

Zbtb7b Cas9-KO Strategy

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Reviewer: Xingkai Xiao

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Overview

Target Gene Name

- *Zbtb7b*

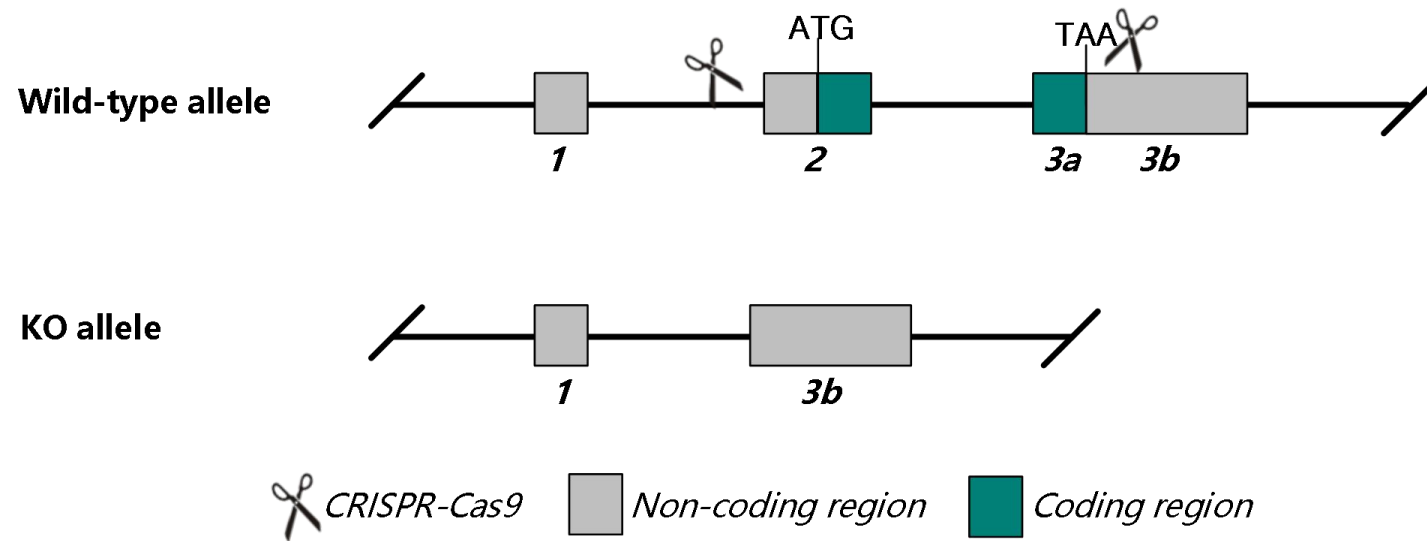
Project Type

- Cas9-KO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Zbtb7b* gene.

Technical Information

- The *Zbtb7b* gene has 9 transcripts. According to the structure of *Zbtb7b* gene, exon 2 and part of exon 3 of *Zbtb7b*-201 (ENSMUST00000029677.9) is recommended as the knockout region. The region contains all of coding sequence. Knockout the region will result in disruption of gene function.
- In this project we use CRISPR-Cas9 technology to modify *Zbtb7b* 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

Zbtb7b zinc finger and BTB domain containing 7B [*Mus musculus* (house mouse)]

[Download Datasets](#)

Gene ID: 22724, updated on 18-May-2023

Summary

Official Symbol Zbtb7b provided by [MGI](#)
Official Full Name zinc finger and BTB domain containing 7B provided by [MGI](#)
Primary source [MGI:MGI:102755](#)
See related [Ensembl:ENSMUSG00000028042](#) [AllianceGenome:MGI:102755](#)
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 Thpok; Zfp67; c-Krox
Summary Enables several functions, including DNA-binding transcription activator activity, RNA polymerase II-specific; histone deacetylase binding activity; and protein homodimerization activity. Involved in several processes, including positive regulation of macromolecule metabolic process; positive regulation of signal transduction; and regulation of alpha-beta T cell activation. Acts upstream of or within regulation of alpha-beta T cell differentiation and regulation of gene expression. Located in nucleus. Is expressed in several structures, including alimentary system; forebrain; genitourinary system; sensory organ; and skin. Orthologous to human ZBTB7B (zinc finger and BTB domain containing 7B). [provided by Alliance of Genome Resources, Apr 2022]
Expression Broad expression in colon adult (RPKM 66.4), duodenum adult (RPKM 59.2) and 18 other tissues [See more](#)
Orthologs [human](#) [all](#)
NEW Try the new [Gene table](#)
Try the new [Transcript table](#)

Genomic context

Location: 3 F1; 3 39.09 cM

See Zbtb7b in [Genome Data Viewer](#)

Exon count: 8

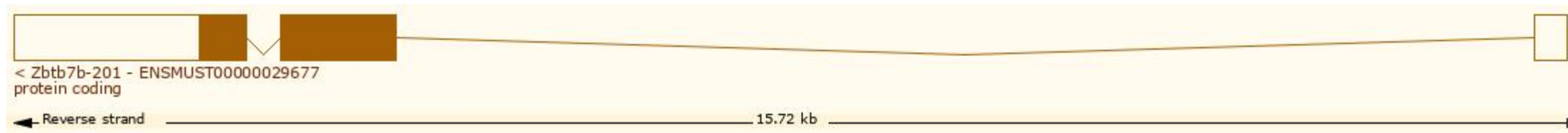
<https://www.ncbi.nlm.nih.gov/gene/22724>

Transcript Information

The gene has 9 transcripts, all transcripts are shown below:

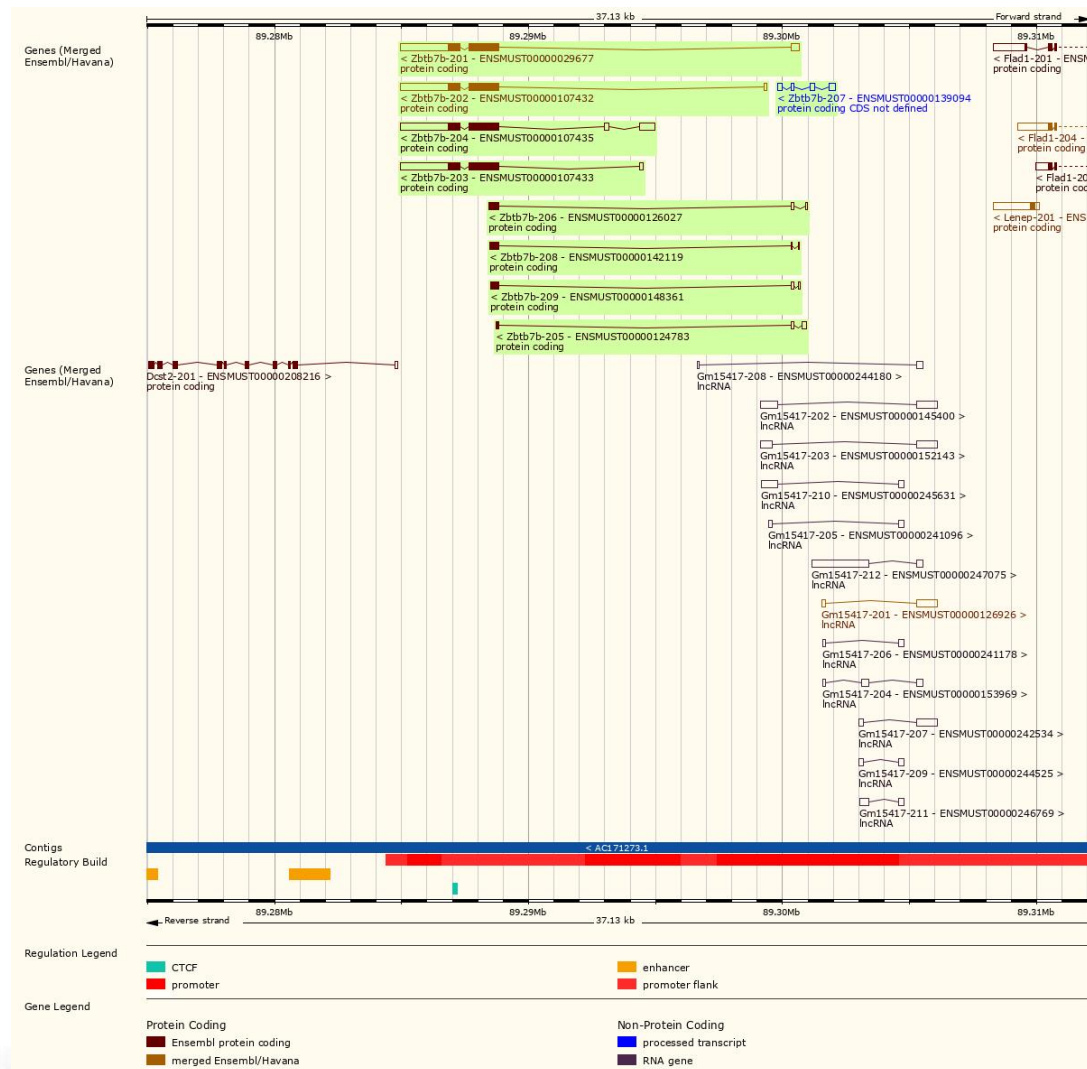
Show/hide columns (1 hidden)					Filter		
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000029677.9	Zbtb7b-201	3851	544aa	Protein coding	CCDS17504	Q5BJ24 Q64321	GENCODE basic APPRIS P1 TSL:1
ENSMUST00000107432.8	Zbtb7b-202	3639	544aa	Protein coding	CCDS17504	Q5BJ24 Q64321	GENCODE basic APPRIS P1 TSL:1
ENSMUST00000107433.8	Zbtb7b-203	3650	544aa	Protein coding	CCDS17504	Q5BJ24 Q64321	GENCODE basic APPRIS P1 TSL:1
ENSMUST00000107435.8	Zbtb7b-204	4333	544aa	Protein coding	CCDS17504	Q5BJ24 Q64321	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1
ENSMUST00000124783.8	Zbtb7b-205	354	29aa	Protein coding		D3YXC8	TSL:3 CDS 3' incomplete
ENSMUST00000126027.8	Zbtb7b-206	544	124aa	Protein coding		D3YZR6	TSL:2 CDS 3' incomplete
ENSMUST00000139094.2	Zbtb7b-207	697	No protein	Protein coding CDS not defined		-	TSL:3
ENSMUST00000142119.2	Zbtb7b-208	403	112aa	Protein coding		D3Z0B5	TSL:3 CDS 3' incomplete
ENSMUST00000148361.8	Zbtb7b-209	481	102aa	Protein coding		D3Z2X8	TSL:2 CDS 3' incomplete

The strategy is based on the design of *Zbtb7b*-201 transcript, the transcription is shown below:

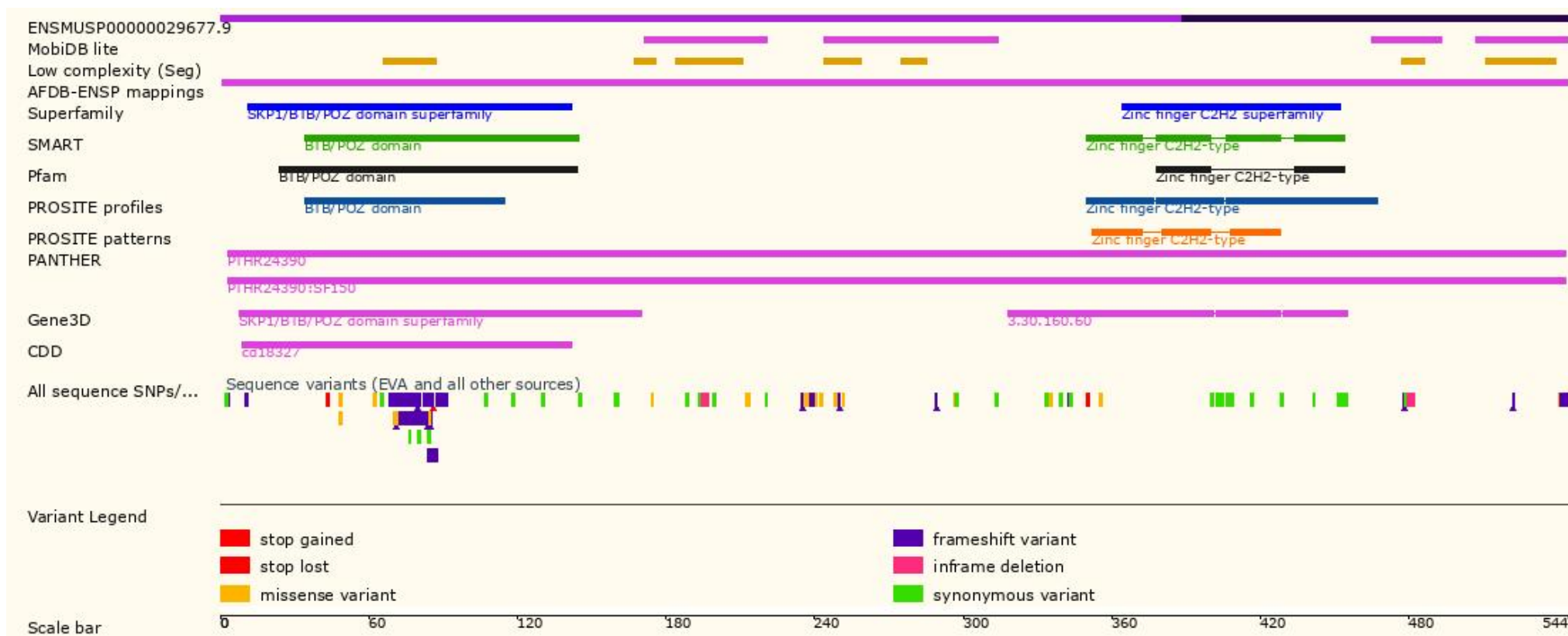


Source: <http://asia.ensembl.org/>

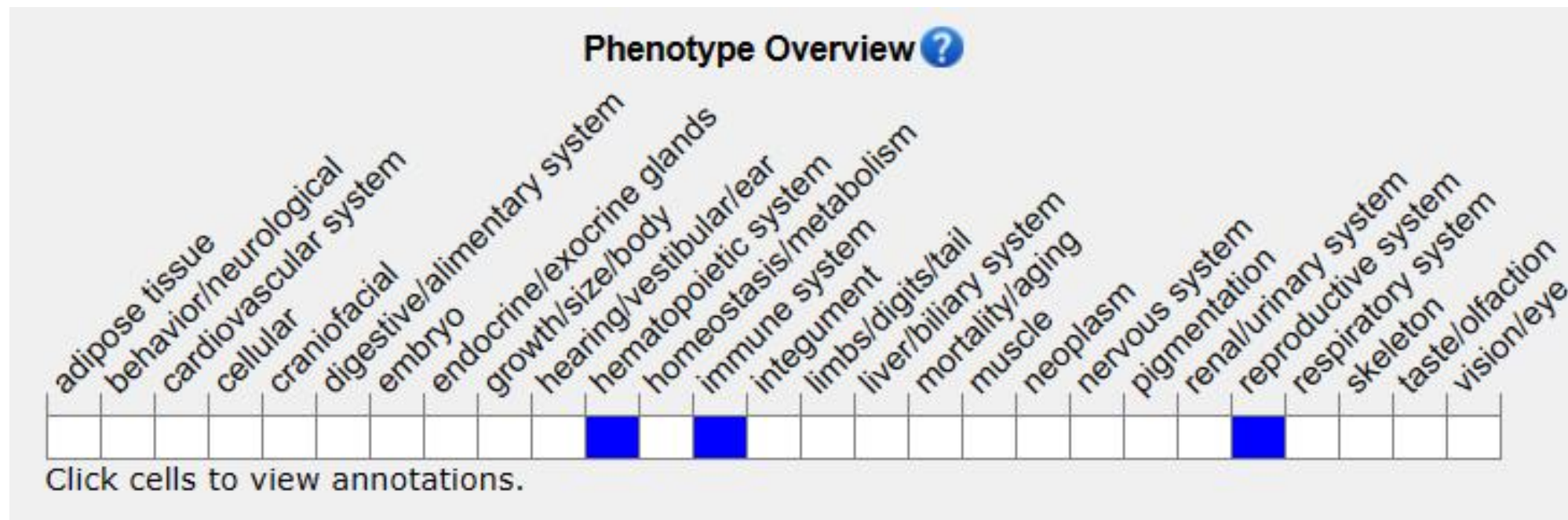
Genomic Information



Protein Information



Mouse Phenotype Information (MGI)

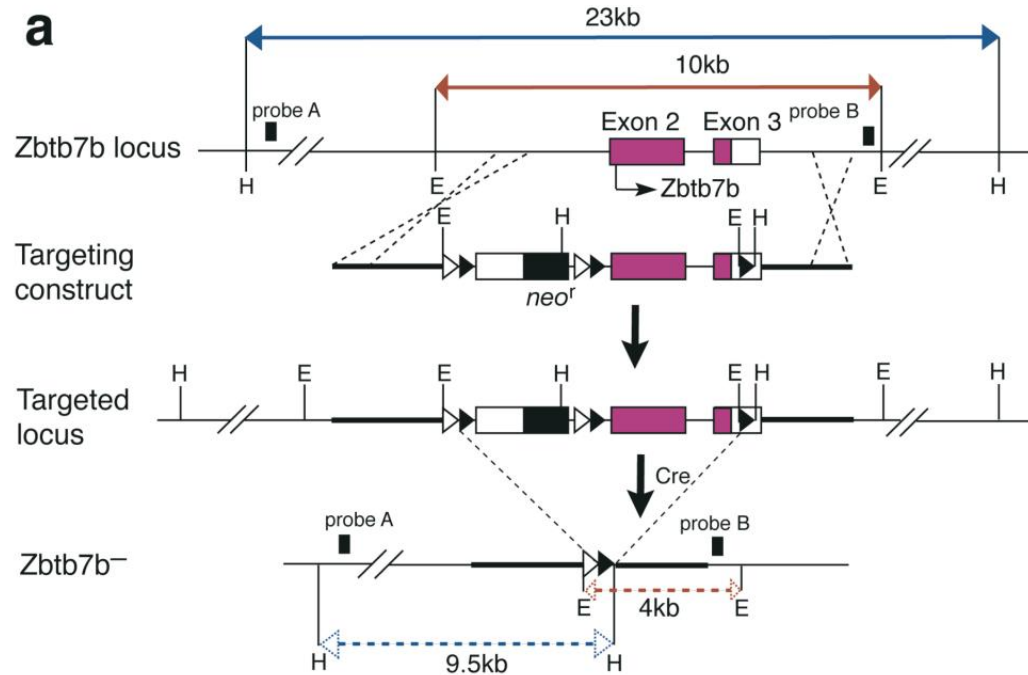


Homozygous mutants exhibit absence of peripheral T cells of the CD4+CD8- MHC class II-restricted T helper subset due to a specific block in thymic development.

Important Information

- According to the existing MGI data, homozygous mutants exhibit absence of peripheral T cells of the CD4+CD8- MHC class II-restricted T helper subset due to a specific block in thymic development.
- The knockout region is about 7 kb away from the 5' of the *Gm15417* gene, which may affect the regulation of this gene.
- The knockout region is about 1 kb away from the 3' of the *Dcst2* gene, which may affect the regulation of this gene.
- This strategy may not affect *Zbtb7b-207* transcript.
- *Zbtb7b* is located on Chr 3. 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.

Reference



(a). The strategy used to target the *Zbtb7b* locus is schematically depicted. A targeting construct was generated by inserting a loxP site (filled triangle) in a non-conserved non-coding region 3' to the *Zbtb7b* ORF (pink filled boxes) and an frt-loxP-*neo*^r-frt-loxP cassette upstream of *Zbtb7b* exon 2 (open triangles: frt sites). The targeted locus was obtained by homologous recombination in ES cells, and the deleted (*Zbtb7b*⁻) allele generated by Cre-mediated deletion of the floxed region including the *neo*^r cassette and entire *Zbtb7b* coding sequence. Relevant restriction sites (E, *Eco*RV; H: *Hinc*II) and the

[1] Wang L, Wildt KF, Zhu J, Zhang X, Feigenbaum L, Tessarollo L, Paul WE, Fowlkes BJ, Bosselut R. Distinct functions for the transcription factors GATA-3 and ThPOK during intrathymic differentiation of CD4(+) T cells. Nat Immunol. 2008 Oct;9(10):1122-30. doi: 10.1038/ni.1647. Epub 2008 Sep 7. PMID: 18776904; PMCID: PMC2805063.