

# Pofut2 Cas9-CKO Strategy

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## **Project Overview**



**Project Name** 

Pofut2

**Project type** 

Cas9-CKO

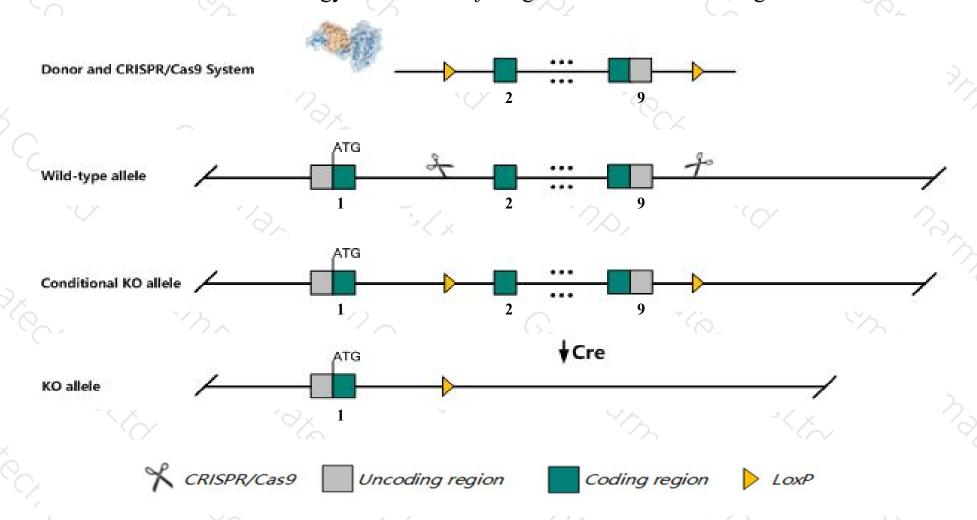
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Pofut2* gene. The schematic diagram is as follows:



### Technical routes



- The *Pofut2* gene has 6 transcripts. According to the structure of *Pofut2* gene, exon2-exon9 of *Pofut2-201* (ENSMUST00000020493.8) transcript is recommended as the knockout region. The region contains most of coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Pofut2* 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.

### **Notice**



- > According to the existing MGI data, Homozygous mutation of this gene results in lethality before weaning.
- ➤ The effect of transcripts 204,206 is unknown.
- $\triangleright$  The flox region is about 1.2 kb away from the 5th end of the Gm10941 gene, and its effect is unknown.
- > The *Pofut2* gene is located on the Chr10. 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)



#### Pofut2 protein O-fucosyltransferase 2 [ Mus musculus (house mouse) ]

Gene ID: 80294, updated on 12-Aug-2019

#### Summary

↑ ?

Official Symbol Pofut2 provided by MGI

Official Full Name protein O-fucosyltransferase 2 provided by MGI

Primary source MGI:MGI:1916863

See related Ensembl: ENSMUSG00000020260

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 FUT13; Al256847; BC003494; C21orf80; 2310011G23Rik

Expression Ubiquitous expression in ovary adult (RPKM 51.4), limb E14.5 (RPKM 44.3) and 28 other tissues See more

Orthologs <u>human</u> all

#### **Genomic context**

Location: 10; 10 C1

Exon count: 9

See Pofut2 in Genome Data Viewer

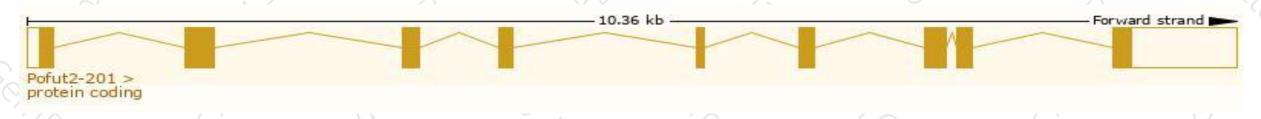
## Transcript information (Ensembl)



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

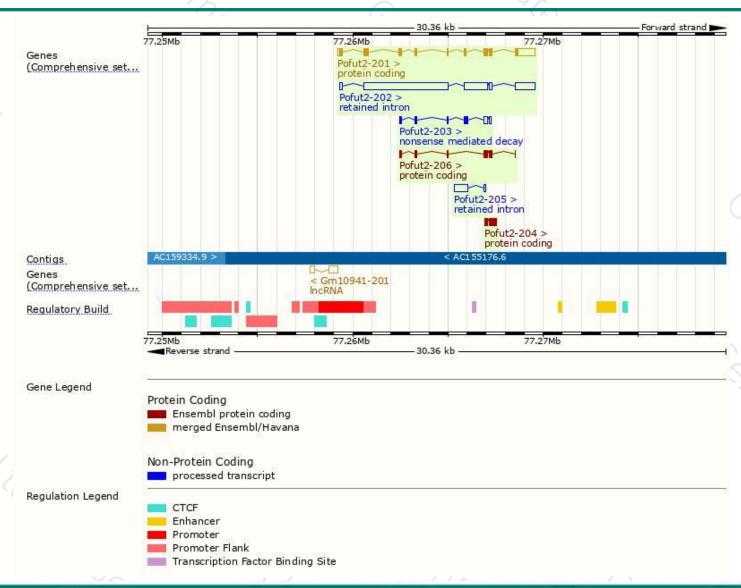
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pofut2-201	ENSMUST00000020493.8	2290	429aa	Protein coding	CCDS23954	B2RV73 Q8VHI3	TSL:1 GENCODE basic APPRIS P1
Pofut2-206	ENSMUST00000219376.1	629	<u>209aa</u>	Protein coding	-	A0A1W2P7Z1	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Pofut2-204	ENSMUST00000218117.1	558	<u>170aa</u>	Protein coding	-	A0A1W2P6H4	CDS 5' incomplete TSL:2
Pofut2-203	ENSMUST00000218064.1	763	<u>164aa</u>	Nonsense mediated decay	92	A0A1W2P844	CDS 5' incomplete TSL:5
Pofut2-202	ENSMUST00000217993.1	7010	No protein	Retained intron	9		TSL:1
Pofut2-205	ENSMUST00000218672.1	756	No protein	Retained intron		-8	TSL:5

The strategy is based on the design of *Pofut2-201* transcript, The transcription is shown below



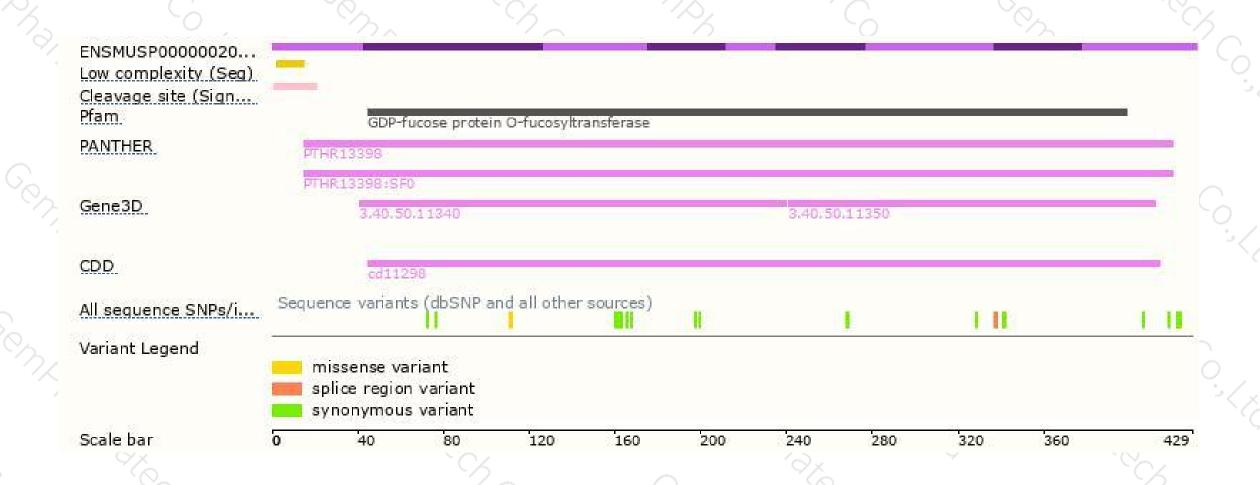
## Genomic location distribution





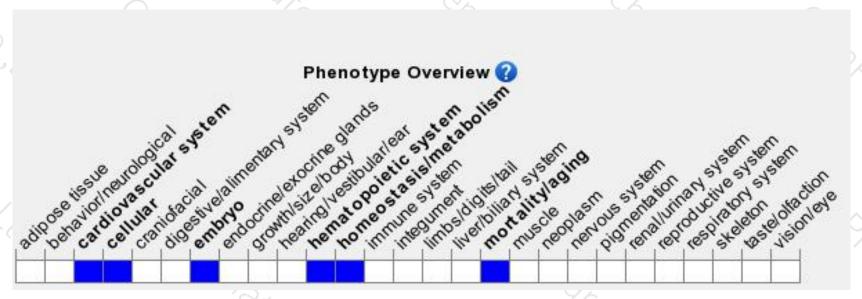
### Protein domain





## Mouse phenotype description(MGI)





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

According to the existing MGI data, Homozygous mutation of this gene results in lethality before weaning.



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





