GemPharmatech™

Slc30a8 Cas9-CKO Strategy

Designer:Xueting Zhang

Reviewer:Ruirui Zhang

Design Date:2023-12-4

Overview

Target Gene Name

- Slc30a8

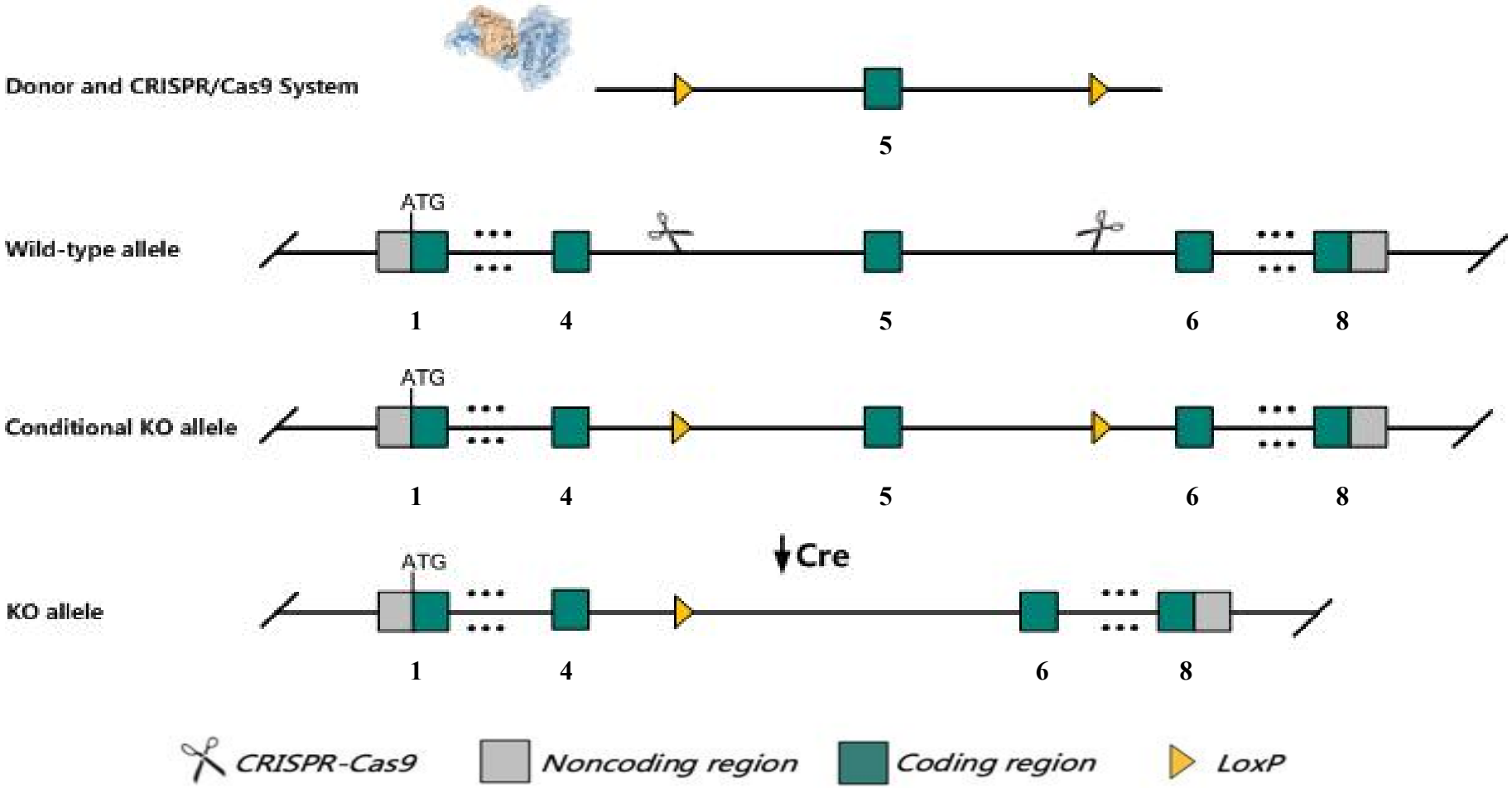
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Slc30a8* gene.

Technical Information

- The *Slc30a8* gene has 1 transcript. According to the structure of *Slc30a8* gene, exon5 of *Slc30a8*-201 (ENSMUST00000037240.3) transcript is recommended as the knockout region. The region contains 151bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Slc30a8* 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

Slc30a8 solute carrier family 30 (zinc transporter), member 8 [Mus musculus (house mouse)]

Gene ID: 239436, updated on 31-Jan-2019

Summary

Official Symbol	Slc30a8 provided by MGI
Official Full Name	solute carrier family 30 (zinc transporter), member 8 provided by MGI
Primary source	MGI:MGI:2442682
See related	Ensembl:ENSMUSG00000022315
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	C820002P14Rik, ZnT-8, ZnT8
Expression	Low expression observed in reference dataset See more
Orthologs	human all

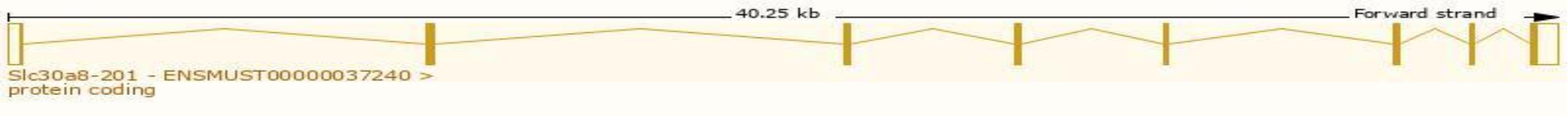
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

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

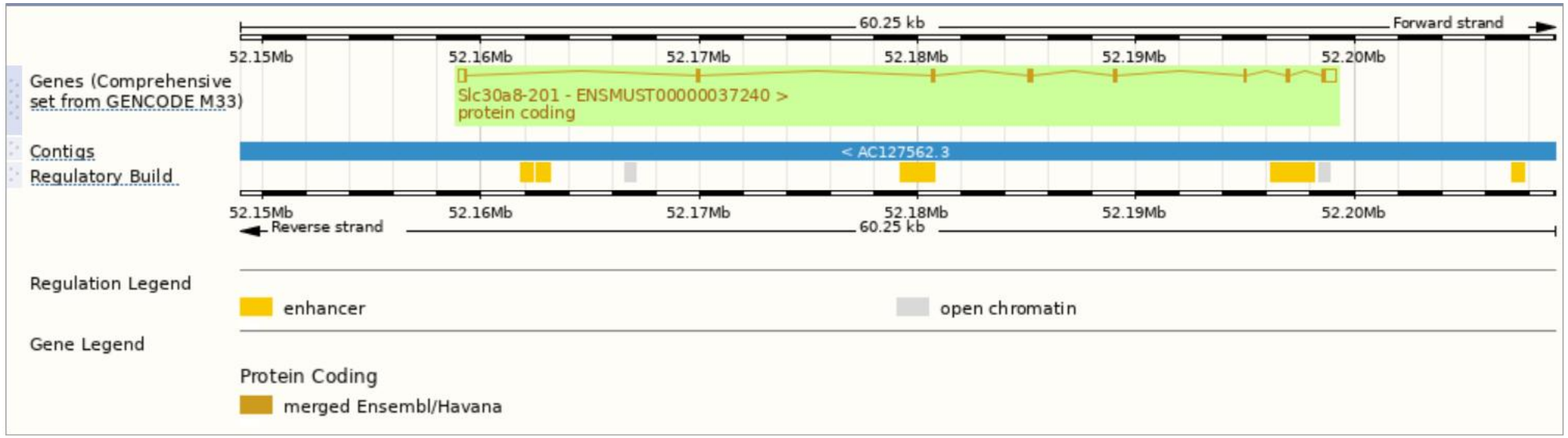
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000037240.3	Slc30a8-201	1971	367aa	Protein coding	CCDS27464	Q8BGG0	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1

The strategy is based on the design of *Slc30a8*-201 transcript, the transcription is shown below:



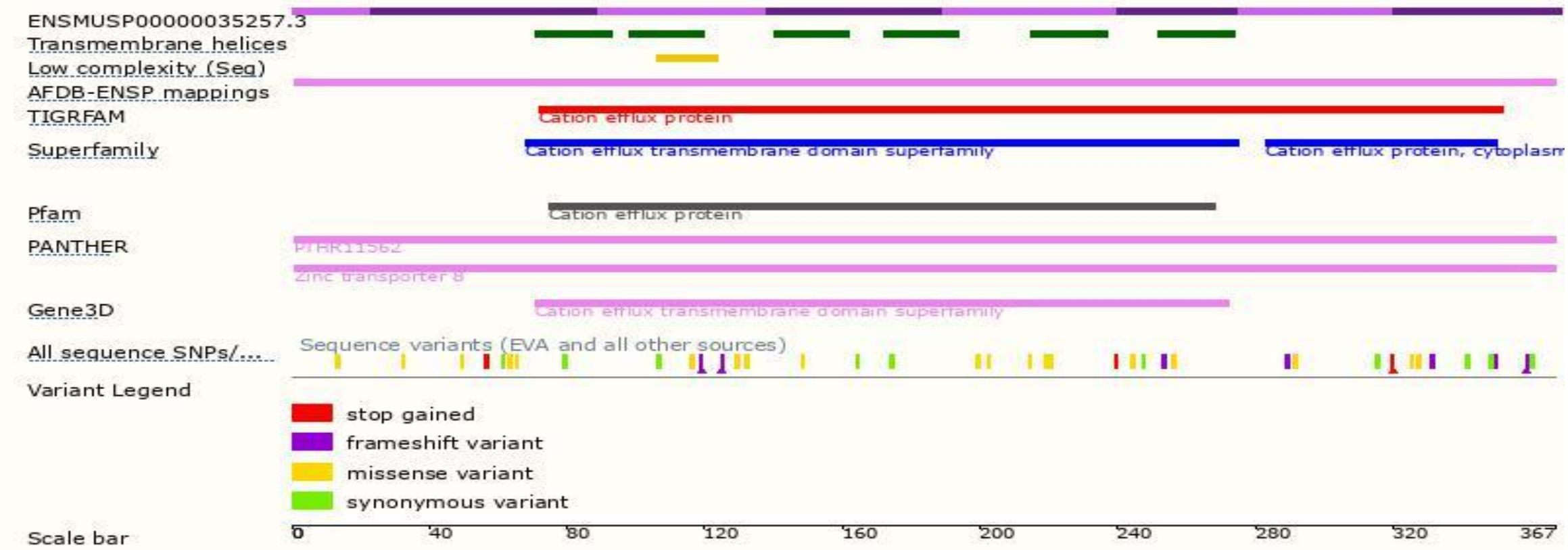
Source: https://www.ensembl.org

Genomic Information



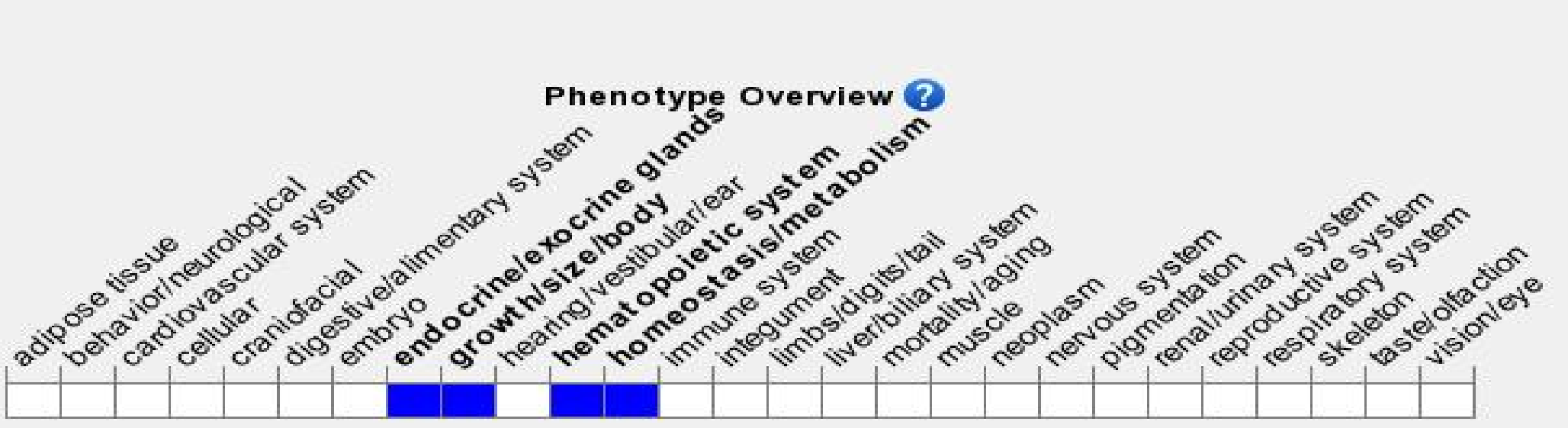
Source: : https://www.ensembl.org

Protein Information



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

Mouse Phenotype Information (MGI)



- Mice homozygous for a knock-out allele exhibit reduced islet zinc levels, circulating insulin levels, and glucose-stimulated insulin secretion.

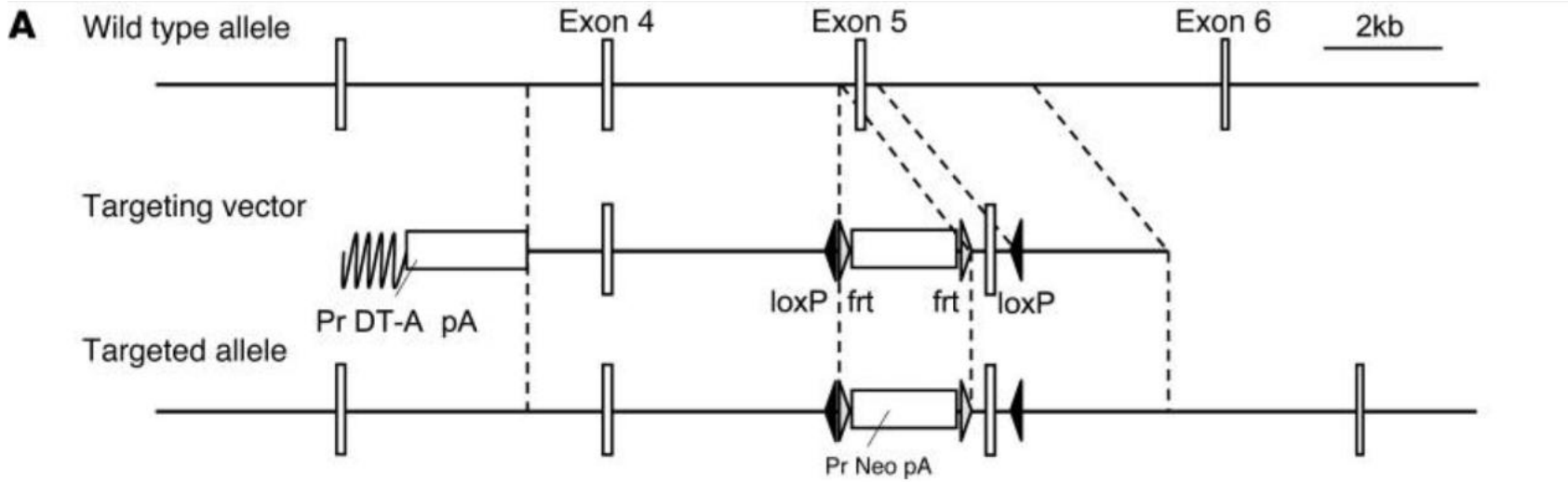
Source: https://www.informatics.jax.org

Important Information

- According to the existing MGI data,mice homozygous for a knock-out allele exhibit reduced islet zinc levels, circulating insulin levels, and glucose-stimulated insulin secretion.
- There are several amino acids will be remained,the effect is unknwon.
- *Slc30a8* is located on Chr15. 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

https://www.mousephenotype.org/data/genes/MGI:2384910



Generation of ZnT8KO mice.

Gene targeting in ES cells was designed to delete exon 5 of the endogenous *Slc30a8* locus (Figure 1 A). The targeting vector contained exon 5 flanked by loxP sites and an *frt*-flanked neocassette (*Pr-Neo pA*) in the 3′-adjacent region. Vector electroporation into TT2 ES cells (72), positive-negative selection, and Southern blot analysis (data not shown) yielded frt-Neo^r heterozygous ES cell clones. These cells were injected into CD-1 8-cell-stage embryos to generate chimeric mutant mice. The neocassette was excised in vivo by crossing the chimeras to mice expressing the Flp recombinase (B6-Tg [CAG-FLPe36]; ref. 73), leading to *Slc30a8*$^{f/+}$ offspring (accession no. CDB0625K; http://www.cdb.riken.jp/arg/mutant%20mice%20list.html). *Slc30a8*$^{f/+}$ mice were then backcrossed onto the C57BL6/J background more than 10 times. The resulting *Slc30a8*$^{f/f}$ mice were bred with *RIP-cre* transgenic mice to generate ZnT8KO mice, with β cell–specific *Slc30a8* deletion. Mice were housed in a specific pathogen–free facility and maintained on normal mouse chow. All mice were housed in specific pathogen–free barrier facilities, maintained under a 12-hour light/12-hour dark cycle, fed standard rodent food (Oriental Yeast) or rodent food containing 60% fat (Research Diet) for the high-fat diet study, and provided water ad libitum.