

# Ankfn1 Cas9-CKO Strategy

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### Overview

#### Target Gene Name

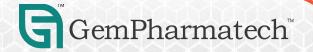
• Ankfn1

### Project Type

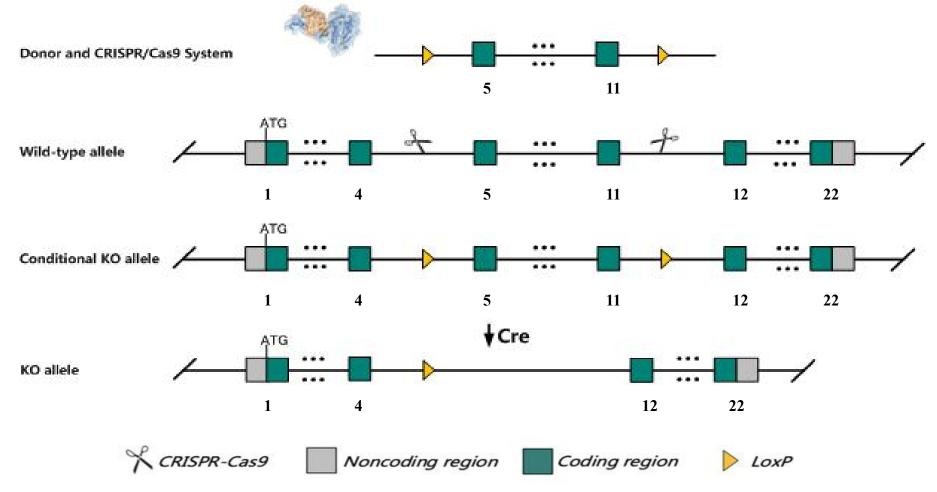
• Cas9-CKO

#### Genetic Background

• C57BL/6JGpt



## Strain Strategy

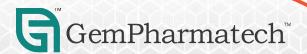


Schematic representation of CRISPR-Cas9 engineering used to edit the Ankfn1 gene.



#### **Technical Information**

- The *Ankfn1* gene has 8 transcripts. According to the structure of *Ankfn1* gene, exon5-exon11 of *Ankfn1*-208 (ENSMUST00000238273.3) transcript is recommended as the knockout region. The region contains 1046bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Ankfn1* 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- 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.



#### Gene Information

#### Ankfn1 ankyrin-repeat and fibronectin type III domain containing 1 [Mus musculus (house mouse)]

Gene ID: 382543, updated on 13-Mar-2020



Source: https://www.ncbi.nlm.nih.gov/

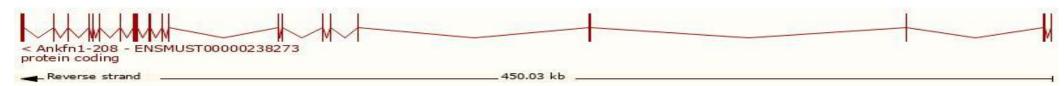


# Transcript Information

The gene has 8 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ankfn1-201	ENSMUST00000050983.1	2665	403aa	Protein coding	CCDS25237	Q8CDJ6	TSL:1 GENCODE basic
Ankfn1-208	ENSMUST00000238273.2	4289	1286aa	Protein coding	-:	e- 1	GENCODE basic
Ankfn1-206	ENSMUST00000207815.1	3250	1083aa	Protein coding	5	A0A140LIW7	CDS 5' incomplete TSL:5
Ankfn1-202	ENSMUST00000128717.8	2391	763aa	Protein coding	-	F6RWQ6	TSL:5 GENCODE basic APPRIS P1
Ankfn1-203	ENSMUST00000169201.1	2232	<u>743aa</u>	Protein coding	21	<u>F6X7B3</u>	TSL:2 GENCODE basic
Ankfn1-205	ENSMUST00000207350.1	523	48aa	Nonsense mediated decay	-	A0A140LHQ2	TSL:5
Ankfn1-207	ENSMUST00000208932.1	2665	No protein	Processed transcript		-	TSL:1
Ankfn1-204	ENSMUST00000207171.1	1032	No protein	Retained intron	-	2	TSL:1

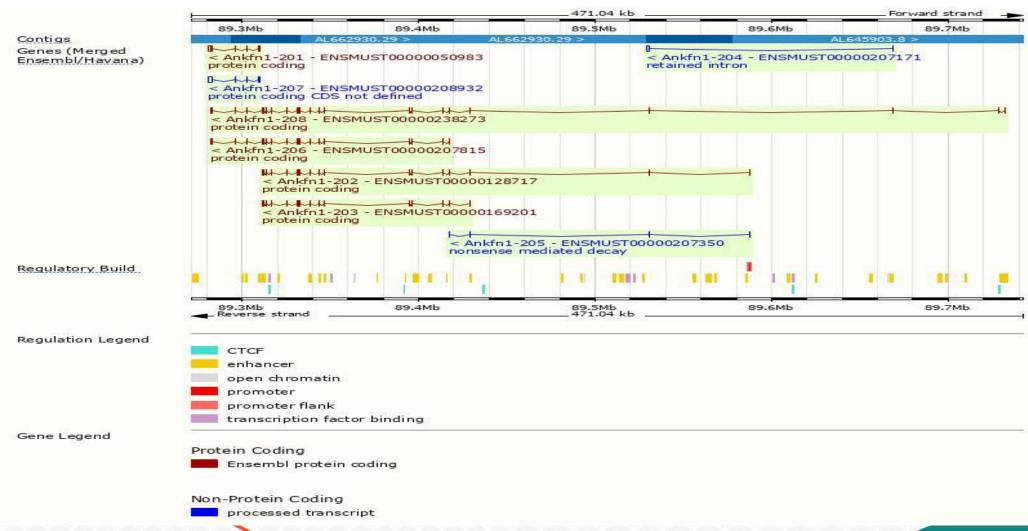
The strategy is based on the design of *Ankfn1*-208 transcript, the transcription is shown below:

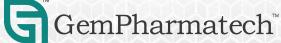


Source: https://www.ensembl.org



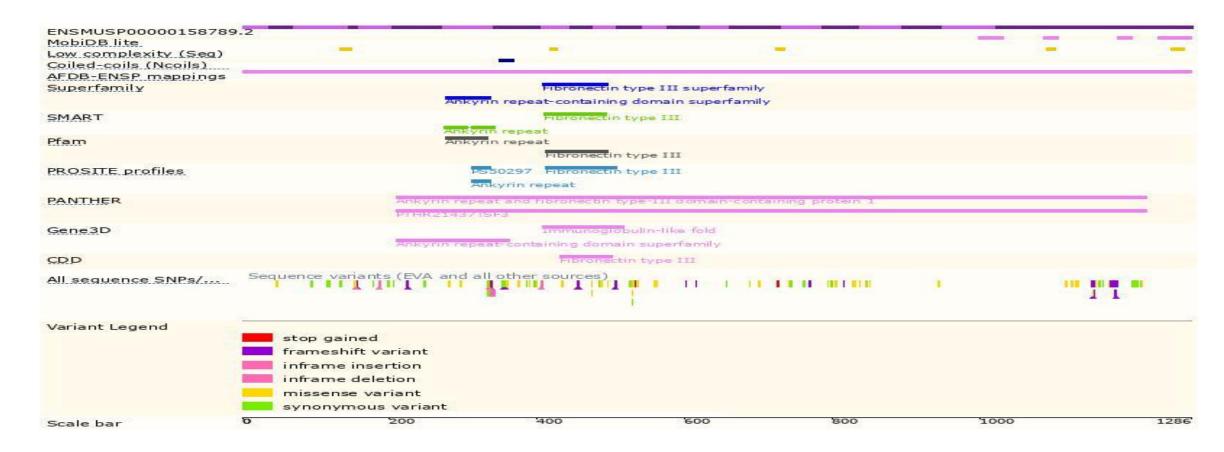
### Genomic Information





Source: : https://www.ensembl.org

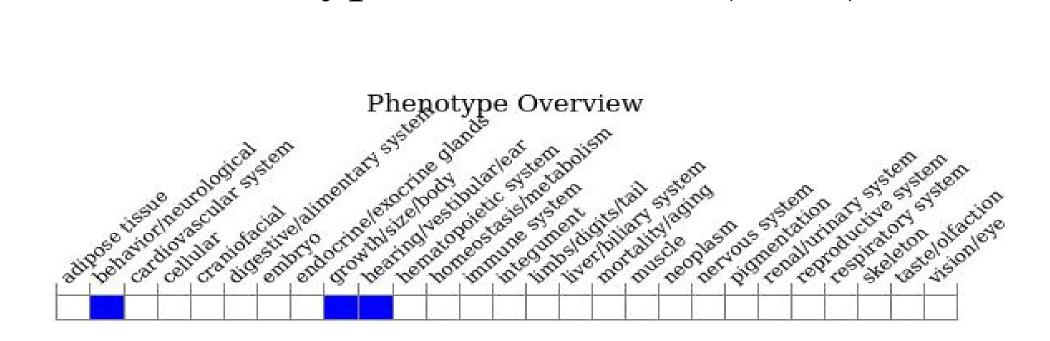
#### Protein Information





Source: : https://www.ensembl.org

# Mouse Phenotype Information (MGI)



• Mutant mice exhibit a variable and subtle head nodding phenotype.



### Important Information

- *Ankfn1* is located on Chr11. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- Transcript-201 may not be affected.
- 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 the existing technology level.

