

# Zfyve16 Cas9-CKO Strategy

Designer:Xueting Zhang

Reviewer: Yanhua Shen

Design Date:2019-9-4

# **Project Overview**



**Project Name** 

Zfyve16

**Project type** 

Cas9-CKO

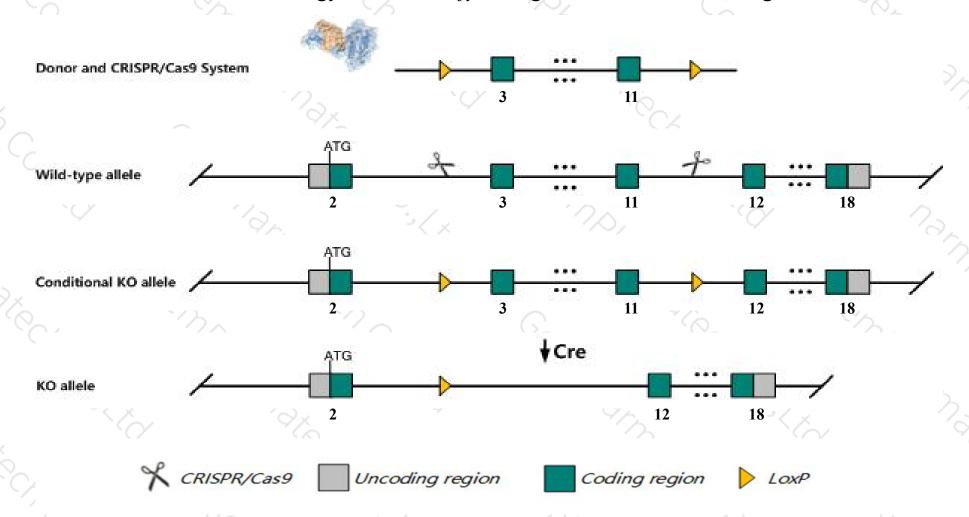
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Zfyve16 gene. The schematic diagram is as follows:



### Technical routes



- The *Zfyve16* gene has 4 transcripts. According to the structure of *Zfyve16* gene, exon3-exon11 of *Zfyve16-201* (ENSMUST00000022217.8) transcript is recommended as the knockout region. The region contains 3584bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Zfyve16* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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**



- > The Zfyve16 gene is located on the Chr13. 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)



#### Zfyve16 zinc finger, FYVE domain containing 16 [ Mus musculus (house mouse) ]

Gene ID: 218441, updated on 12-Aug-2019

#### Summary

☆ ?

Official Symbol Zfyve16 provided by MGI

Official Full Name zinc finger, FYVE domain containing 16 provided by MGI

Primary source MGI:MGI:2145181

See related Ensembl: ENSMUSG00000021706

Gene type protein coding
RefSeq status PROVISIONAL
Organism Mus musculus

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;

Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus

Also known as Al035632; mKIAA0305; B130031L15; B130024H06Rik

Expression Ubiquitous expression in CNS E18 (RPKM 4.3), CNS E14 (RPKM 3.7) and 25 other tissues See more

Orthologs human all

#### Genomic context



Location: 13; 13 C3

See Zfyve16 in Genome Data Viewer

Exon count: 18

| Annotation release | Status            | Assembly                     | Chr | Location                                   |  |
|--------------------|-------------------|------------------------------|-----|--|--|
| <u>108</u>         | current           | GRCm38.p6 (GCF_000001635.26) | 13  | NC_000079.6 (9248774992530810, complement) |  |
| Build 37.2         | previous assembly | MGSCv37 (GCF_000001635.18)   | 13  | NC_000079.5 (9325770493300765, complement) |  |

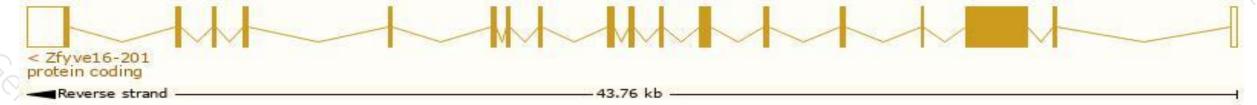
# Transcript information (Ensembl)



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

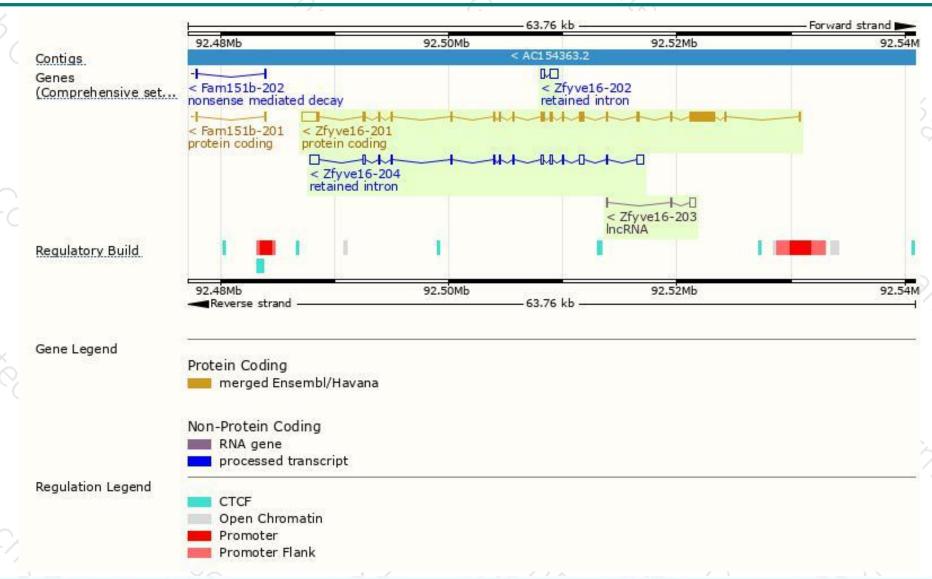
| Name        | Transcript ID        | bp   | Protein    | Biotype         | CCDS      | UniProt | Flags                         |
|-------------|----------------------|------|------------|-----------------|-----------|---------|-------------------------------|
| Zfyve16-201 | ENSMUST00000022217.8 | 6150 | 1528aa     | Protein coding  | CCDS26682 | Q80U44  | TSL:1 GENCODE basic APPRIS P1 |
| Zfyve16-204 | ENSMUST00000156586.7 | 3146 | No protein | Retained intron | -         | -       | TSL:1                         |
| Zfyve16-202 | ENSMUST00000136135.1 | 929  | No protein | Retained intron | 49        |         | TSL:3                         |
| Zfyve16-203 | ENSMUST00000154850.1 | 783  | No protein | IncRNA          | 29        | 10 T    | TSL:3                         |

The strategy is based on the design of Zfyve16-201 transcript, The transcription is shown below



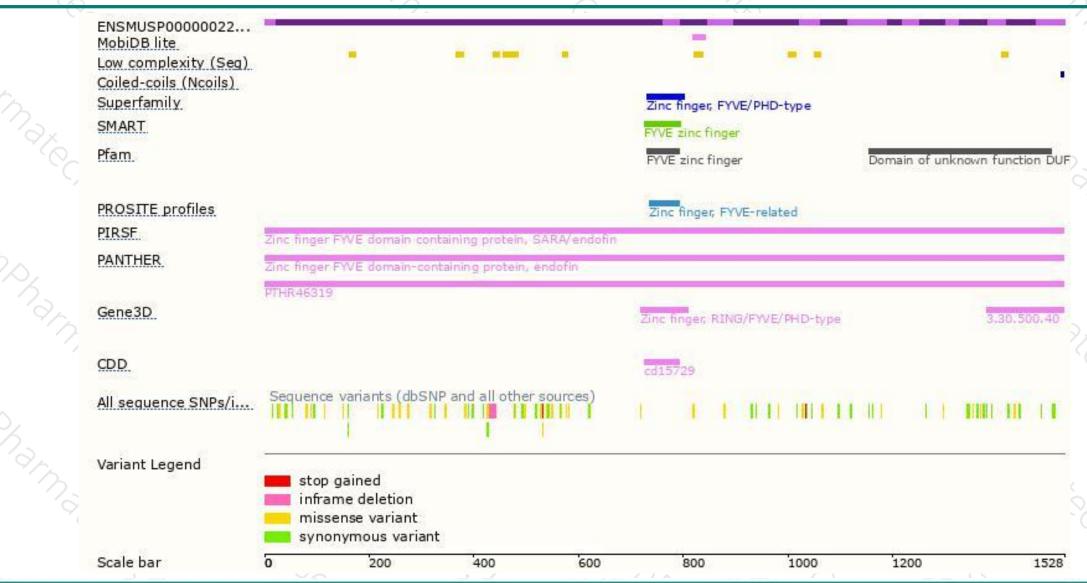
### Genomic location distribution





### Protein domain







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





