GemPharmatech™

Slc25a19 Cas9-CKO Strategy

Designer: Longuyn Hu

Reviewer: Rui Xiong

Design Date: 2023-12-29

Overview

Target Gene Name

- Slc25a19

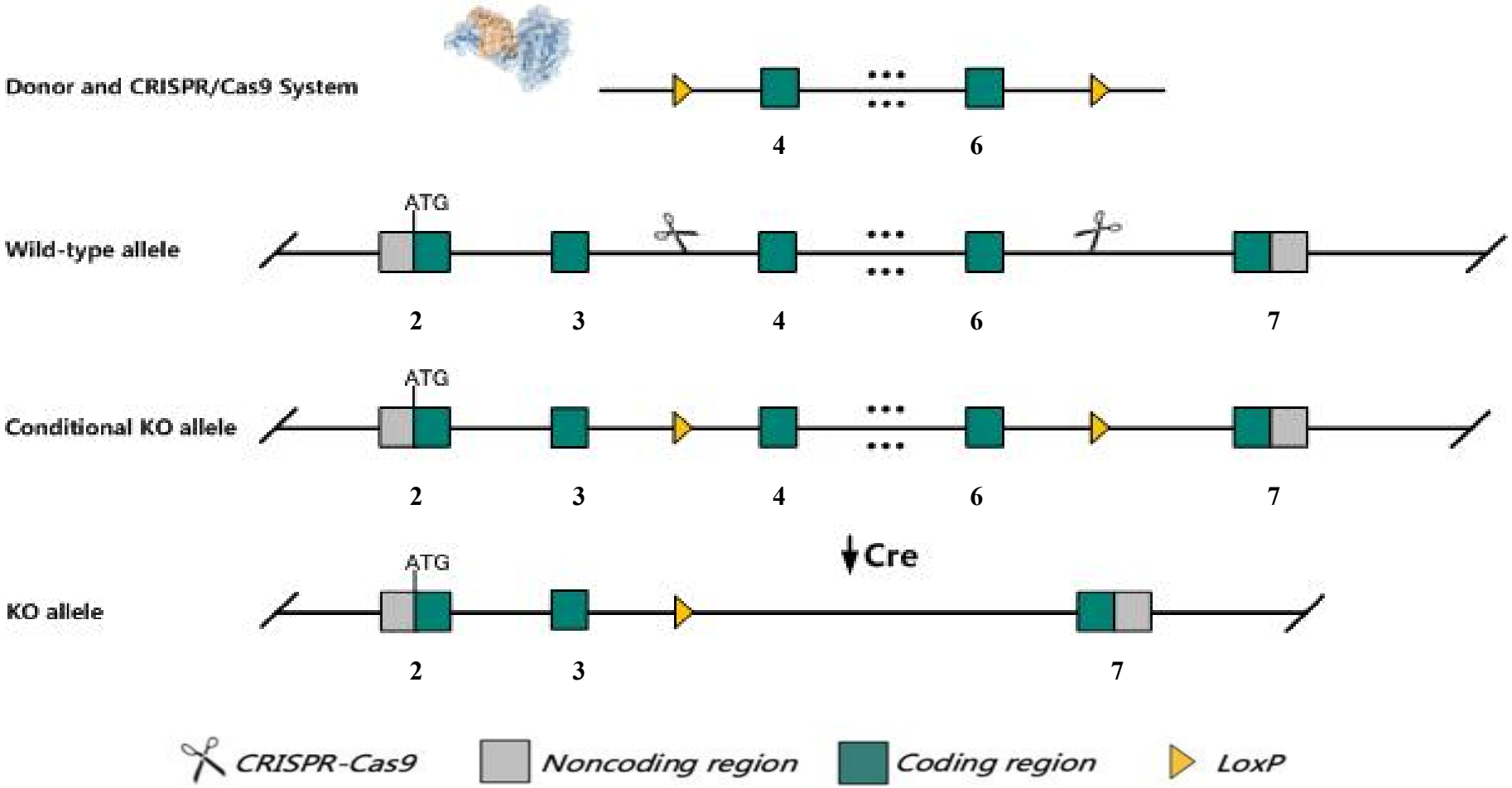
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Slc25a19* gene.

Technical Information

- The *Slc25a19* gene has 12 transcripts. According to the structure of *Slc25a19* gene, exon4-exon6 of *Slc25a19*-201 (ENSMUST00000021089.11) transcript is recommended as the knockout region. The region contains 486bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Slc25a19* 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

Slc25a19 solute carrier family 25 (mitochondrial thiamine pyrophosphate carrier), member 19 [*Mus musculus* (house mouse)]

Gene ID: 67283, updated on 23-Nov-2023

Download Datasets

Summary

Official Symbol	Slc25a19 provided by MGI
Official Full Name	solute carrier family 25 (mitochondrial thiamine pyrophosphate carrier), member 19 provided by MGI
Primary source	MGI:MGI:1914533
See related	Ensembl:ENSMUSG00000020744 AllianceGenome:MGI:1914533
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	DNC; TPC; MUP1; 2900089E13Rik
Summary	Predicted to enable thiamine transmembrane transporter activity. Predicted to be involved in thiamine pyrophosphate transmembrane transport. Located in mitochondrion. Is expressed in central nervous system; embryo; retina inner nuclear layer; retina layer; and retina outer nuclear layer. Human ortholog(s) of this gene implicated in inherited metabolic disorder and microcephaly. Orthologous to human SLC25A19 (solute carrier family 25 member 19). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Ubiquitous expression in subcutaneous fat pad adult (RPKM 38.7), mammary gland adult (RPKM 32.6) and 28 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/

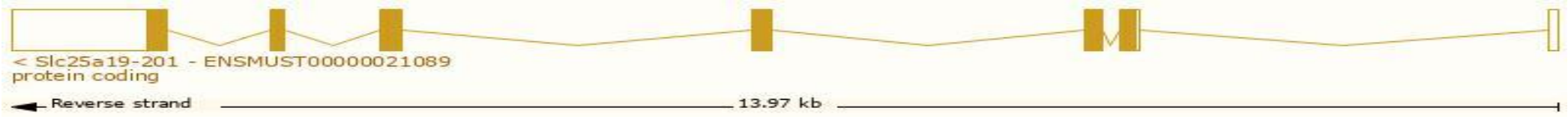
Transcript Information

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

Show/hide columns (1 hidden) Filter

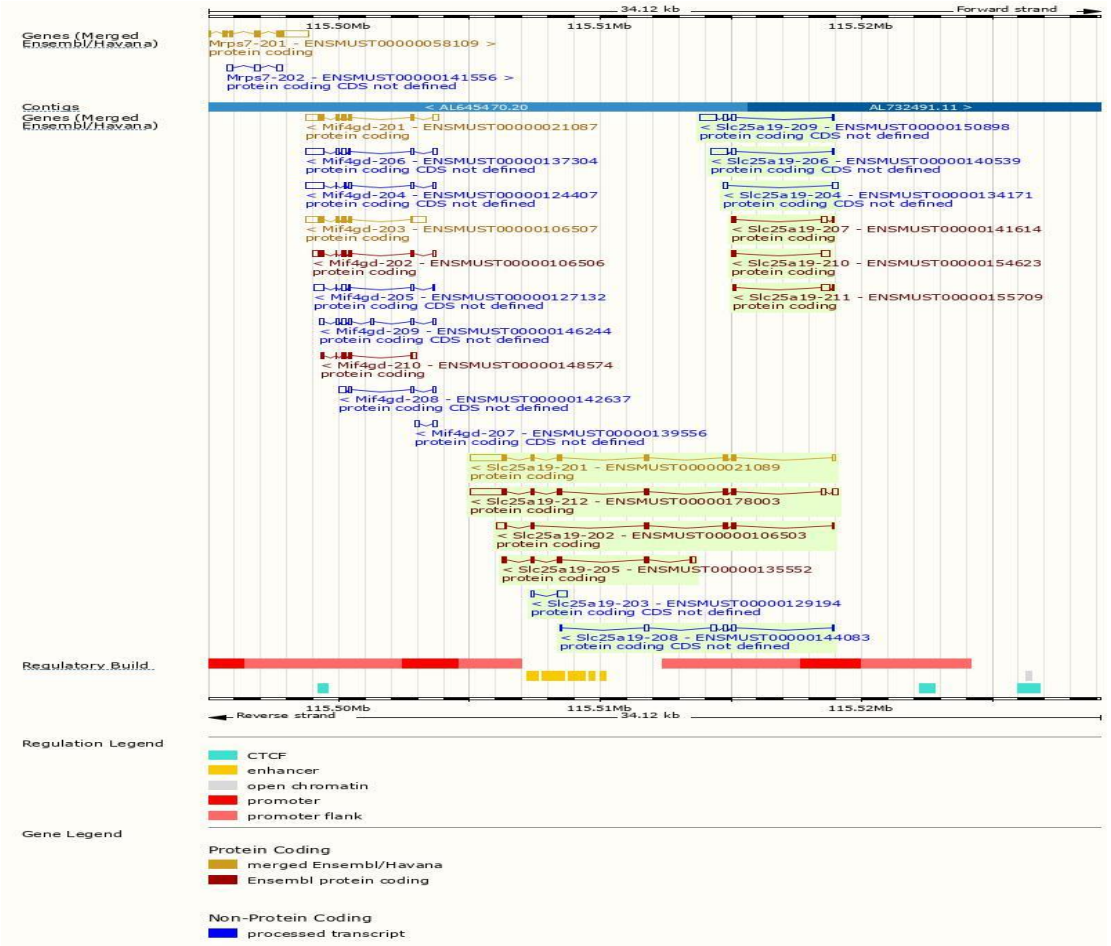
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match ▲	Flags
ENSMUST00000155709.2	Slc25a19-211	453	18aa	Protein coding		A2A9V2	TSL:3 CDS 3' incomplete
ENSMUST00000154623.2	Slc25a19-210	461	37aa	Protein coding		A2A9V3	TSL:2 CDS 3' incomplete
ENSMUST00000141614.3	Slc25a19-207	455	44aa	Protein coding		A2A9V4	TSL:3 CDS 3' incomplete
ENSMUST00000106503.10	Slc25a19-202	1055	227aa	Protein coding	CCDS56819	A2A9V5	TSL:1
ENSMUST00000135552.2	Slc25a19-205	826	225aa	Protein coding		A2A9V7	TSL:3 CDS 3' incomplete
ENSMUST00000178003.8	Slc25a19-212	2614	318aa	Protein coding	CCDS25644	Q9DAM5	Ensembl Canonical GENCODE basic APPRIS P1 TSL:5
ENSMUST00000021089.11	Slc25a19-201	2307	318aa	Protein coding	CCDS25644	Q9DAM5	GENCODE basic APPRIS P1 TSL:1
ENSMUST00000150898.8	Slc25a19-209	954	No protein	Protein coding CDS not defined		-	TSL:5
ENSMUST00000140539.8	Slc25a19-206	834	No protein	Protein coding CDS not defined		-	TSL:2
ENSMUST00000144083.8	Slc25a19-208	755	No protein	Protein coding CDS not defined		-	TSL:3
ENSMUST00000129194.2	Slc25a19-203	491	No protein	Protein coding CDS not defined		-	TSL:2
ENSMUST00000134171.2	Slc25a19-204	368	No protein	Protein coding CDS not defined		-	TSL:2

The strategy is based on the design of *Slc25a19*-201 transcript, the transcription is shown below:



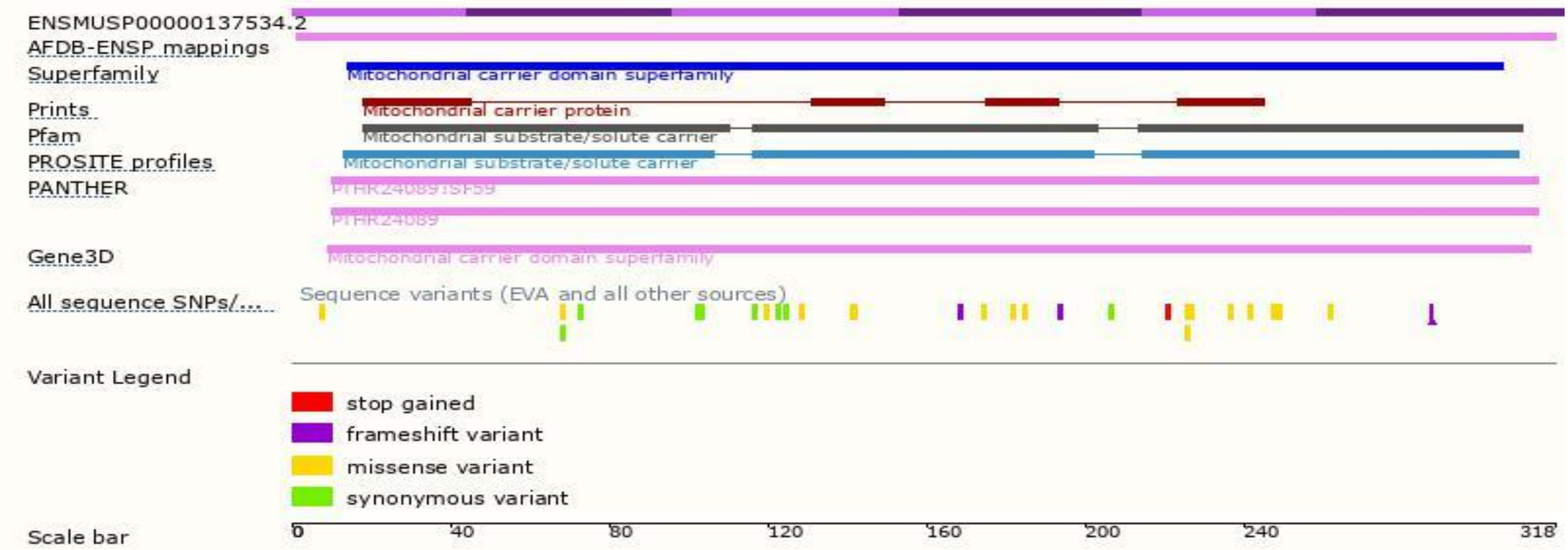
Source: https://www.ensembl.org

Genomic Information



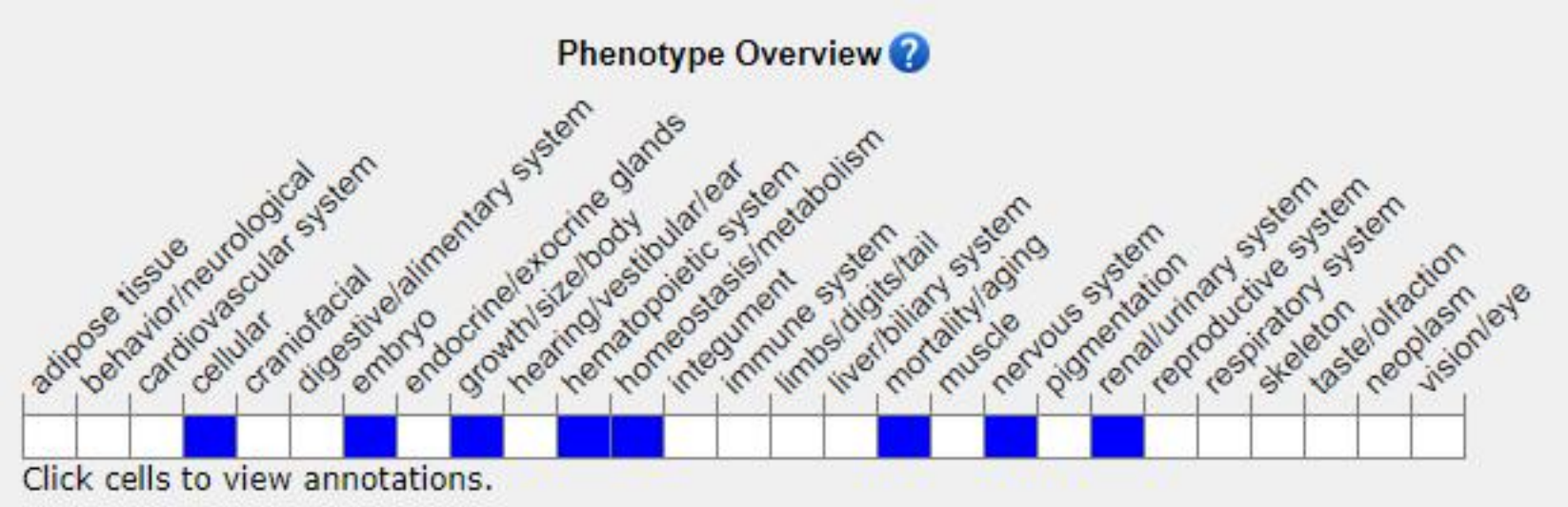
Source: : https://www.ensembl.org

Protein Information



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

Mouse Phenotype Information (MGI)



- Homozygous mutation of this gene results in lethality by E12, neural tube closure defects resulting in exencephaly and microcephaly, growth arrest, anemia, elevated alpha-ketoglutarate in amniotic fluid, and reduced thiamine pyrophosphate content in mitochondria.

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

Important Information

- According to the existing MGI data, Homozygous mutation of this gene results in lethality by E12, neural tube closure defects resulting in exencephaly and microcephaly, growth arrest, anemia, elevated alpha-ketoglutarate in amniotic fluid, and reduced thiamine pyrophosphate content in mitochondria.
- The KO region deletes part of the coding sequence, but does not result in frameshift.
- *Slc25a19* is located on Chr11. 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

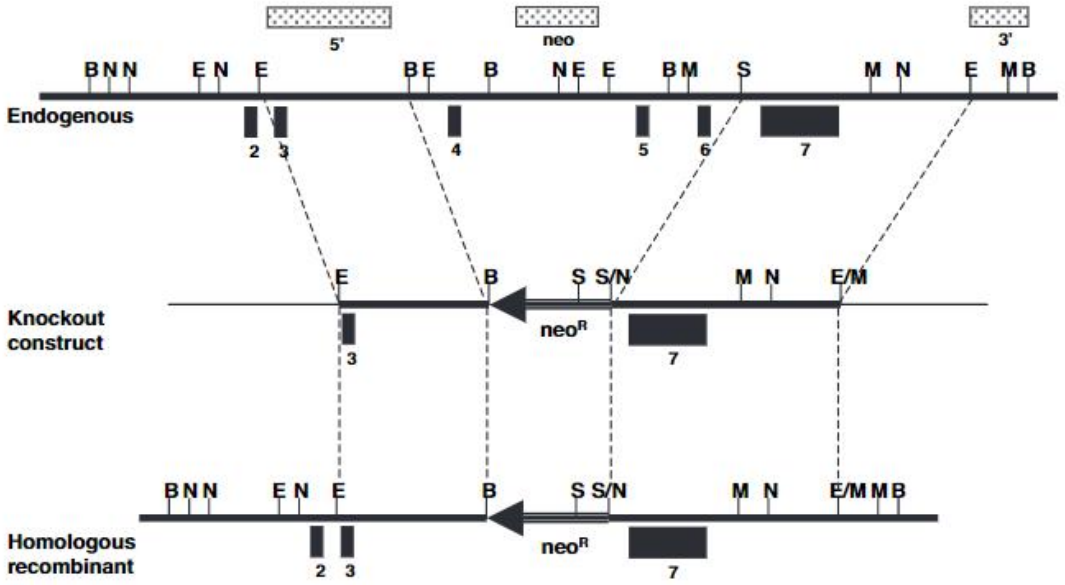


Fig. 1. Diagram of the *Slc25a19* knockout strategy. A 2.5-kb EcoRI (E)/BamHI (B) fragment (5′) and a 3.9-kb SpeI (S)/EcoRI (3′) fragment were cloned into pPNT-far. The construct is missing exons 4–6. Transfected ES cell clones were digested with either BamHI (B) (3′ and neo probes) or NheI (N) (5′ probe) to test for homologous recombination. Thick lines represent mouse sequence, thin lines represent vector sequence, and the arrow represents the Neo^R cassette. The stippled boxes represent the Southern blot analysis probes. Black numbered boxes are exons (the translation start site is in exon 2).

Lindhurst MJ, Fiermonte G, Song S, Struys E, De Leonardis F, Schwartzberg PL, Chen A, Castegna A, Verhoeven N, Mathews CK, Palmieri F, Biesecker LG. Knockout of Slc25a19 causes mitochondrial thiamine pyrophosphate depletion, embryonic lethality, CNS malformations, and anemia. Proc Natl Acad Sci U S A. 2006 Oct 24;103(43):15927-32. doi: 10.1073/pnas.0607661103. Epub 2006 Oct 11. PMID: 17035501; PMCID: PMC1595310.