GemPharmatech™

Gfi1 Cas9-CKO Strategy

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Overview

Target Gene Name

- Gfi1

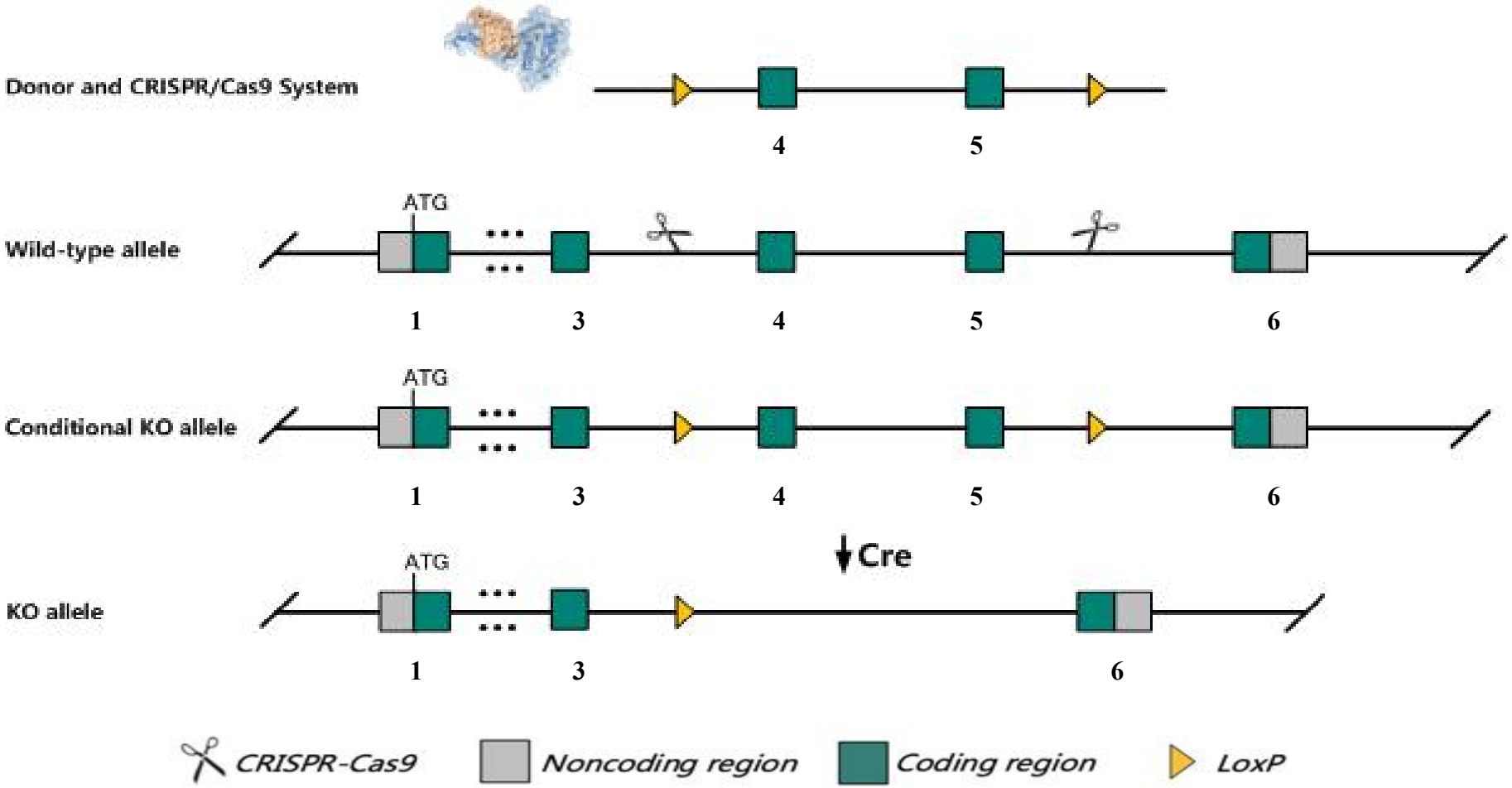
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Gfi1* gene.

Technical Information

- The *Gfi1* gene has 4 transcripts. According to the structure of *Gfi1* gene, exon4-exon5 of *Gfi1*-201 (ENSMUST00000031205.16) transcript is recommended as the knockout region. The region contains 304bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Gfi1* 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

Gfi1 growth factor independent 1 transcription repressor [Mus musculus (house mouse)]

Gene ID: 14581, updated on 16-May-2023

Summary

Official Symbol	Gfi1 provided by MGI
Official Full Name	growth factor independent 1 transcription repressor provided byMGI
Primary source	MGI:MGI:103170
See related	Ensembl:ENSMUSG00000029275
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	Gfi-1, Pal-1, Pal1
Summary	Predicted to enable DNA-binding transcription repressor activity, RNA polymerase II-specific and RNA polymerase II cis-regulatory region sequence-specific DNA binding activity. Involved in negative regulation of NF-kappaB transcription factor activity and negative regulation of transcription by RNA polymerase II. Acts upstream of or within several processes, including cellular response to lipopolysaccharide; inner ear development; and regulation of cell fate specification. Predicted to be located in nuclear body and nuclear matrix. Predicted to be part of transcription repressor complex. Is expressed in several structures, including gut epithelium; hemolymphoid system; liver; nervous system; and sensory organ. Used to study severe congenital neutropenia. Human ortholog(s) of this gene implicated in acute myeloid leukemia and severe congenital neutropenia 2. Orthologous to human GFI1 (growth factor independent 1 transcriptional repressor). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Biased expression in thymus adult (RPKM 40.7), colon adult (RPKM 6.0) and 3 other tissuesSee more
Orthologs	human all

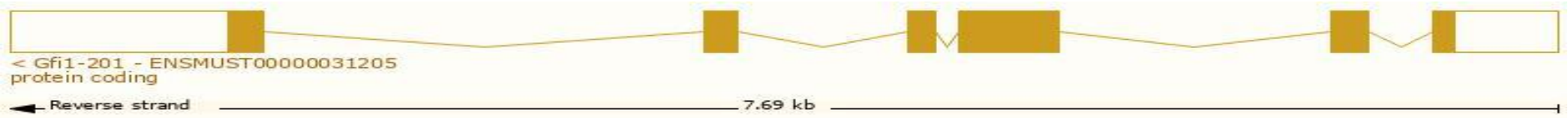
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

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

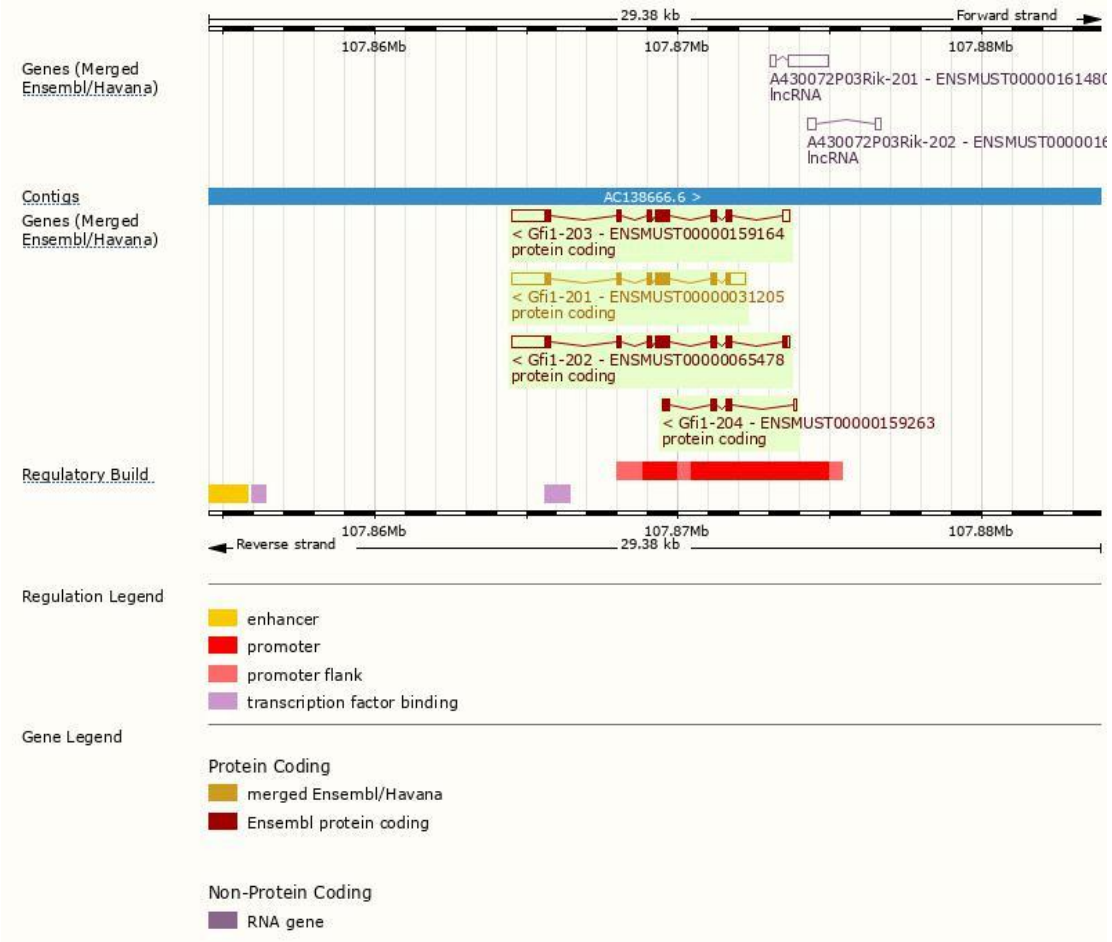
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gfi1-202	ENSMUST00000065478.12	2639	493aa	Protein coding	CCDS59680		A single transcript chosen for a gene which is the most conserved, most highly expressed, has the longest coding sequence and is represented in other key resources, such as NCBI and UniProt. This is defined in detail on http://www.ensembl.org/info/genome/genebuild/canonical.html Ensembl Canonical , The GENCODE set is the gene set for human and mouse. GENCODE basic , TSL:1 ,
Gfi1-201	ENSMUST00000031205.16	2860	423aa	Protein coding	CCDS57371		The GENCODE set is the gene set for human and mouse. GENCODE basic , APPRIS P1 , TSL:1 ,
Gfi1-203	ENSMUST00000159164.9	2648	423aa	Protein coding	CCDS57371		The GENCODE set is the gene set for human and mouse. GENCODE basic , APPRIS P1 , TSL:1 ,
Gfi1-204	ENSMUST00000159263.3	689	186aa	Protein coding			TSL:3 , CDS 3' incomplete ,

The strategy is based on the design of *Gfi1*-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org

Genomic Information



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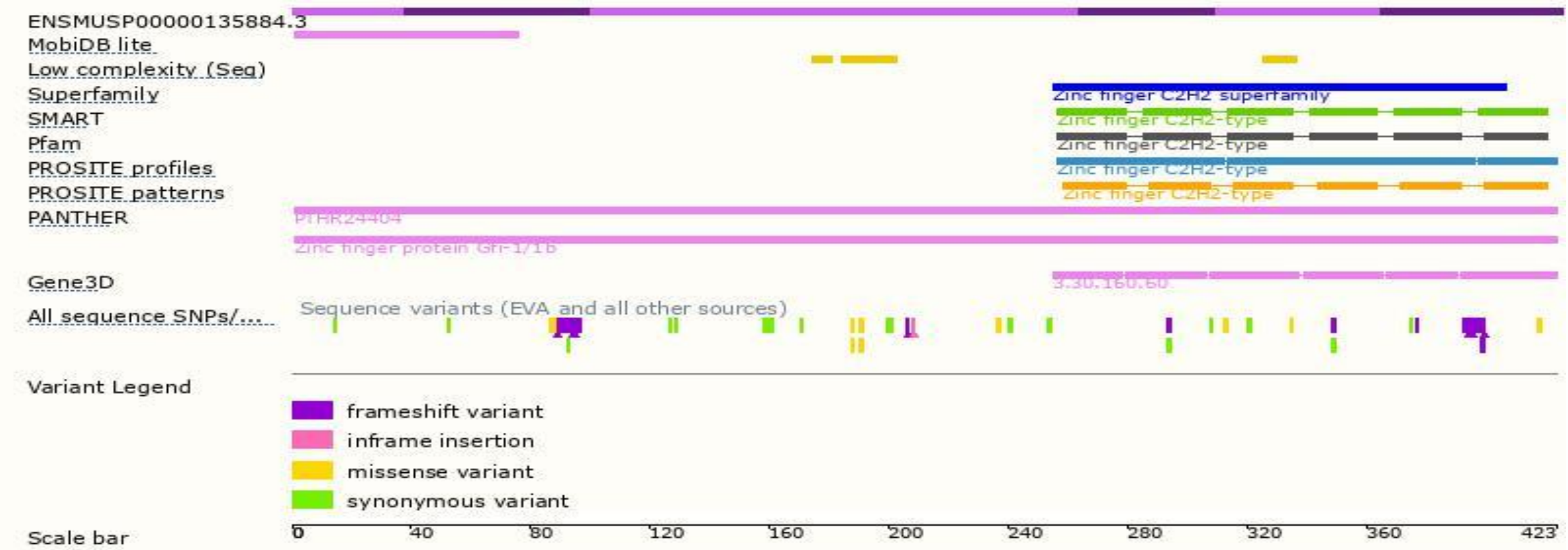
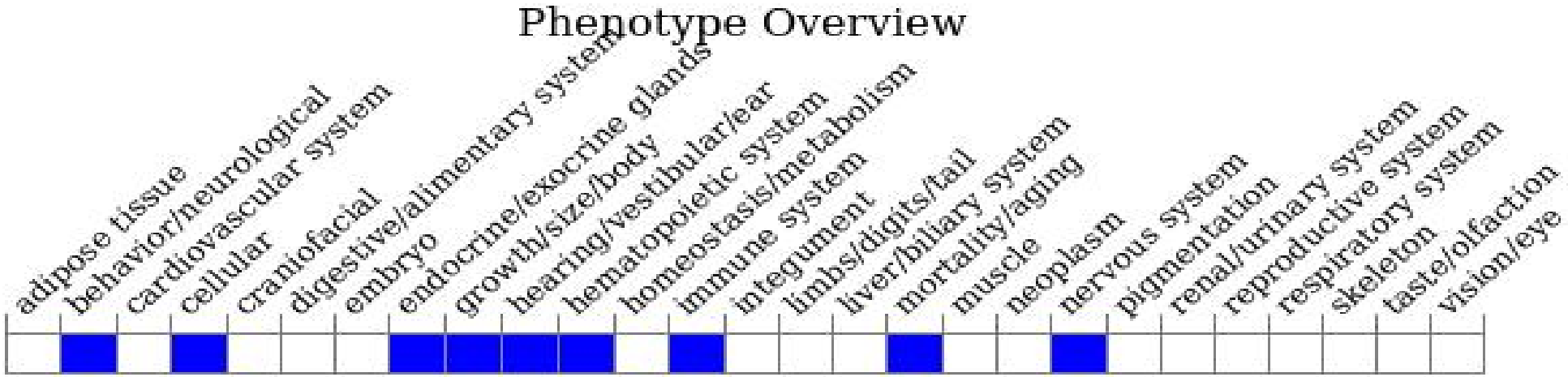
Source: : https://www.ensembl.org

Protein Information



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

Mouse Phenotype Information (MGI)



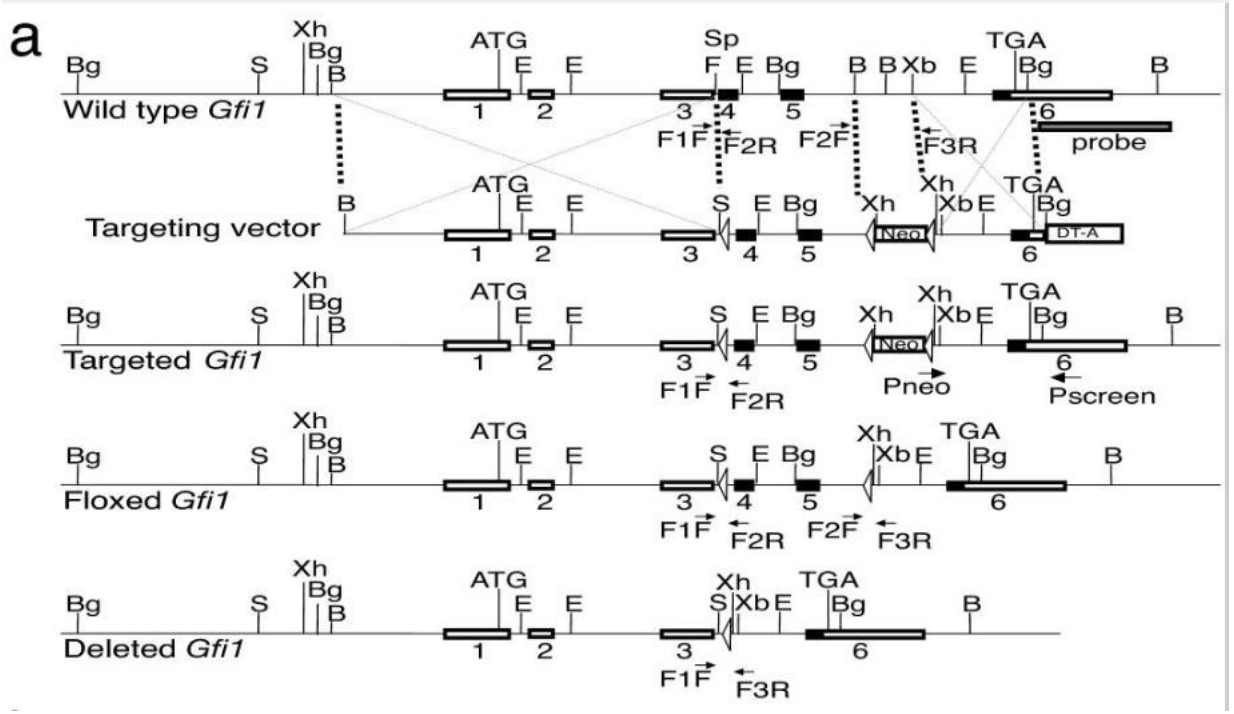
- Homozygotes for targeted null mutations exhibit loss of inner ear hair cells, ataxia, circling, and deafness. Mutants also show a block in granulocyte and neutrophil maturation, and are hypersensitive to endotoxin stimulation.

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

Important Information

- Intron 3-4 is only 118 bp, and the insertion of loxp may affect the normal splicing of the target gene.
- The knockout position of this strategy is approximately 3.9 kb from the 5-terminal of *A430072P03Rik* gene, which may affect the 5-terminal regulation of *A430072P03Rik* gene.
- *Gfi1* is located on Chr5. 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.

References



Zhu J, Jankovic D, Grinberg A, Guo L, Paul WE. Gfi-1 plays an important role in IL-2-mediated Th2 cell expansion. Proc Natl Acad Sci U S A. 2006 Nov 28;103(48):18214-9. doi: 10.1073/pnas.0608981103. Epub 2006 Nov 20. PMID: 17116877; PMCID: PMC1654136.