

# Tgfbr3 Cas9-CKO Strategy

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Reviewer: Ruirui Zhang

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## **Project Overview**



**Project Name** 

Tgfbr3

**Project type** 

Cas9-CKO

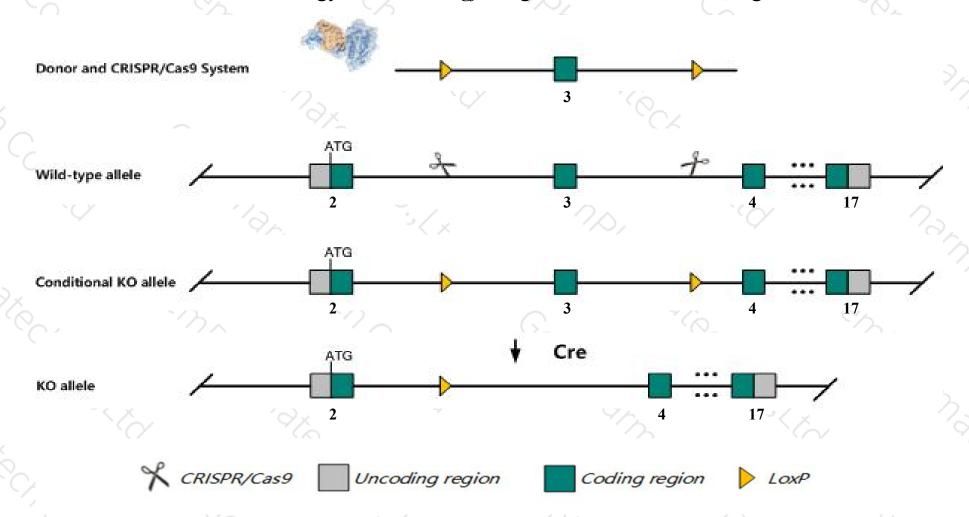
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Tgfbr3* gene. The schematic diagram is as follows:



### Technical routes



- The *Tgfbr3* gene has 4 transcripts. According to the structure of *Tgfbr3* gene, exon3 of *Tgfbr3-201*(ENSMUST00000031224.14) transcript is recommended as the knockout region. The region contains 185bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Tgfbr3* 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, Mice homozygous for disruptions in this gene usually die as embryos. The very few individuals that survive are poorly fertile with abnormalities of the spleen, liver, heart, and skeletal system.
- > The *Tgfbr3* gene is located on the Chr5. 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)



#### Tgfbr3 transforming growth factor, beta receptor III [ Mus musculus (house mouse) ]

Gene ID: 21814, updated on 12-Aug-2019

#### Summary

☆ ?

Official Symbol Tgfbr3 provided by MGI

Official Full Name transforming growth factor, beta receptor III provided by MGI

Primary source MGI:MGI:104637

See related Ensembl:ENSMUSG00000029287

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 TBRIII; AU015626; AW215636; 1110036H20Rik

Expression Broad expression in adrenal adult (RPKM 38.6), bladder adult (RPKM 20.1) and 20 other tissues See more

Orthologs <u>human</u> all

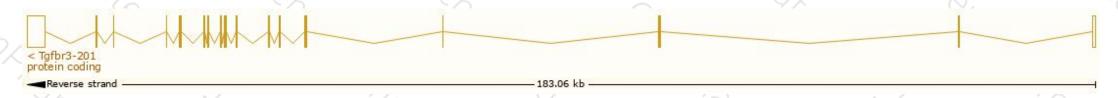
## Transcript information (Ensembl)



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

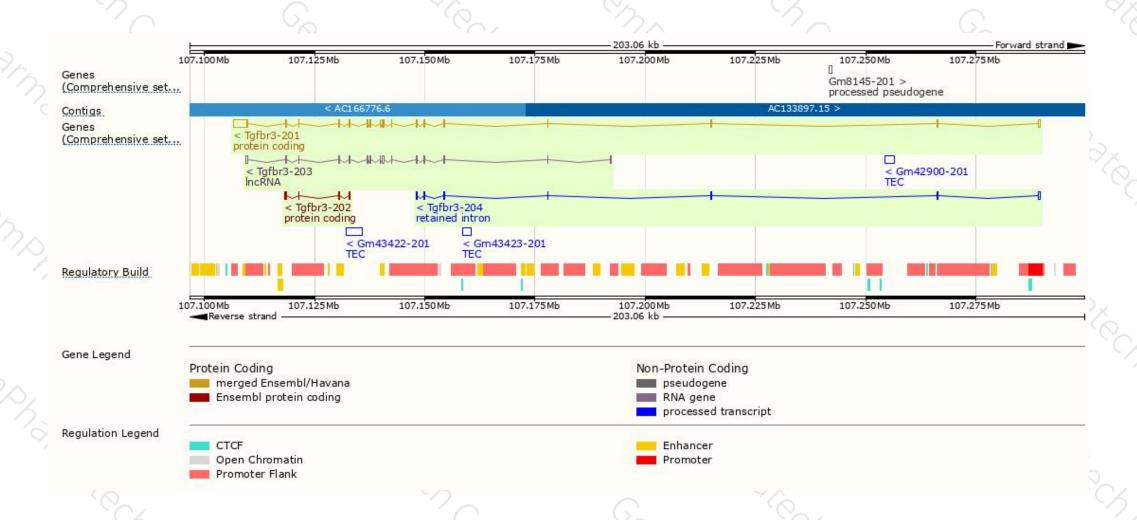
Name 🍦	Transcript ID	bp 🛊	Protein	Biotype	CCDS	UniProt	Flags
Tgfbr3-201	ENSMUST00000031224.14	6087	850aa	Protein coding	CCDS19499₽	<u>A0A0R4J097</u> ₽	TSL:1 GENCODE basic APPRIS P1
Tgfbr3-202	ENSMUST00000136882.1	687	<u>156aa</u>	Protein coding	628	F6VPT9₽	CDS 5' incomplete TSL:2
Tgfbr3-204	ENSMUST00000146591.1	1368	No protein	Retained intron		520	TSL:1
Tgfbr3-203	ENSMUST00000138469.7	2849	No protein	IncRNA	155	-	TSL:1

The strategy is based on the design of Tgfbr3-201 transcript, The transcription is shown below



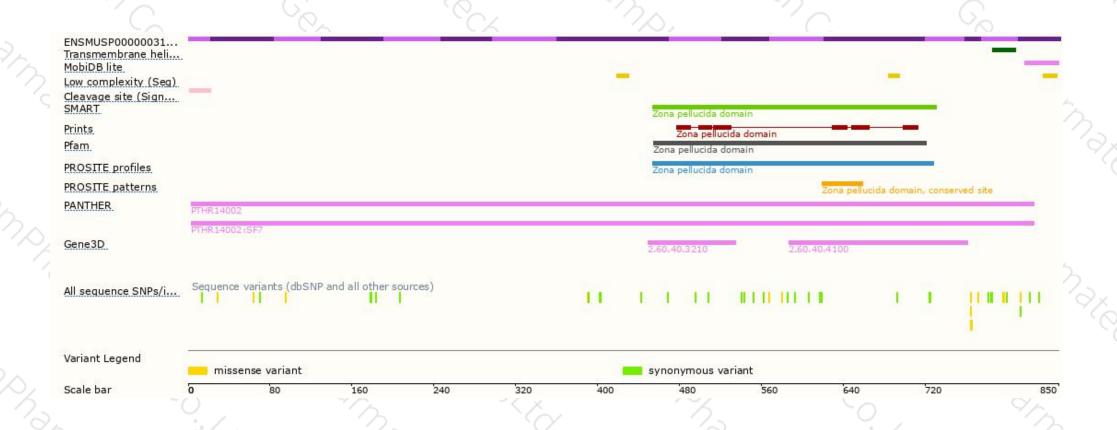
### Genomic location distribution





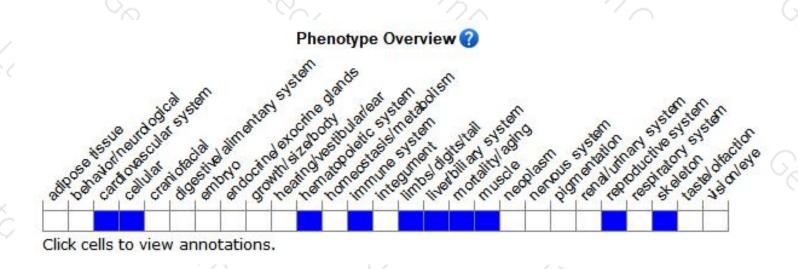
## Protein domain





## 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, Mice homozygous for disruptions in this gene usually die as embryos. The very few individuals that survive are poorly fertile with abnormalities of the spleen, liver, heart, and skeletal system.



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





