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Fadd Cas9-CKO Strategy

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Reviewer:Jinling Wang

Design Date:2023-11-3

Overview

Target Gene Name

- Fadd

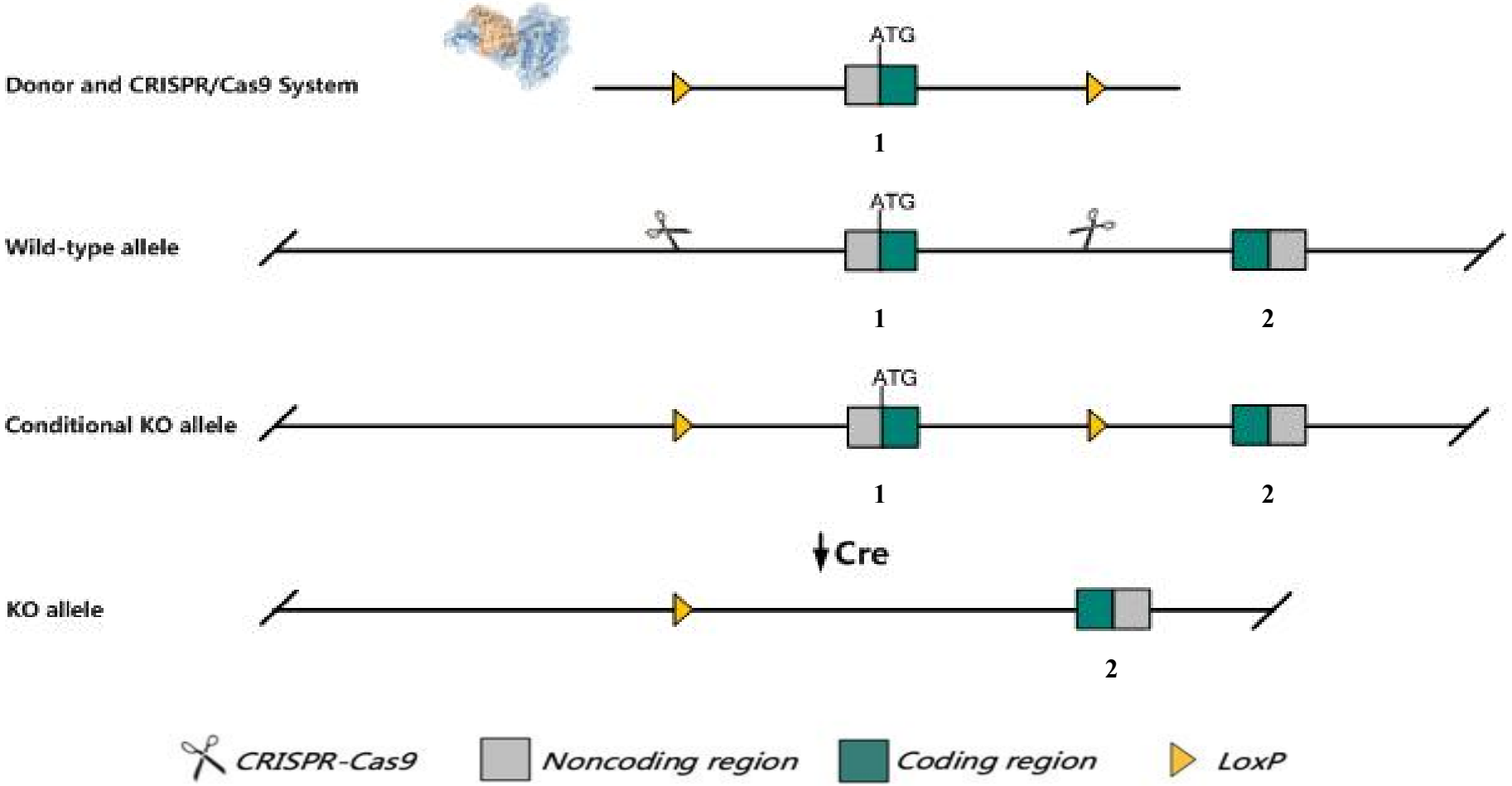
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Fadd* gene.

Technical Information

- The *Fadd* gene has 1 transcript. According to the structure of *Fadd* gene, exon1 of *Fadd*-201 (ENSMUST00000033394.8) transcript is recommended as the knockout region. The region contains start codon ATG. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Fadd* 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 on-target 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.
- 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.

Gene Information

Fadd Fas associated via death domain [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 14082, updated on 15-Oct-2023

Summary

Official Symbol	Fadd provided by MGI
Official Full Name	Fas associated via death domain provided by MGI
Primary source	MGI:MGI:109324
See related	Ensembl:ENSMUSG00000031077 AllianceGenome:MGI:109324
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	Mort1/FADD
Summary	Predicted to enable several functions, including caspase binding activity; death effector domain binding activity; and tumor necrosis factor receptor superfamily binding activity. Involved in several processes, including hematopoietic or lymphoid organ development; negative regulation of activation-induced cell death of T cells; and positive regulation of CD8-positive, alpha-beta cytotoxic T cell extravasation. Acts upstream of or within extrinsic apoptotic signaling pathway in absence of ligand; motor neuron apoptotic process; and regulation of programmed cell death. Predicted to be located in several cellular components, including cell body; cytosol; and membrane raft. Predicted to be part of CD95 death-inducing signaling complex and ripoptosome. Predicted to be active in cytoplasm. Is expressed in several structures, including alimentary system; brain; genitourinary system; hemolymphoid system gland; and liver and biliary system. Human ortholog(s) of this gene implicated in leukemia. Orthologous to human FADD (Fas associated via death domain). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Ubiquitous expression in ovary adult (RPKM 10.0), large intestine adult (RPKM 9.9) and 28 other tissues See more
Orthologs	human all

NEW Try the new Gene table
Try the new Transcript table

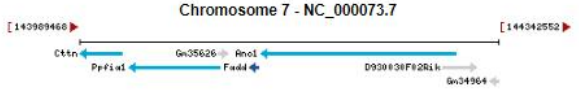
Genomic context

Location: 7 F5; 7 88.85 cM — See Fadd in Genome Data Viewer

Exon count: 2

Annotation release	Status	Assembly	Chr	Location
RS_2023_04	current	GRCm39 (GCF_000001635.27)	7	NC_000073.7 (144132060..144136178, complement)
108.20200622	previous assembly	GRCm38.p6 (GCF_000001635.26)	7	NC_000073.6 (144578323..144582441, complement)

Chromosome 7 - NC_000073.7



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

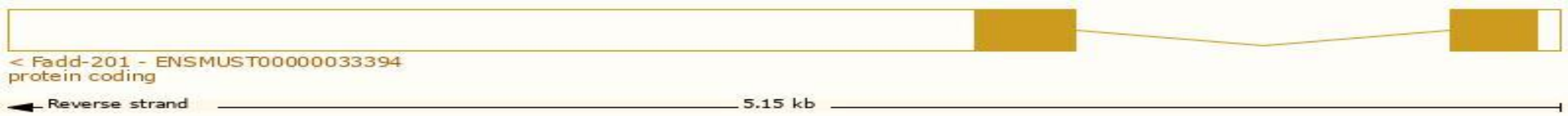
Transcript Information

The gene has 1 transcript,and the transcript is shown below:

Show/hide columns (1 hidden) Filter

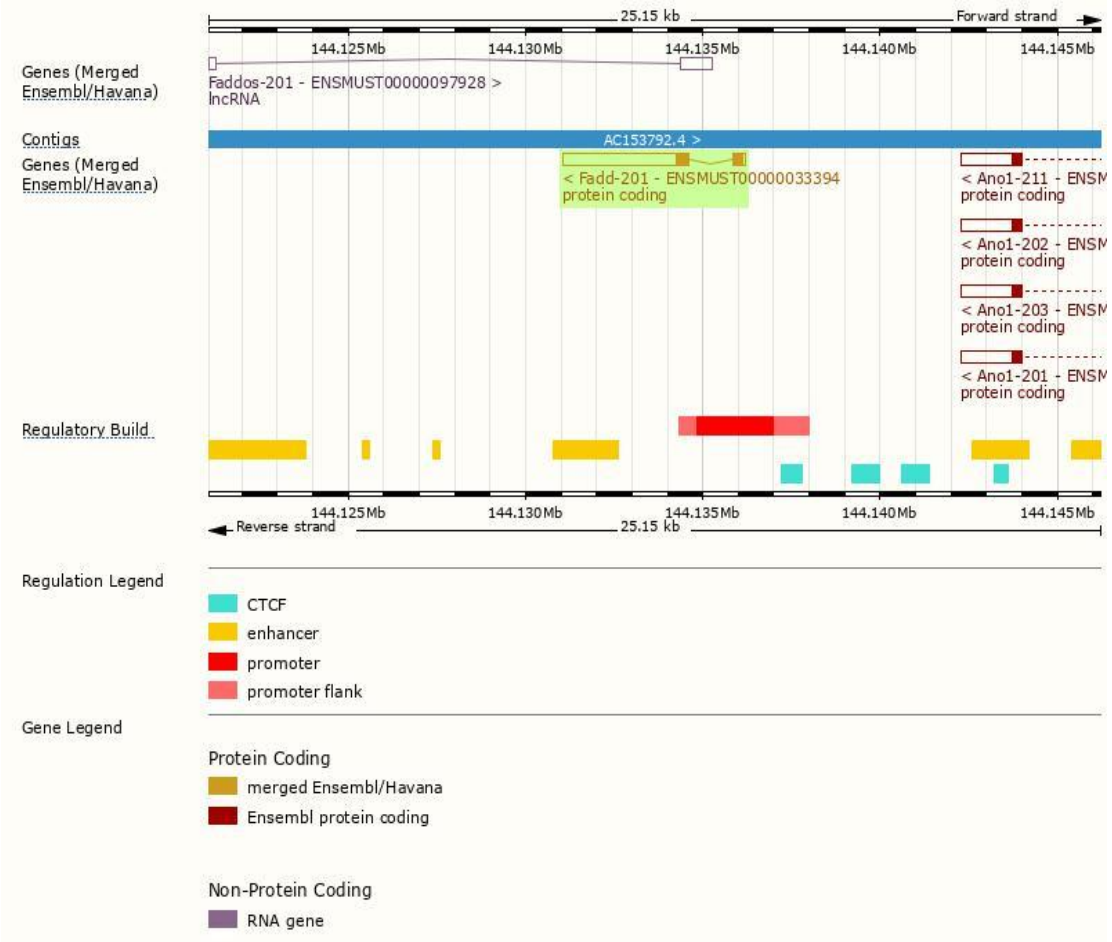
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000033394.8	Fadd-201	3903	205aa	Protein coding	CCDS22050	Q61160	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1

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



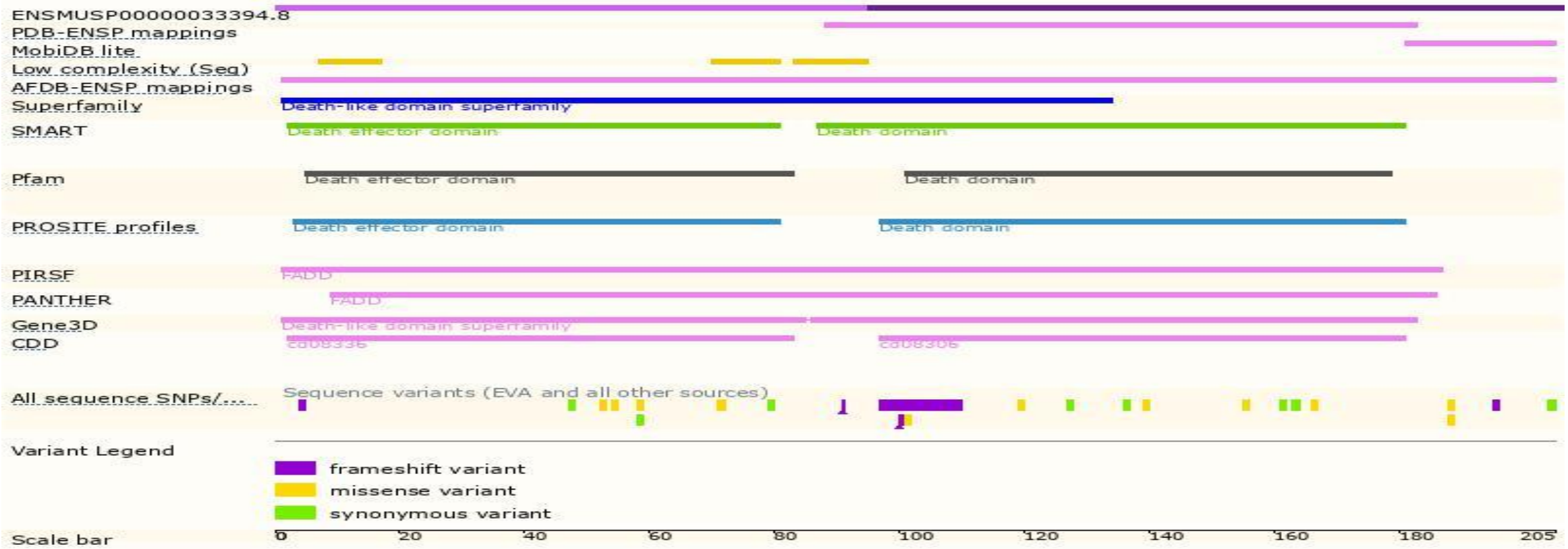
Source: https://www.ensembl.org

Genomic Information



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

Protein Information



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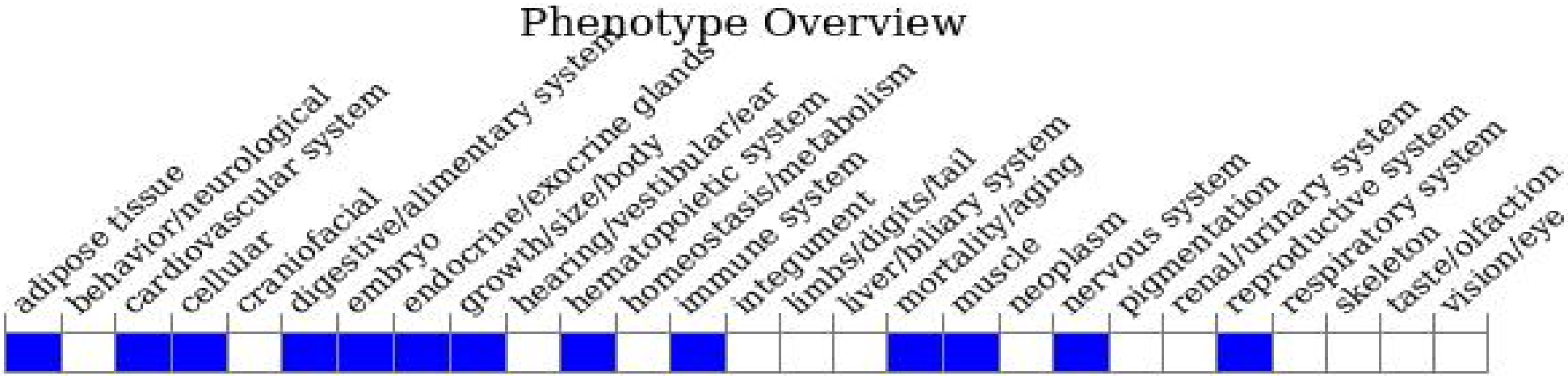
Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



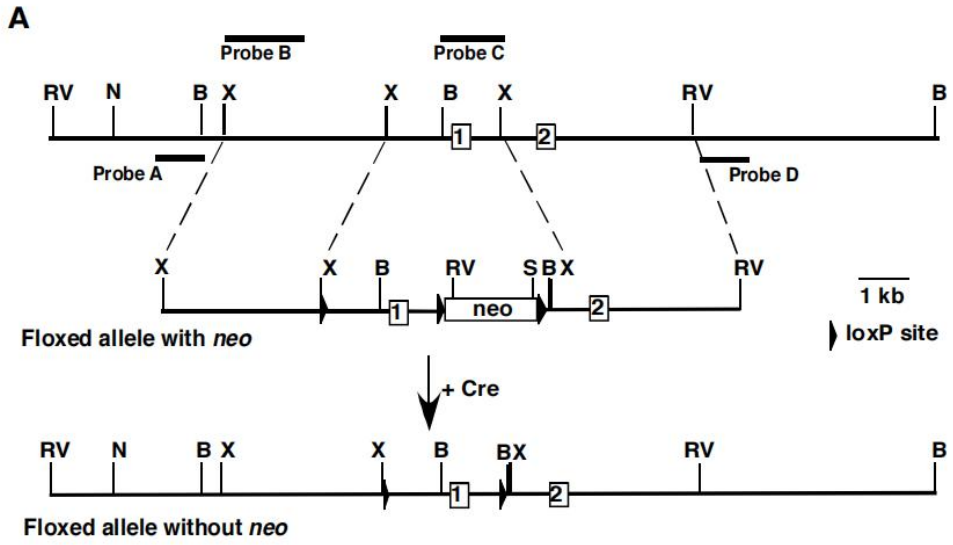
- Mice homozygous for a knock-out allele exhibit embryonic lethality associated with abnormal embryogenesis.

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

Important Information

- According to MGI information,Mice homozygous for a knock-out allele exhibit embryonic lethality associated with abnormal embryogenesis.Mice homozygous for a knock-out allele exhibit embryonic lethality associated with abnormal embryogenesis.
- *Fadd* is located on Chr7. 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.
- 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 the existing technology level.

Reference



Legends to Supplementary Figures:

Fig. S1. Generation of a T cell-specific FADD conditional knockout mouse. (A). A schematic diagram of the genomic *FADD* locus, the knockout allele with the neomycin (neo) gene, and the knockout allele post-neo excision by the Cre system. The probes and enzymes used for screening are also represented. RV=EcoR V, N=Nhe I, B=BamHI, X=Xho I, S=Spe I. Boxes with numbers denote exons and triangles denote loxP sites. (B) Potential knockout mice were analyzed by southern blot analysis for evidence of the knockout allele. The 9.1kB band represents the knockout allele containing the neomycin gene. (C) Mice containing the knockout allele were mated to EIIa Cre transgenic mice and mosaic male offspring were analyzed by southern blot analysis for all combinations of deletions. Offspring from mosaic males with the corrected deletion were mated to C57BL/6 females. They were then analyzed by southern blot analysis for the presence of the flox allele.

Osborn SL, Diehl G, Han SJ, Xue L, Kurd N, Hsieh K, Cado D, Robey EA, Winoto A. Fas-associated death domain (FADD) is a negative regulator of T-cell receptor-mediated necroptosis. Proc Natl Acad Sci U S A. 2010 Jul 20;107(29):13034-9. doi: 10.1073/pnas.1005997107. Epub 2010 Jul 6. PMID: 20615958; PMCID: PMC2919948.