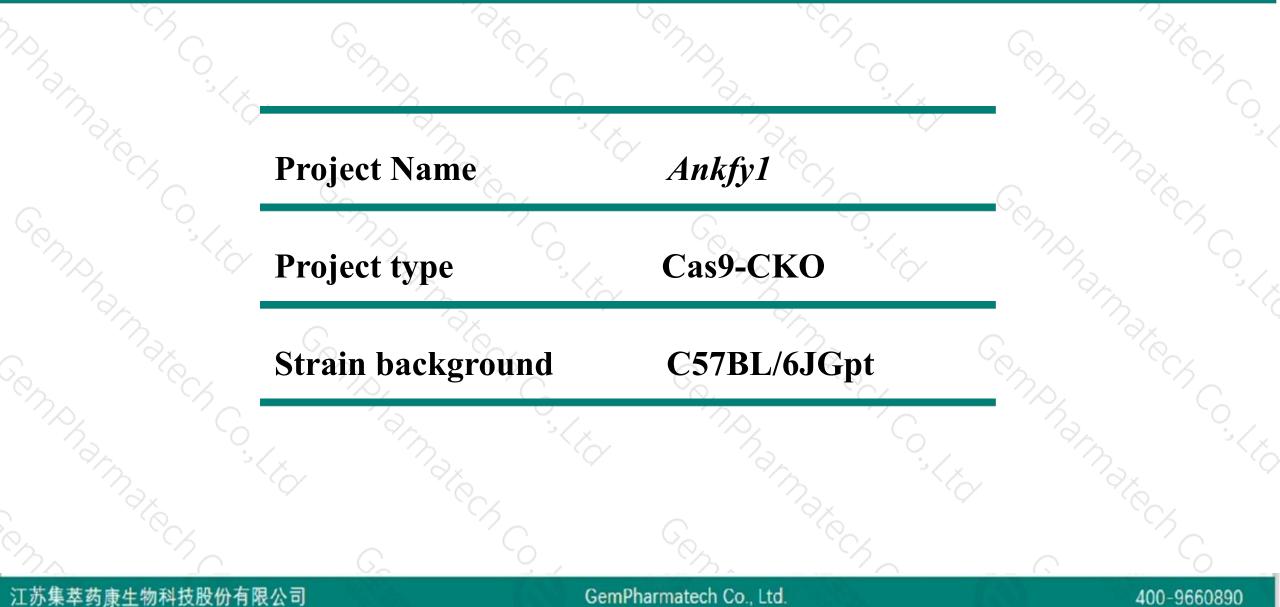
Designer: Reviewer:

**Design Date:** 

Daohua Xu Huimin Su 2019-9-26

## **Project Overview**

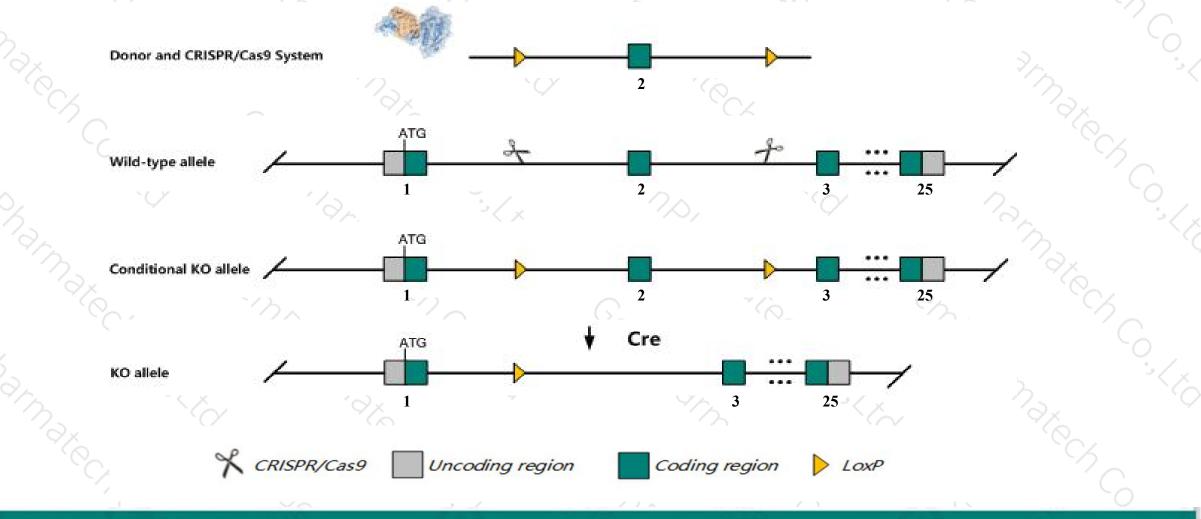




## **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the *Ankfy1* gene. The schematic diagram is as follows:



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The Ankfy1 gene has 6 transcripts. According to the structure of Ankfy1 gene, exon2 of Ankfy1-206 (ENSMUST00000155998.1) transcript is recommended as the knockout region. The region contains 193bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Ankfy1* 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.



- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit partial embryonic lethality with no apparent neural developmental defects on a mixed genetic background but show complete embryonic lethality on highly homogenous genetic backgrounds.
- The Ankfy1 gene is located on the Chr11. 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)**



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#### Ankfy1 ankyrin repeat and FYVE domain containing 1 [Mus musculus (house mouse)]

Gene ID: 11736, updated on 31-Jan-2019

#### Summary

Official Symbol	Ankfy1 provided by MGI
Official Full Name	ankyrin repeat and FYVE domain containing 1 provided by MGI
Primary source	MGI:MGI:1337008
See related	Ensembl:ENSMUSG0000020790
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	Ankhzn, ZFYVE14, mKIAA1255
Expression	Ubiquitous expression in CNS E18 (RPKM 14.1), large intestine adult (RPKM 13.9) and 28 other tissues See more
Orthologs	human all

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The gene has 6 transcripts, all transcripts are shown below:

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Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ankfy1-206	ENSMUST00000155998.1	8030	<u>1169aa</u>	Protein coding	CCDS24989	Q810B6	TSL:1 GENCODE basic APPRIS P1
Ankfy1-202	ENSMUST00000127610.7	7154	<u>439aa</u>	Nonsense mediated decay	19 <del>.</del> 0	Q810B6	TSL:1
Ankfy1-205	ENSMUST00000150172.7	2894	No protein	Retained intron	13 <b>2</b> 5	-	TSL:1
Ankfy1-204	ENSMUST00000147195.1	742	No protein	Retained intron	121		TSL:2
Ankfy1-203	ENSMUST00000132889.1	642	No protein	Retained intron	1.7	10	TSL:2
Ankfy1-201	ENSMUST00000102548.10	3324	No protein	IncRNA	-		TSL:5

82.14 kb

The strategy is based on the design of Ankfy1-206 transcript, The transcription is shown below

Ankfy1-206 > protein coding

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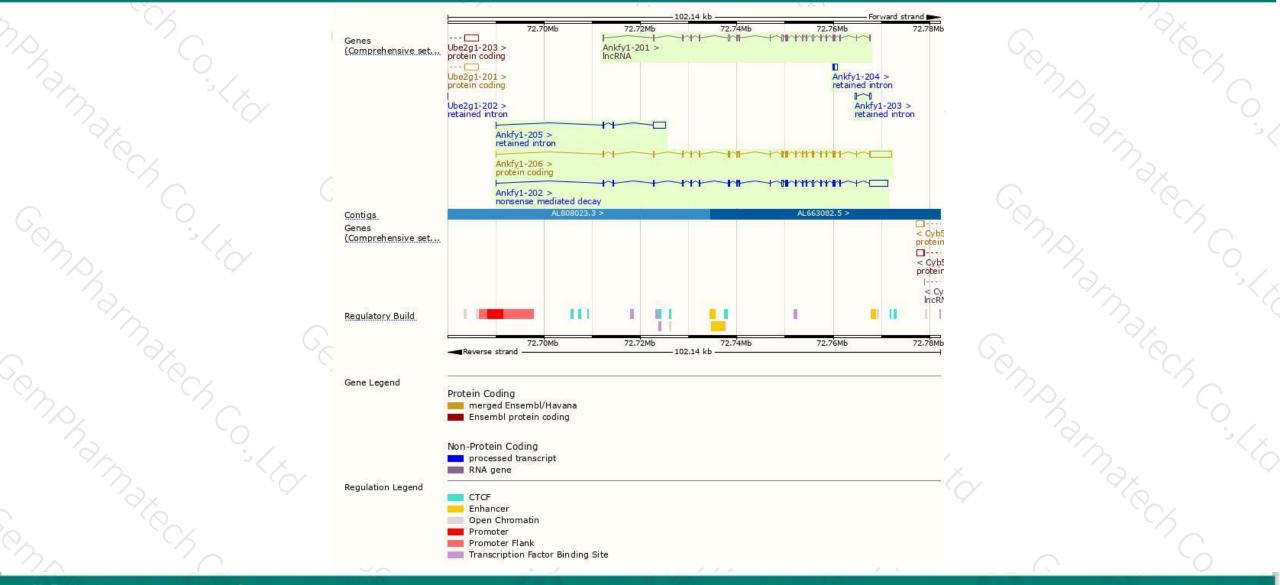
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Forward strand

### **Genomic location distribution**



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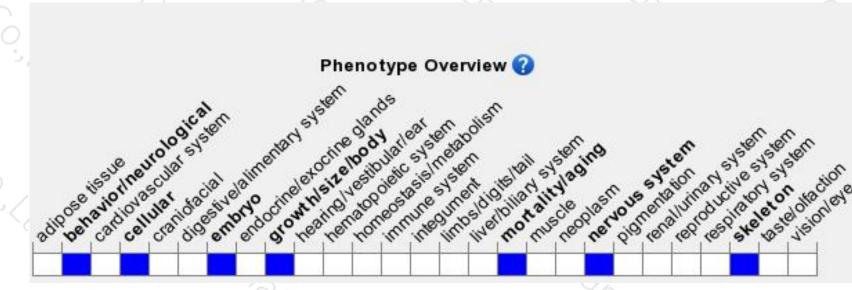
## **Protein domain**



ns,	ENSMUSP00000118 Low complexity (Seg) Coiled-coils (Ncoils)		
	Superfamily	SKP1/BTB/POZ domain superfamily	Zinc fing
armare.	SMART	Ankyrin repeat-containing domain superfamily BTB/POZ domain Ankyrin repeat	PYVE zint
	Prints	Ankyrin repeat	
	Pfam.	BTB/POZ domain Ankyrin repeat PF13857	FYVE zir
Ch Bh	PROSITE profiles	PF13637 Ankyrin repeat-containing domain BTB/POZ domain Ankyrin repeat	Zinc fing
- Pro-	PANTHER	PTHR24123:SF86	
300	Gene3D	PTHR24123 3.30.710.10 Ankyrin repeat-containing domain superfamily	Zinc fing
$\gamma_{\mathcal{O}_{\mathcal{L}}}$	CDD	Ankyrin repeat-containing domain	cd15728
Par .	All sequence SNPs/i	Sequence variants (dbSNP and all other sources)	-< ×
	Variant Legend	missense variant	
	Scale bar	0 100 200 300 400 500 600 700 800 900 1000	1169
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## 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 partial embryonic lethality with no apparent neural developmental defects on a mixed genetic background but show complete embryonic lethality on highly homogenous genetic backgrounds.

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If you have any questions, you are welcome to inquire. Tel: 400-9660890



