

Fndc3a Cas9-KO Strategy

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Design Date: 2023-11-23

Overview

Target Gene Name

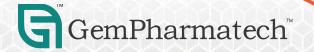
• Fndc3a

Project Type

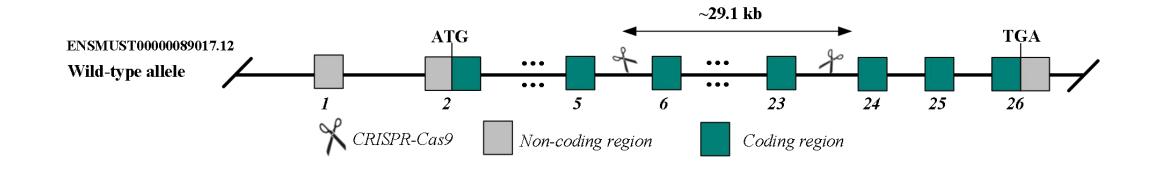
• Cas9-KO

Genetic Background

• C57BL/6JGpt



Strain Strategy



Technical Information

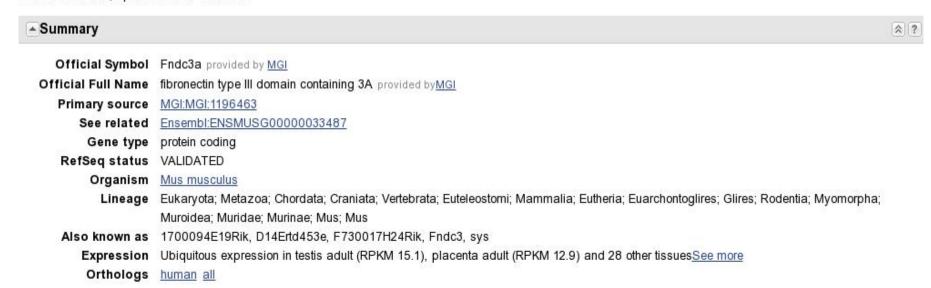
- The *Fndc3a* gene has 7 transcripts. According to the structure of *Fndc3a* gene, exon6-exon23 of *Fndc3a*-201 (ENSMUST00000089017.12) transcript is recommended as the knockout region. The region contains 2497 bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Fndc3a* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs 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 ontarget amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.



Gene Information

Fndc3a fibronectin type III domain containing 3A [Mus musculus (house mouse)]

Gene ID: 319448, updated on 31-Jan-2019



Source: https://www.ncbi.nlm.nih.gov/



Transcript Information

The gene has 7 transcripts, all transcripts are shown below:

Transcript ID 🗼	Name A	bp 🛊	Protein 崇	Biotype	CCDS	UniProt Match 🛊	Flags
ENSMUST00000089017.12	Fndc3a-201	6143	<u>1198aa</u>	Protein coding	CCDS27264₺	Q8BX90 ₽	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1
ENSMUST00000159144.2	Fndc3a-202	452	No protein	Retained intron		-	TSL:3
ENSMUST00000161550.2	Fndc3a-203	491	No protein	Protein coding CDS not defined		-	TSL:3
ENSMUST00000162478.8	Fndc3a-204	1808	540aa	Protein coding		E0CXY0₽	TSL:5 CDS 3' incomplete
ENSMUST00000162825.8	Fndc3a-205	4100	<u>1154aa</u>	Protein coding		F6TLV3 ₺	TSL:5 CDS 5' incomplete
ENSMUST00000162922.8	Fndc3a-206	1262	No protein	Retained intron		-	TSL:1
ENSMUST00000163013.2	Fndc3a-207	3109	No protein	Retained intron		8	TSL:1

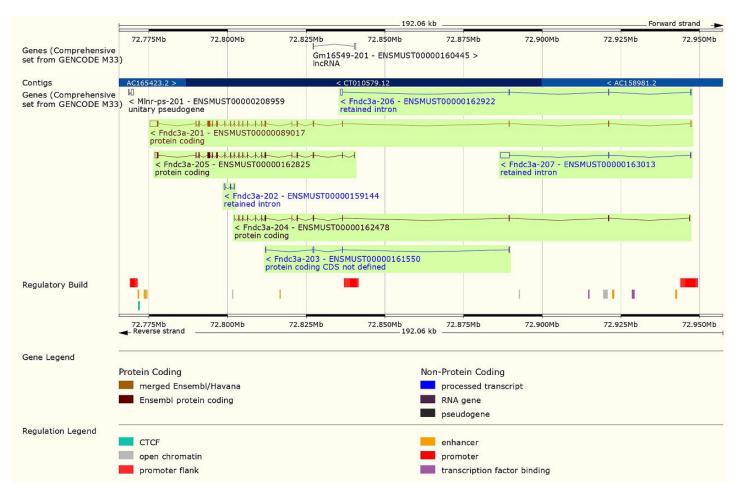
The strategy is based on the design of *Fndc3a*-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org



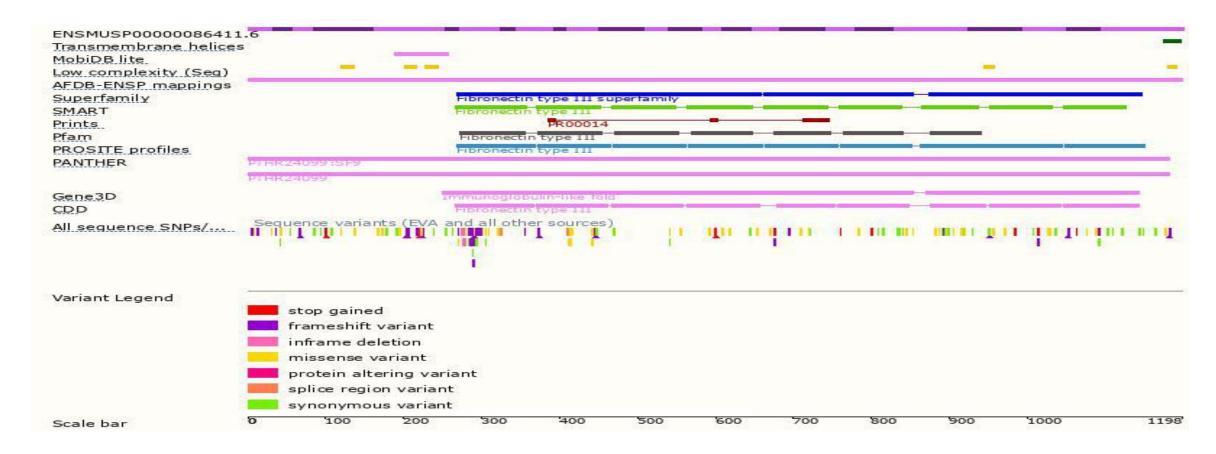
Genomic Information

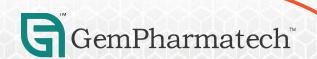




Source: : https://www.ensembl.org

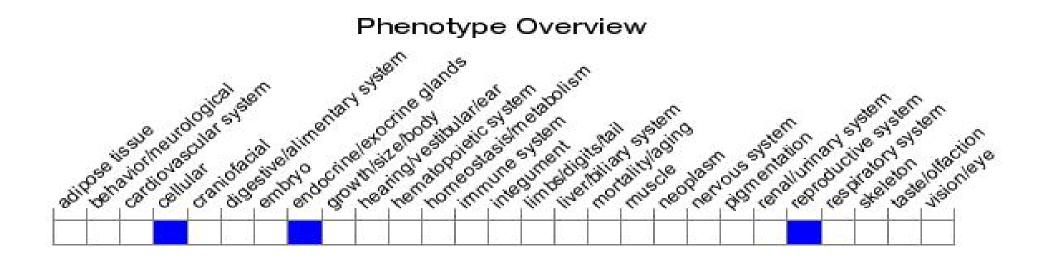
Protein Information





Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



• Males homozygous for an insertional mutation are sterile; females are fertile. In mutant males, spermatids form multinucleated syncytia and fail to mature, while Sertoli cells exhibit abnormal cytoplasmic vacuoles.



Source: https://www.informatics.jax.org

Important Information

- *Fndc3a* is located on Chr14. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- The knockout region of this strategy is about 4.2 kb away from the *Gm16549*-201 gene, which may affect its 5-terminal regulatory function.
- The *Fndc3a*-204 and *Fndc3a*-205 transcript is incomplete, and the impact of this strategy on it is unknown.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

