GemPharmatech™

Zeb1 Cas9-CKO Strategy

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Reviewer: Jinling Wang

Design Date: 2023-12-29

Overview

Target Gene Name

- Zeb1

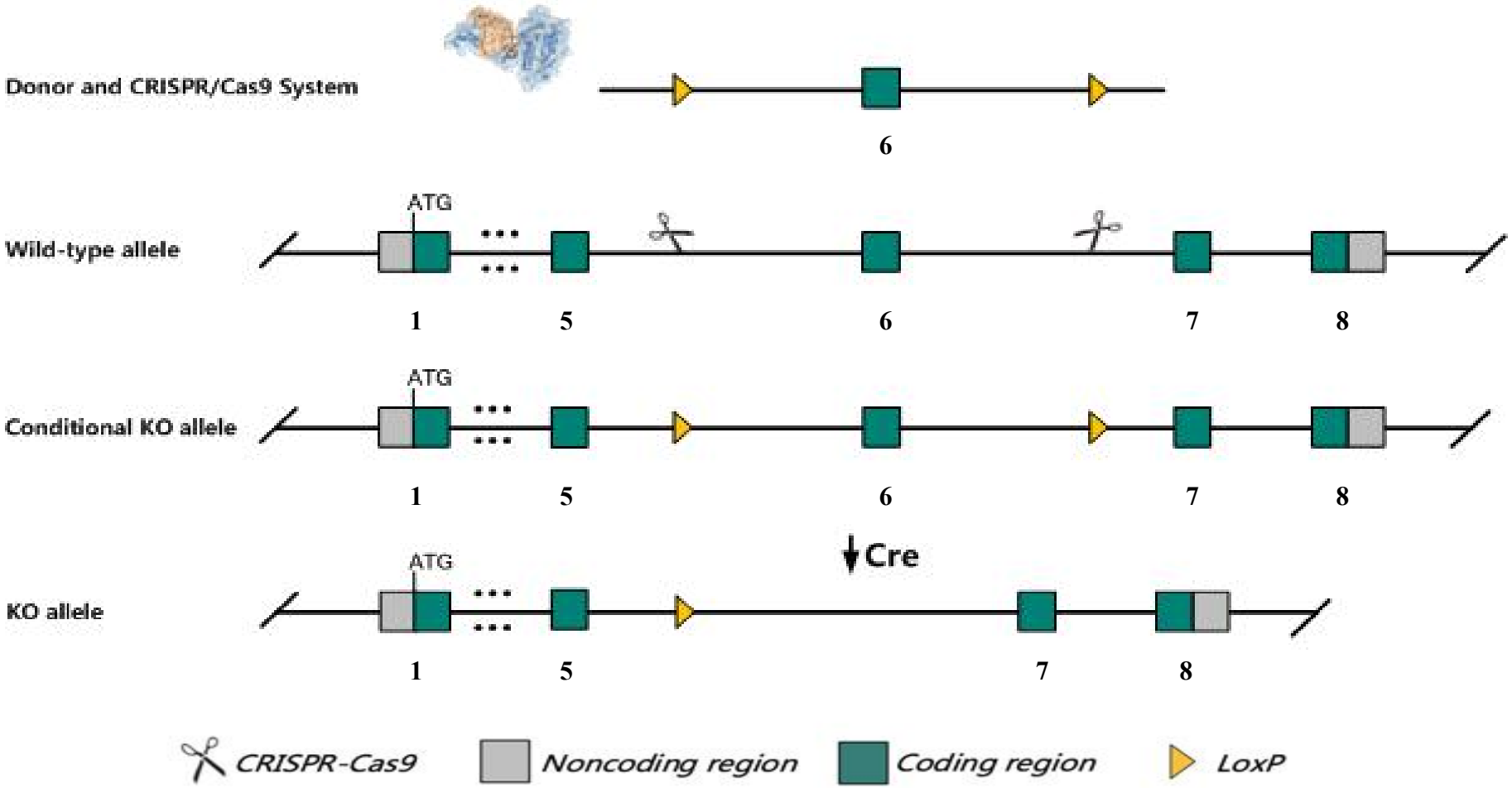
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy

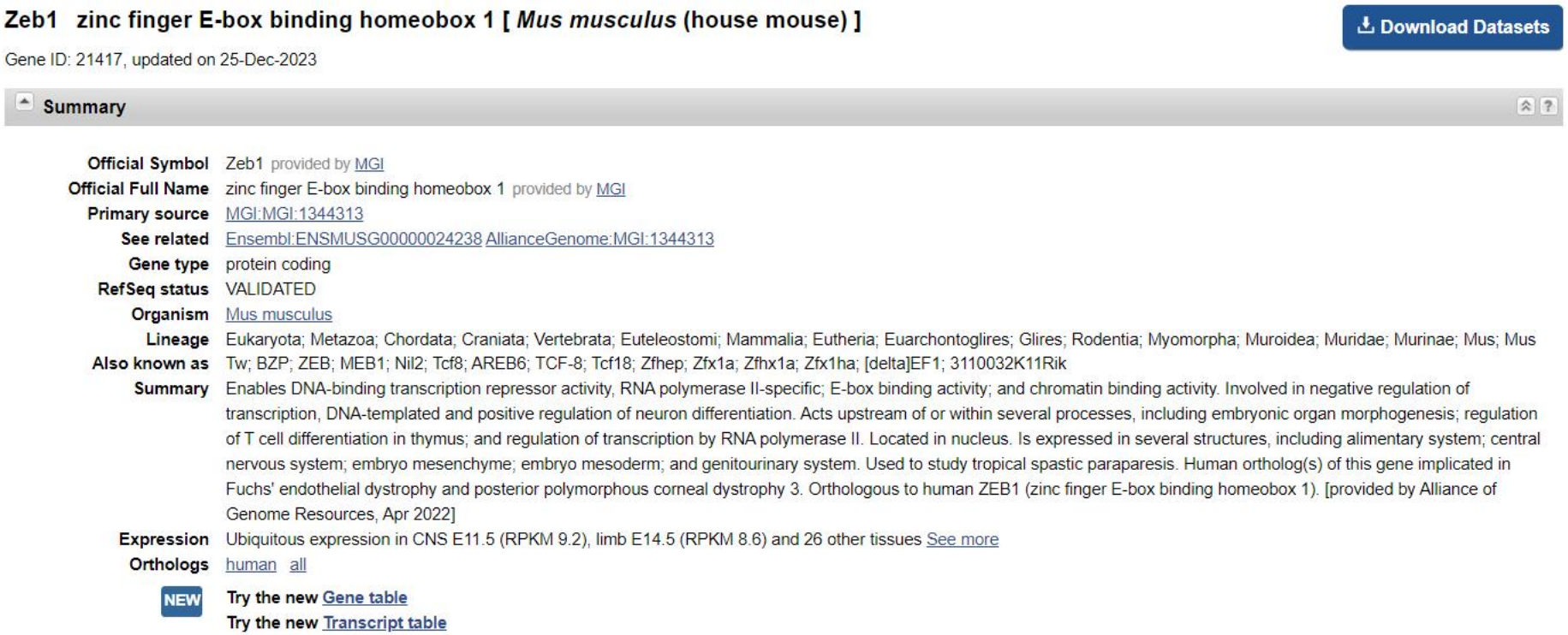


Schematic representation of CRISPR-Cas9 engineering used to edit the *Zeb1* gene.

Technical Information

- The *Zeb1* gene has 12 transcripts. According to the structure of *Zeb1* gene, exon6 of *Zeb1*-201 (ENSMUST00000025081.13) transcript is recommended as the knockout region. The region contains 1805bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Zeb1* 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



Zeb1 zinc finger E-box binding homeobox 1 [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 21417, updated on 25-Dec-2023

Summary

Official Symbol	Zeb1 provided by MGI
Official Full Name	zinc finger E-box binding homeobox 1 provided by MGI
Primary source	MGI:MGI:1344313
See related	Ensembl:ENSMUSG00000024238 AllianceGenome:MGI:1344313
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	Tw; BZP; ZEB; MEB1; Nil2; Tcf8; AREB6; TCF-8; Tcf18; Zfhep; Zfx1a; Zfhx1a; Zfx1ha; [delta]EF1; 3110032K11Rik
Summary	Enables DNA-binding transcription repressor activity, RNA polymerase II-specific; E-box binding activity; and chromatin binding activity. Involved in negative regulation of transcription, DNA-templated and positive regulation of neuron differentiation. Acts upstream of or within several processes, including embryonic organ morphogenesis; regulation of T cell differentiation in thymus; and regulation of transcription by RNA polymerase II. Located in nucleus. Is expressed in several structures, including alimentary system; central nervous system; embryo mesenchyme; embryo mesoderm; and genitourinary system. Used to study tropical spastic paraparesis. Human ortholog(s) of this gene implicated in Fuchs' endothelial dystrophy and posterior polymorphous corneal dystrophy 3. Orthologous to human ZEB1 (zinc finger E-box binding homeobox 1). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Ubiquitous expression in CNS E11.5 (RPKM 9.2), limb E14.5 (RPKM 8.6) and 26 other tissues See more
Orthologs	human all

NEW **Try the new Gene table**

Try the new Transcript table

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

Transcript Information

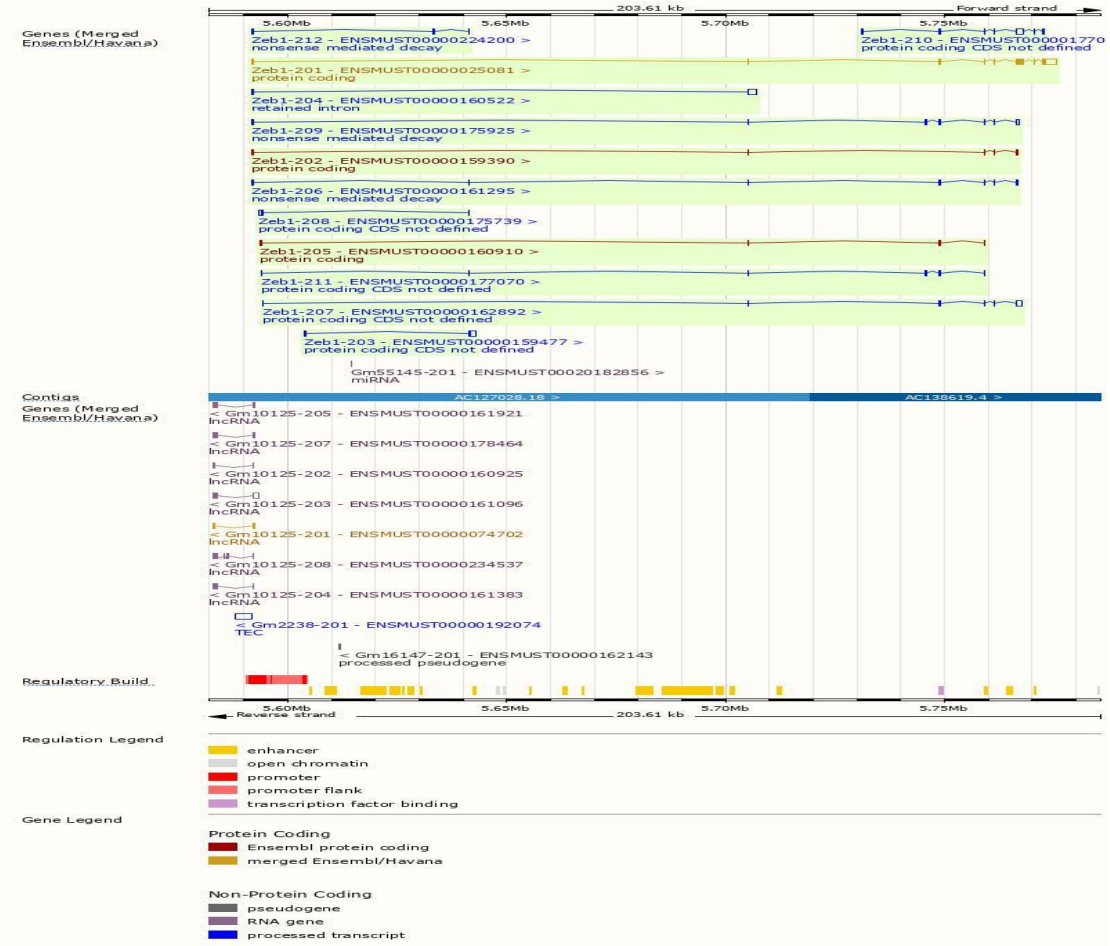
The gene has 12 transcripts,all transcripts are shown below:

Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000025081.13	Zeb1-201	5801	1117aa	Protein coding	CCDS29039	Q64318	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1
ENSMUST00000159390.8	Zeb1-202	900	293aa	Protein coding		E9PXY5	TSL:3 CDS 3' incomplete
ENSMUST00000160910.2	Zeb1-205	774	154aa	Protein coding		E0CX96	TSL:3 CDS 3' incomplete
ENSMUST00000175925.8	Zeb1-209	1485	105aa	Nonsense mediated decay		H3BJU2	TSL:5
ENSMUST00000161295.8	Zeb1-206	1215	20aa	Nonsense mediated decay		E0CY37	TSL:5 CDS 5' incomplete
ENSMUST00000224200.3	Zeb1-212	266	37aa	Nonsense mediated decay		A0A3Q4EHM2	-
ENSMUST00000177030.2	Zeb1-210	2768	No protein	Protein coding CDS not defined		-	TSL:5
ENSMUST00000162892.8	Zeb1-207	2464	No protein	Protein coding CDS not defined		-	TSL:1
ENSMUST00000159477.2	Zeb1-203	1610	No protein	Protein coding CDS not defined		-	TSL:1
ENSMUST00000177070.8	Zeb1-211	819	No protein	Protein coding CDS not defined		-	TSL:5
ENSMUST00000175739.2	Zeb1-208	790	No protein	Protein coding CDS not defined		-	TSL:3
ENSMUST00000160522.2	Zeb1-204	1938	No protein	Retained intron		-	TSL:1

The strategy is based on the design of *Zeb1*-201 transcript, the transcription is shown below:

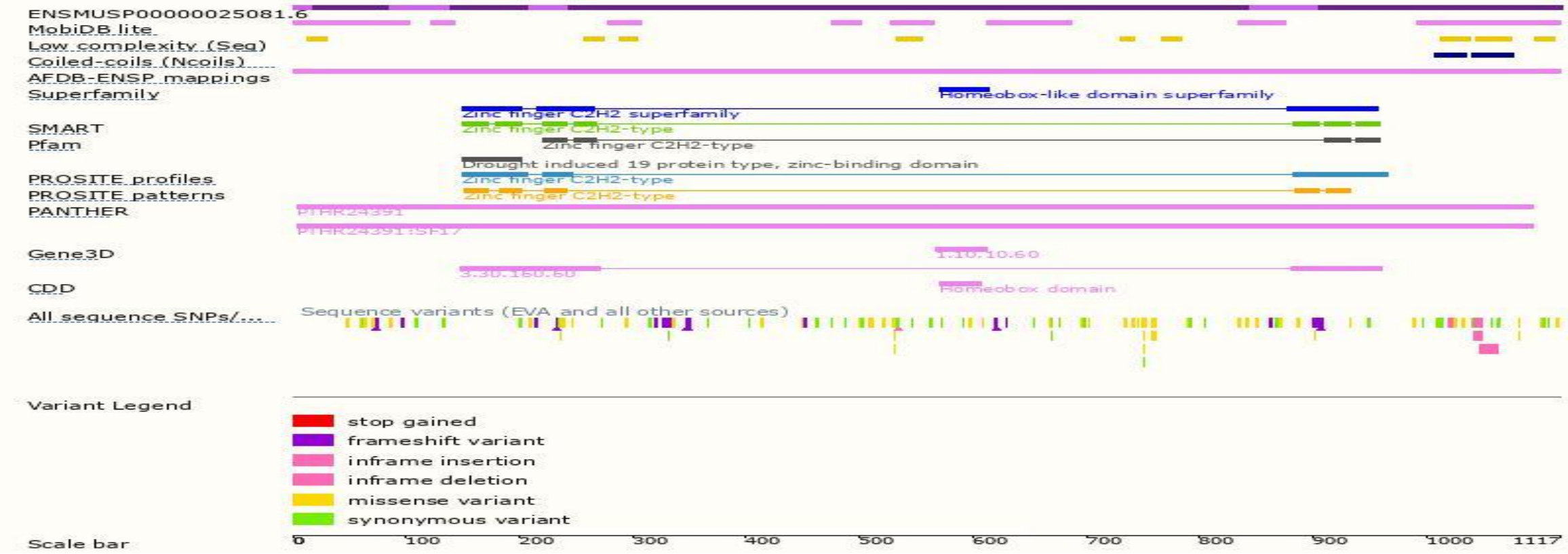
Source: https://www.ensembl.org

Genomic Information



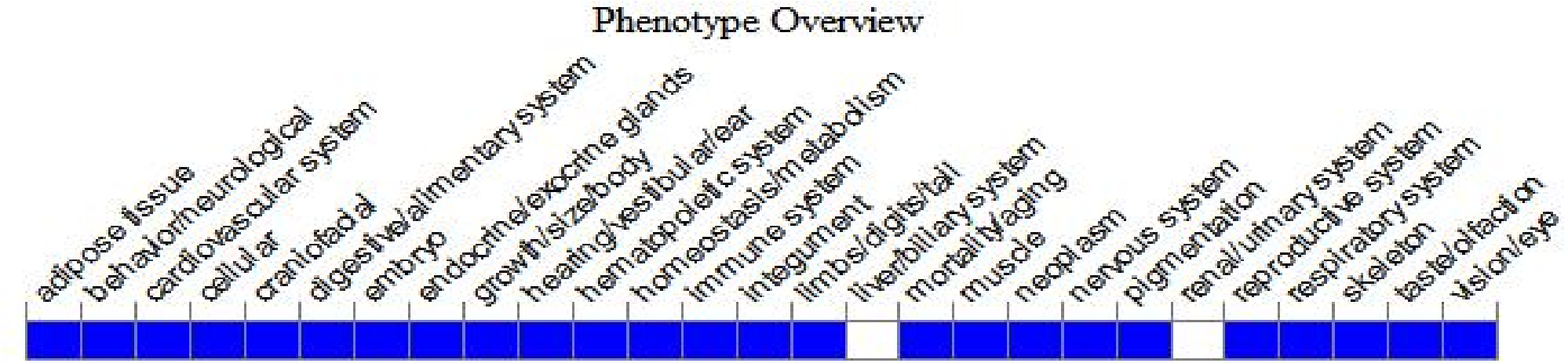
Source: : https://www.ensembl.org

Protein Information



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

Mouse Phenotype Information (MGI)



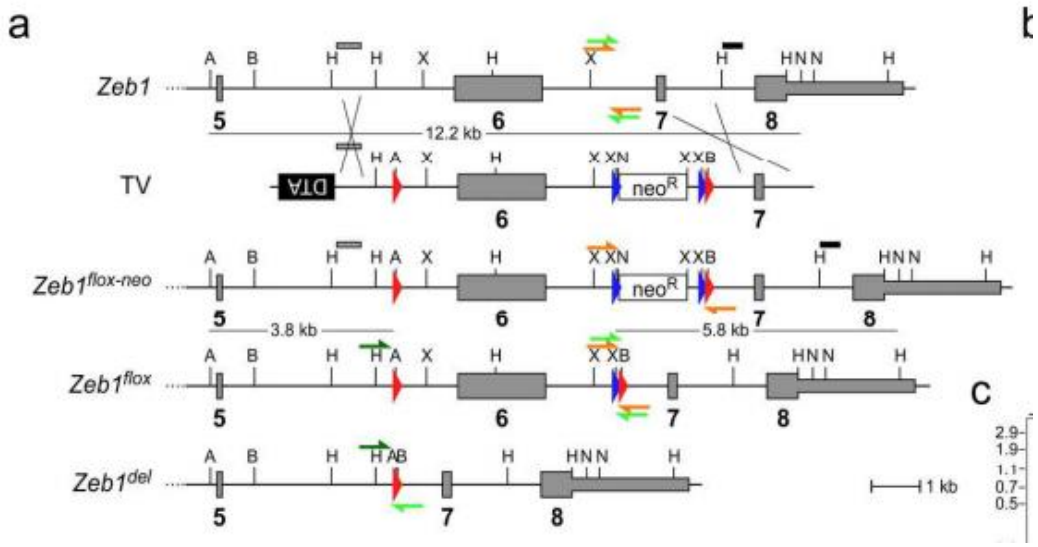
- Mutations at this locus affect thymus organization and homozygotes exhibit severe thymic T cell deficiency. Some mutations result in eye anomalies and extensive skeletal abnormalities. Homozygotes generally die at birth due to respiratory failure.

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

Important Information

- According to MGI information,mutations at this locus affect thymus organization and homozygotes exhibit severe thymic T cell deficiency. Some mutations result in eye anomalies and extensive skeletal abnormalities. Homozygotes generally die at birth due to respiratory failure.
- The effect of this strategy on the transcripts of *Zeb1*-203, *Zeb1*-204, *Zeb1*-205, *Zeb1*-208, *Zeb1*-211, and *Zeb1*-212 is unknown.
- *Zeb1* is located on Chr18. 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



C57BL/6 derived genomic region of the *Zeb1* locus ranging from +172.0 to +179.4 kb using standard molecular cloning techniques. The initial 5.5 kb sequences containing exon 6 and the downstream 2 kb sequences containing exon 7 were used as long and short homologous arms, respectively. An FRT-PGKneo-FRT-loxP cassette was inserted 1.4 kb downstream of exon 6 into intronic sequences at position +177.6 kb. Similarly, a proximal loxP site was inserted 1.3 kb upstream of exon 6 at +173.1 kb together with an exogenous *Avr*II site. A diphtheria toxin A cassette (DTA) was cloned in reverse orientation upstream of the *Zeb1* genomic sequences for negative selection. The linearized vector was electroporated into C57BL/6 ES cells with the support of Genoway and selected with 250 µg/ml G418 as described previously (Bedzhov *et al.*, 2012). Resistant ES-cell clones were analyzed for homologous

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Brabletz S, Lasierra Losada M, Schmalhofer O, Mitschke J, Krebs A, Brabletz T, Stemmler MP. Generation and characterization of mice for conditional inactivation of Zeb1. Genesis. 2017 Apr;55(4). doi: 10.1002/dvg.23024. Epub 2017 Feb 23. PMID: 28176446.