

# Gfus Cas9-KO Strategy

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**Reviewer: Shuang Zhang** 

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## **Project Overview**

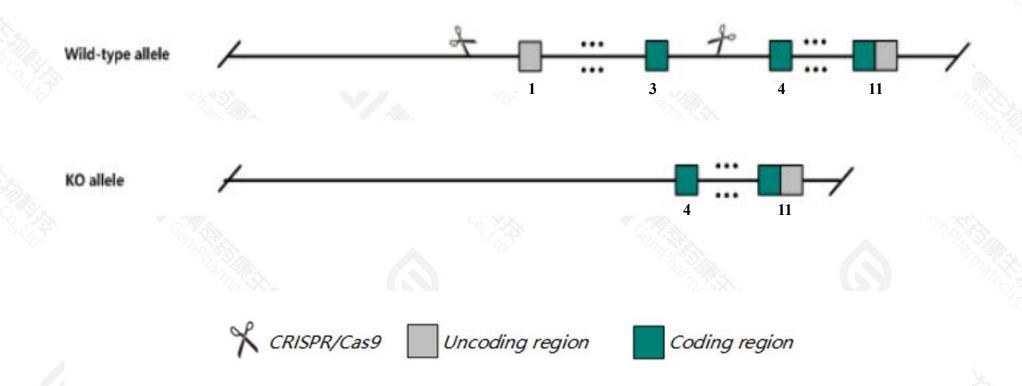


Project Name	Gfus
Project type	Cas9-KO
Strain background	C57BL/6JGpt

## **Knockout strategy**



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



### **Technical routes**



- The *Gfus* gene has 14 transcripts. According to the structure of *Gfus* gene, exon1-exon3 of *Tsta3-204*(ENSMUST00000229641.1) transcript is recommended as the knockout region. The region contains 261bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Gfus* gene. The brief process is as follows: CRISPR/Cas9 system 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.

### **Notice**



- > According to the existing MGI data, homozygotes for a targeted null mutation frequently die in utero, while survivors are smaller than normal, show colon abnormalities, have reduced fertility, and frequently die before weaning. Heterozygotes also show some embryonic loss.
- > The *Gfus* gene is located on the Chr15. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

### Gene information (NCBI)



#### Gfus GDP-L-fucose synthase [Mus musculus (house mouse)]

Gene ID: 22122, updated on 17-Dec-2020

#### Summary

☆ ?

Official Symbol Gfus provided by MGI

Official Full Name GDP-L-fucose synthase provided by MGI

Primary source MGI:MGI:98857

See related Ensembl:ENSMUSG00000022570

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 Al256181, FX, Tst, Tsta3, Tstap, Tstap35b

Expression Ubiquitous expression in colon adult (RPKM 74.6), large intestine adult (RPKM 59.5) and 28 other tissuesSee more

Orthologs <u>human all</u>

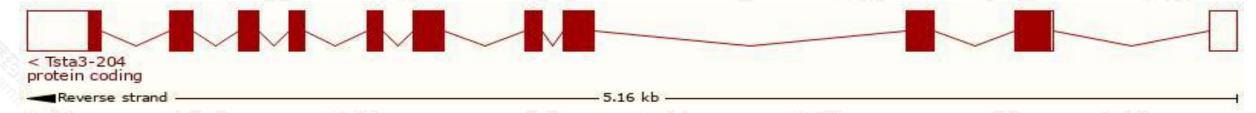
## Transcript information (Ensembl)



The gene has 14 transcripts, all transcripts are shown below:

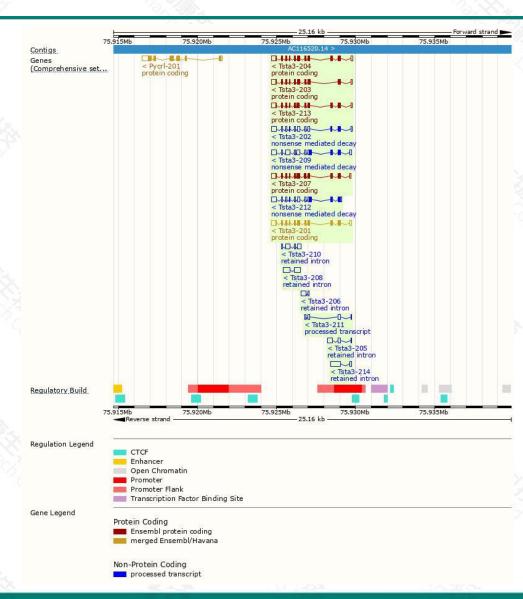
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Tsta3-204	ENSMUST00000229641.1	1355	321aa	Protein coding	CCDS27555	P23591 Q5HZI6	GENCODE basic APPRIS P1	
Tsta3-201	ENSMUST00000023231.6	1335	321aa	Protein coding	CCDS27555	P23591 Q5HZI6	TSL:1 GENCODE basic APPRIS P	
Tsta3-207	ENSMUST00000229951.1	1405	351aa	Protein coding		A0A2R8VI39	GENCODE basic	
Tsta3-213	ENSMUST00000230736.1	1319	327aa	Protein coding		A0A2R8W6P6	GENCODE basic	
Tsta3-203	ENSMUST00000229289.1	1223	<u>292aa</u>	Protein coding	=	A0A2R8VHD0	GENCODE basic	
Tsta3-209	ENSMUST00000230364.1	1603	128aa	Nonsense mediated decay		A0A2R8VKL9		
Tsta3-212	ENSMUST00000230610.1	1431	128aa	Nonsense mediated decay	-	A0A2R8VKL9		
Tsta3-202	ENSMUST00000229085.1	1270	<u>62aa</u>	Nonsense mediated decay	-	A0A2R8W6N0		
Tsta3-211	ENSMUST00000230485.1	383	No protein	Processed transcript	-	6 <del>.</del> 6		
Tsta3-208	ENSMUST00000229997.1	817	No protein	Retained intron	-	042		
Tsta3-214	ENSMUST00000230884.1	723	No protein	Retained intron		-		
Tsta3-210	ENSMUST00000230454.1	649	No protein	Retained intron		-		
Tsta3-205	ENSMUST00000229668.1	523	No protein	Retained intron	2	927		
Tsta3-206	ENSMUST00000229786.1	415	No protein	Retained intron		9.54		

The strategy is based on the design of *Tsta3-204* transcript, the transcription is shown below:



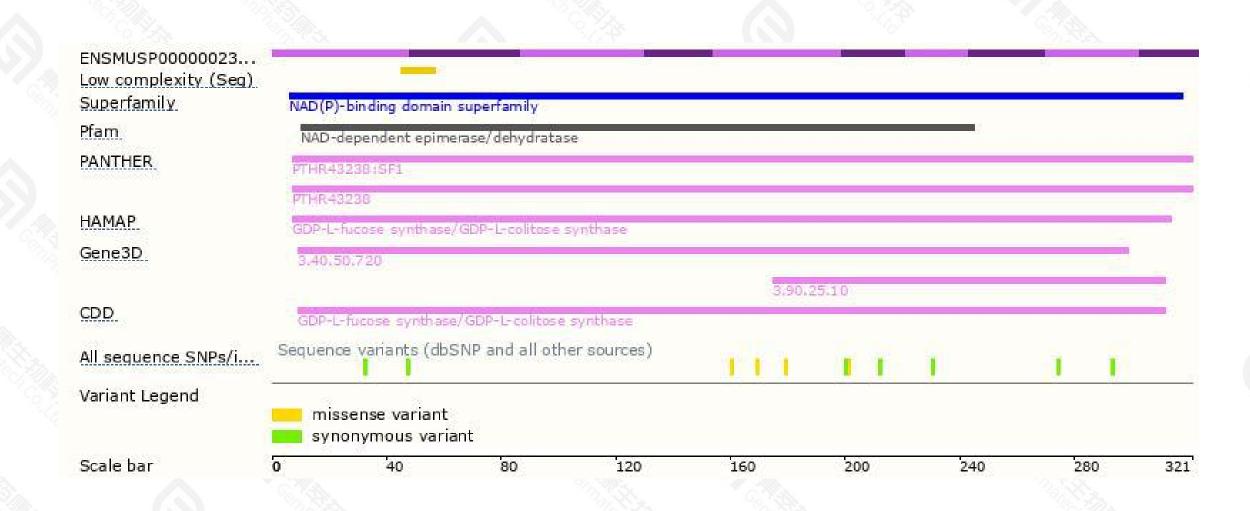
### Genomic location distribution





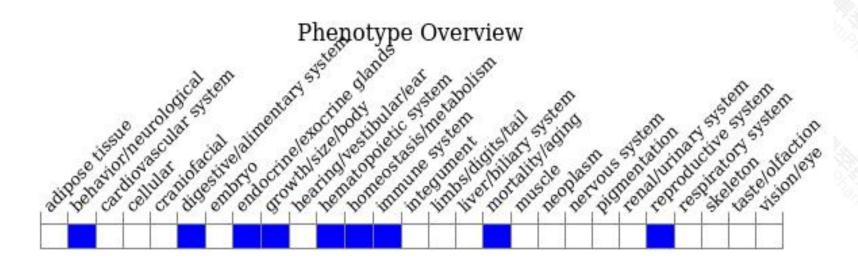
### Protein domain





## 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, homozygotes for a targeted null mutation frequently die in utero, while survivors are smaller than normal, show colon abnormalities, have reduced fertility, and frequently die before weaning. Heterozygotes also show some embryonic loss.



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

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