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Fance Cas9-KO Strategy

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Reviewer: Jing Chen

Design Date: 2023.12.25

Overview

Target Gene Name

- Fance

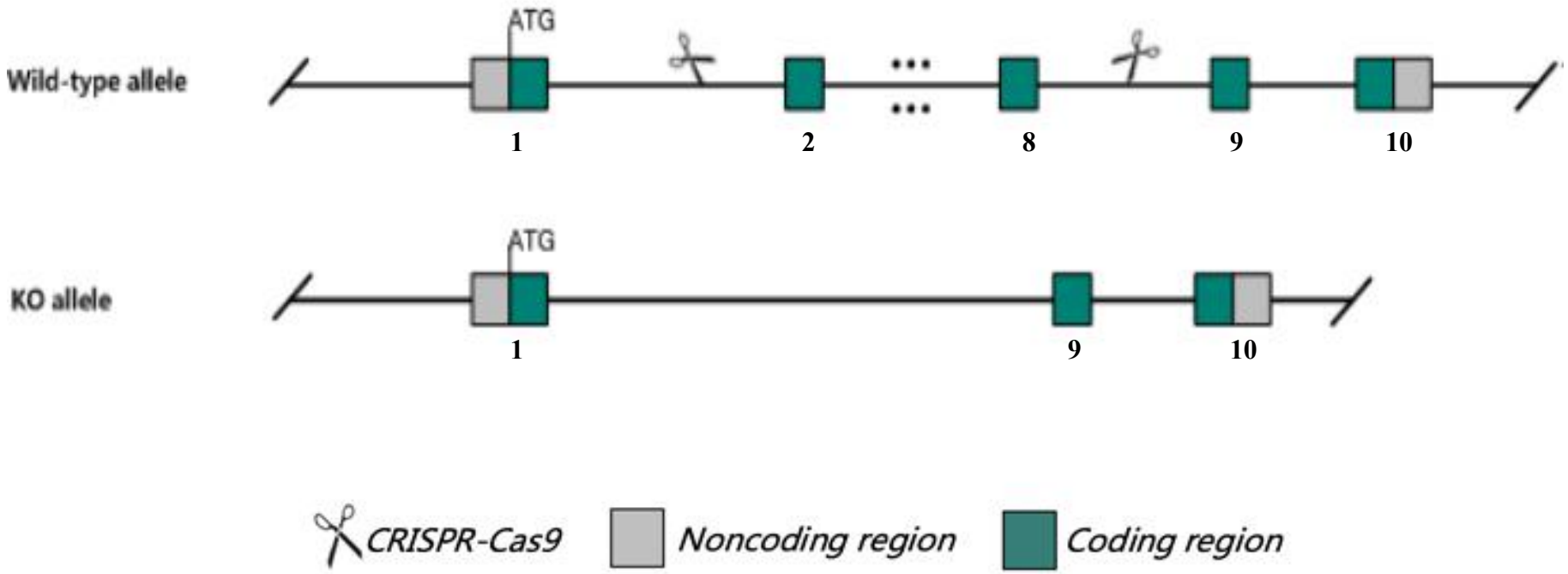
Project Type

- Cas9-KO

Genetic Background

- C57BL/6JGpt

Strain Strategy



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

Technical Information

- The *Fance* gene has 16 transcripts. According to the structure of *Fance* gene, exon2-exon8 of *Fance*-203 (ENSMUST00000114803.9) transcript is recommended as the knockout region. The region contains 1114bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Fance* 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 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.

Gene Information

Fance Fanconi anemia, complementation group E [Mus musculus (house mouse)]

Gene ID: 72775, updated on 12-Apr-2023

Summary

Official Symbol	Fance provided by MGI
Official Full Name	Fanconi anemia, complementation group E provided byMGI
Primary source	MGI:MGI:1920025
See related	Ensembl:ENSMUSG00000007570
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2810451D06Rik
Summary	This gene encodes the complementation group E subunit of the multimeric Fanconi anemia (FA) nuclear complex composed of proteins encoded by over ten Fanconi anemia complementation (FANC) group genes: FANCA, FANCB, FANCC, FANCD1 (also called BRCA2), FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ (also called BRIP1), FANCL, FANCM and FANCN (also called PALB2). The FA complex is necessary for protection against DNA damage. This gene product is required for the nuclear accumulation of FANCC and provides a critical bridge between the FA complex and FANCD2. Defects in the related human gene are a cause of Fanconi anemia, a genetically heterogeneous recessive disorder characterized by cytogenetic instability, hypersensitivity to DNA crosslinking agents, increased chromosomal breakage, and defective DNA repair. Translation of this protein is initiated at a non-AUG (CUG) start codon, which is inferred from the related human gene and the notion that this protein is functionally indispensable. Multiple transcript variants encoding different isoforms have been identified. [provided by RefSeq, Aug 2009]
Expression	Ubiquitous expression in adrenal adult (RPKM 8.9), ovary adult (RPKM 7.6) and 28 other tissuesSee more
Orthologs	human all

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

Transcript Information

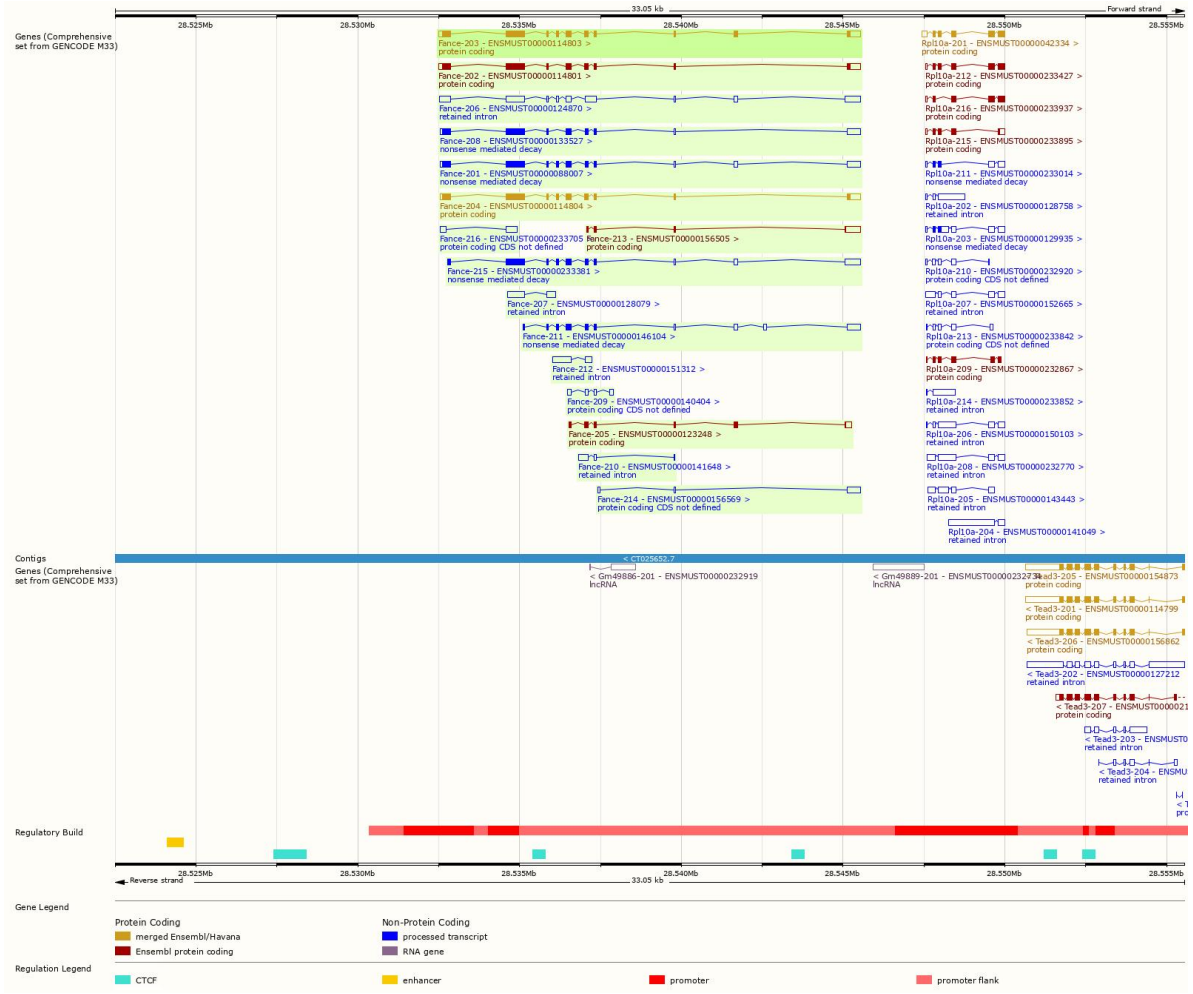
The gene has 16 transcripts,all transcripts are shown below:

Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000088007.12	Fance-201	1942	406aa	Nonsense mediated decay		B8JJD6	TSL:5
ENSMUST00000114801.9	Fance-202	1825	462aa	Protein coding		B8JJD5	GENCODE basic TSL:1
ENSMUST00000114803.9	Fance-203	2025	526aa	Protein coding		B8JJD3	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1
ENSMUST00000114804.11	Fance-204	1842	484aa	Protein coding		F7DAL6	GENCODE basic TSL:1
ENSMUST00000123248.8	Fance-205	638	154aa	Protein coding		B8JJD7	TSL:3 CDS 5' incomplete
ENSMUST00000124870.8	Fance-206	2193	No protein	Retained intron		-	TSL:2
ENSMUST00000128079.3	Fance-207	816	No protein	Retained intron		-	TSL:1
ENSMUST00000133527.9	Fance-208	1751	384aa	Nonsense mediated decay		B8JJD8	TSL:5
ENSMUST00000140404.2	Fance-209	398	No protein	Protein coding CDS not defined		-	TSL:3
ENSMUST00000141648.3	Fance-210	404	No protein	Retained intron		-	TSL:5
ENSMUST00000146104.3	Fance-211	1197	151aa	Nonsense mediated decay		B8JJD1	TSL:3 CDS 5' incomplete
ENSMUST00000151312.2	Fance-212	770	No protein	Retained intron		-	TSL:3
ENSMUST00000156505.9	Fance-213	672	71aa	Protein coding		B8JJD2	TSL:2 CDS 5' incomplete
ENSMUST00000156569.2	Fance-214	536	No protein	Protein coding CDS not defined		-	TSL:5
ENSMUST00000233381.2	Fance-215	1774	355aa	Nonsense mediated decay		A0A3B2W3J1	CDS 5' incomplete
ENSMUST00000233705.2	Fance-216	531	No protein	Protein coding CDS not defined		-	-

The strategy is based on the design of *Fance*-203 transcript, the transcription is shown below:

Source: https://www.ensembl.org

Genomic Information



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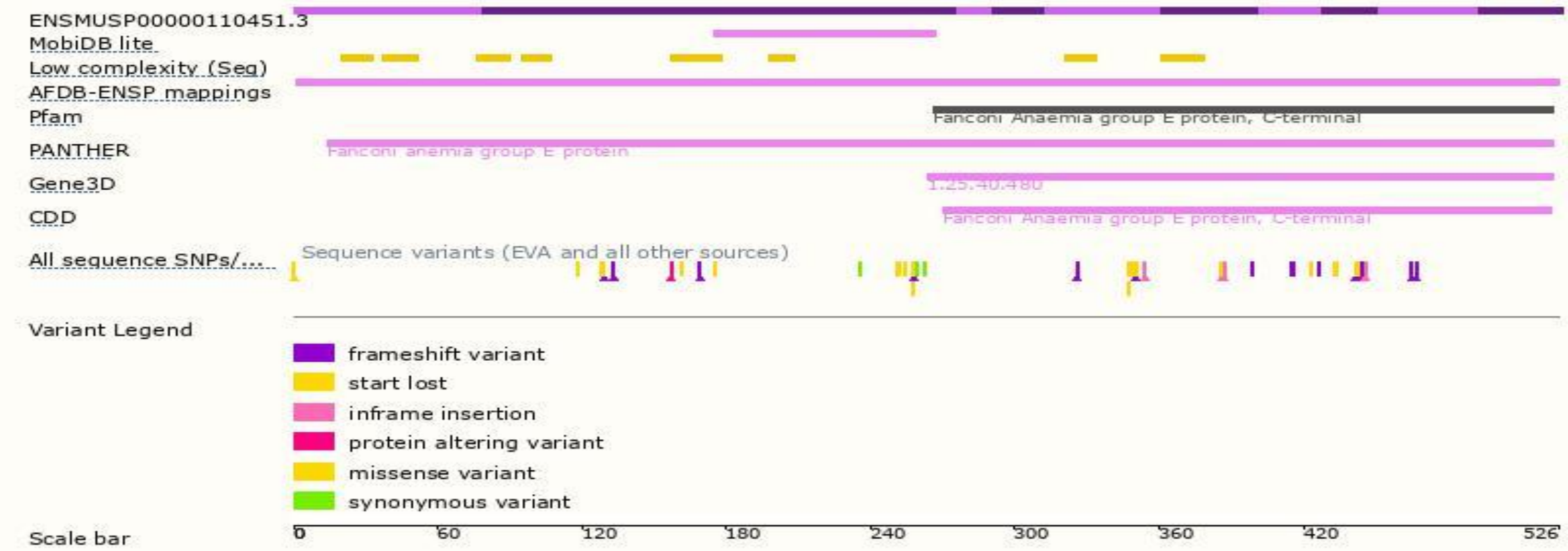
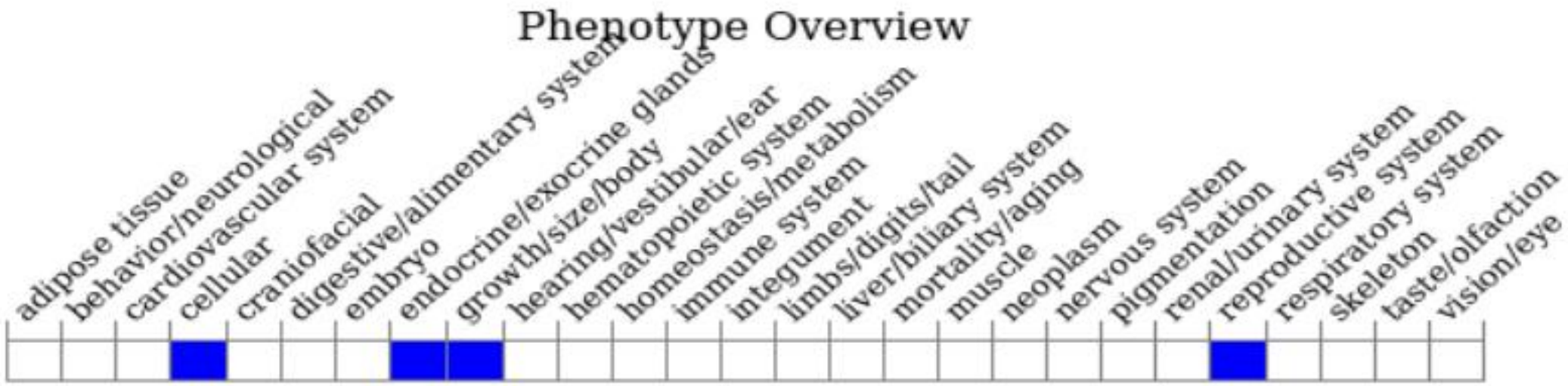
Source: : https://www.ensembl.org

Protein Information



Source: https://www.ensembl.org

Mouse Phenotype Information (MGI)



- Homozygous knockout causes reduced male fertility.

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

Important Information

- According to MGI, homozygous knockout causes reduced male fertility.
- The knockout region is approximately 7.0kb away from the 5’ End of the gene *Rpl10a,* which may affect the regulation of the 5-terminal.
- Knockout regions overlap with gene *LncRNA Gm49886-201*, with unknown impact.
- *Fance* is located on Chr17. 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 risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.