

# Fgd3 Cas9-CKO Strategy

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Reviewer: Ruirui Zhang

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## **Project Overview**



Project Name Fgd3

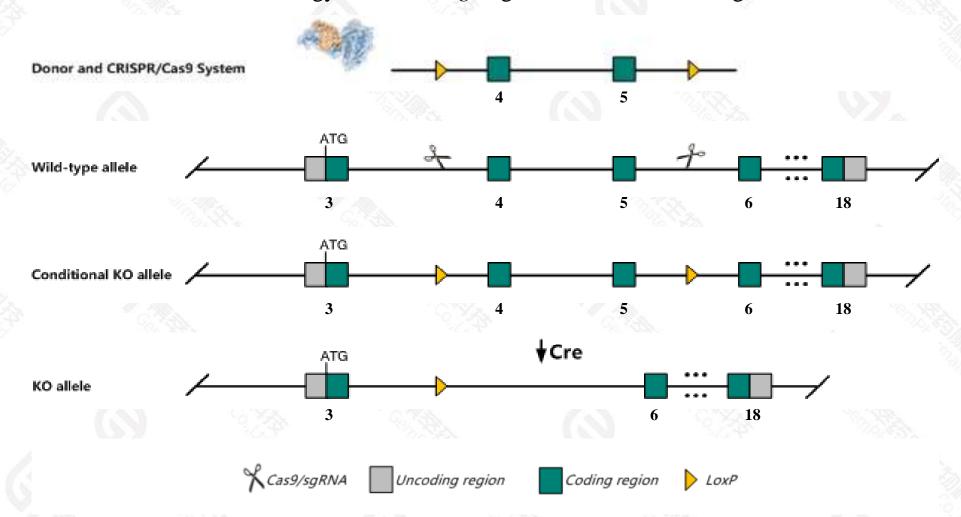
Project type Cas9-CKO

Strain background C57BL/6JGpt

## **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the Fgd3 gene. The schematic diagram is as follows:



### **Technical routes**



- The Fgd3 gene has 3 transcripts. According to the structure of Fgd3 gene, exon4-exon5 of Fgd3203(ENSMUST00000110087.8) transcript is recommended as the knockout region. The region contains 227bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Fgd3* 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.

### **Notice**



- $\succ$  The Fgd3 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)

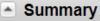


#### Fgd3 FYVE, RhoGEF and PH domain containing 3 [ Mus musculus (house mouse) ]

**♣** Download Datasets

☆ ?

Gene ID: 30938, updated on 14-Jan-2021



Official Symbol Fgd3 provided by MGI

Official Full Name FYVE, RhoGEF and PH domain containing 3 provided by MGI

Primary source MGI:MGI:1353657

See related Ensembl:ENSMUSG00000037946

Gene type protein coding
RefSeq status 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 ZFYVE; ZFYVE5; 5830461L01Rik

Expression Broad expression in spleen adult (RPKM 7.4), thymus adult (RPKM 4.4) and 20 other tissues See more

Orthologs <u>human</u> all

Try the new Gene table

Try the new Transcript table

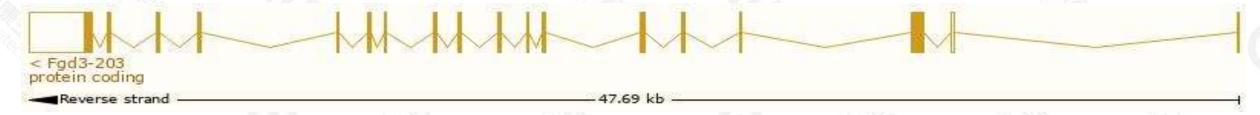
## Transcript information (Ensembl)



The gene has 3 transcripts, all transcripts are shown below:

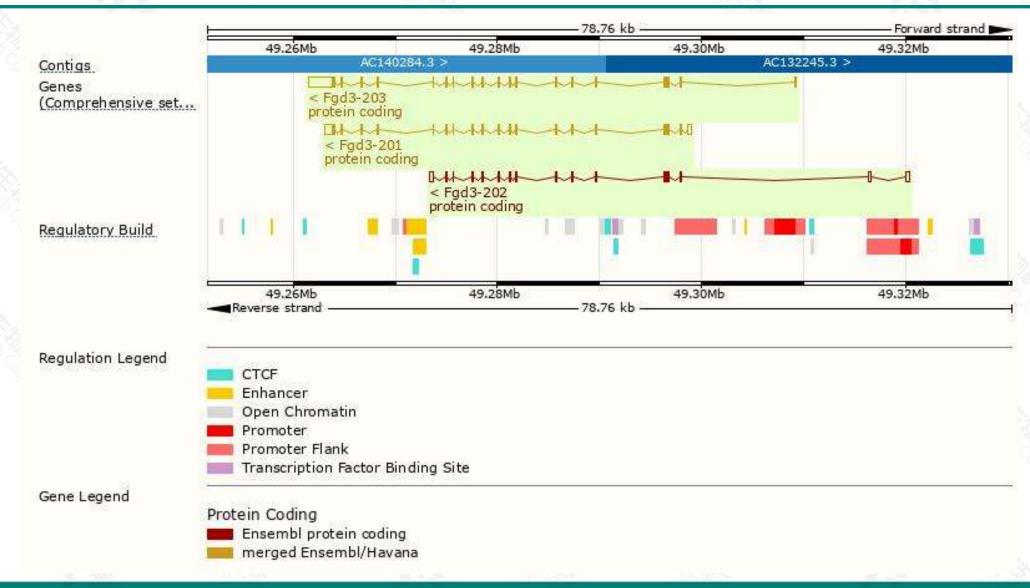
|          |                       |      | - 1/0/-      |                |           |            |                               |
|----------|-----------------------|------|--------------|----------------|-----------|------------|-------------------------------|
| Name     | Transcript ID         | bp   | Protein      | Biotype        | CCDS      | UniProt    | Flags                         |
| Fgd3-203 | ENSMUST00000110087.8  | 4698 | <u>733aa</u> | Protein coding | CCDS26499 | Q3TNB8     | TSL:1 GENCODE basic APPRIS P1 |
| Fgd3-201 | ENSMUST00000048716.10 | 3519 | <u>733aa</u> | Protein coding | CCDS26499 | Q3TNB8     | TSL:1 GENCODE basic APPRIS P1 |
| Fgd3-202 | ENSMUST00000110086.1  | 2628 | <u>532aa</u> | Protein coding | 9         | A0A0R4J1D9 | TSL:1 GENCODE basic           |

The strategy is based on the design of Fgd3-203 transcript, the transcription is shown below:



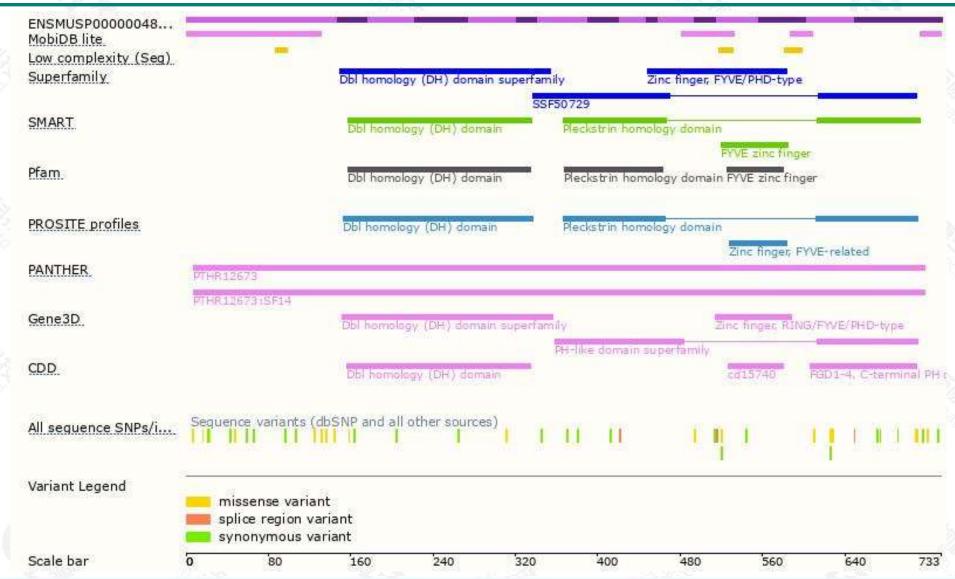
### Genomic location distribution





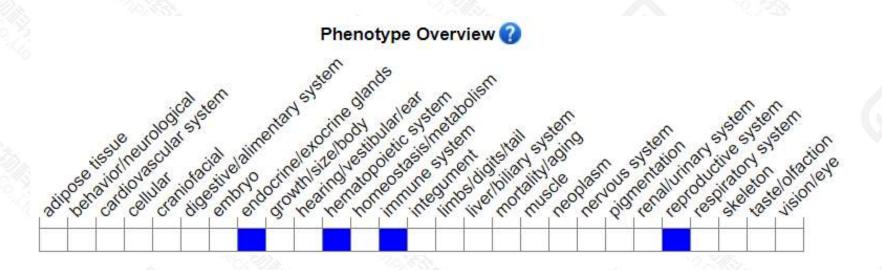
### Protein domain





## Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).



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

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