GemPharmatech™

Slc43a2 Cas9-CKO Strategy

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Design Date: 2023-12-13

Overview

Target Gene Name

- Slc43a2

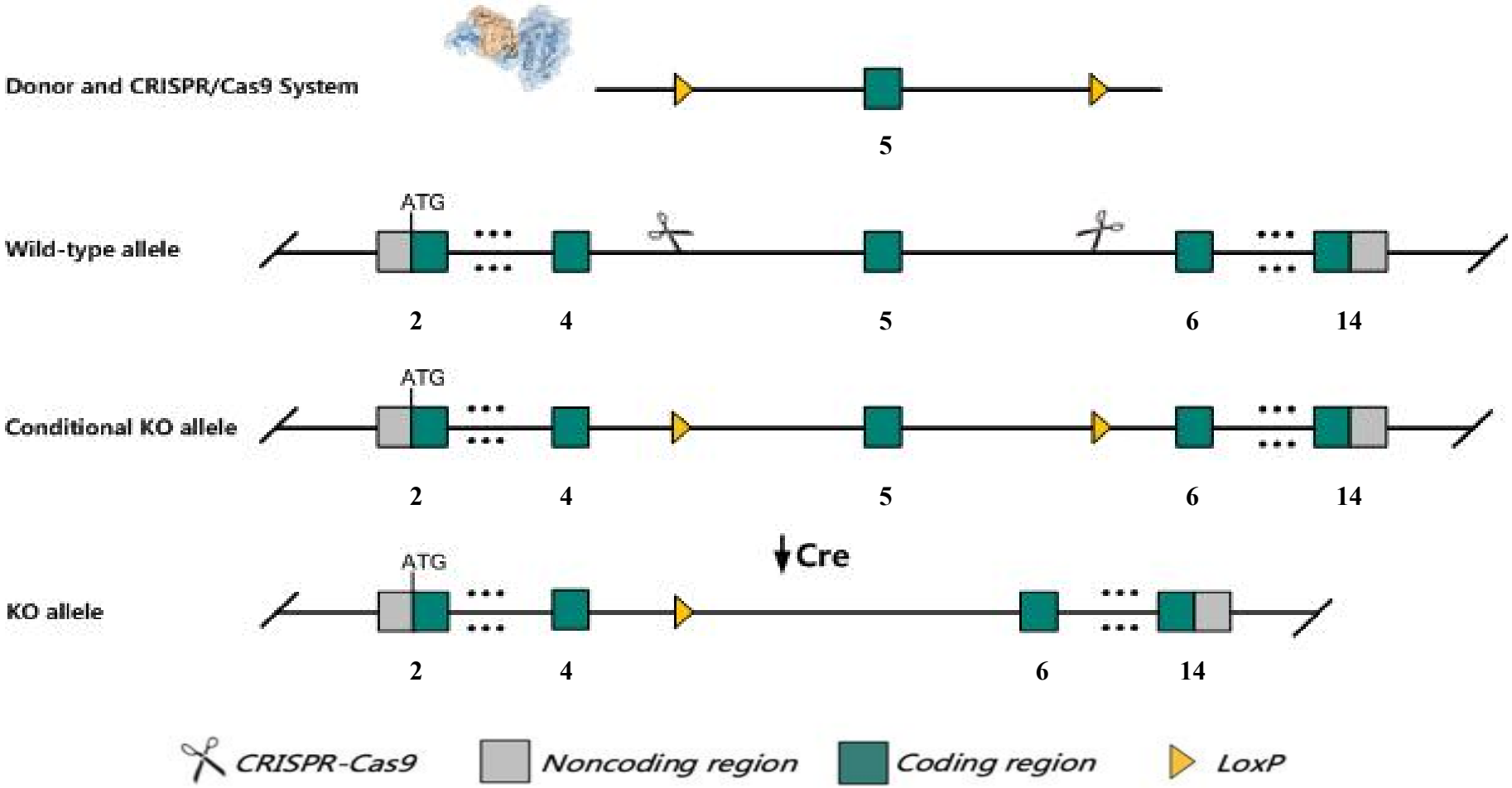
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Slc43a2* gene.

Technical Information

- The *Slc43a2* gene has 11 transcripts. According to the structure of *Slc43a2* gene, exon5 of *Slc43a2*-201 (ENSMUST00000042561.14) transcript is recommended as the knockout region. The region contains 77bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Slc43a2* 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

Slc43a2 solute carrier family 43, member 2 [Mus musculus (house mouse)]

Gene ID: 215113, updated on 17-Dec-2020

Summary

Official Symbol	Slc43a2 provided by MGI
Official Full Name	solute carrier family 43, member 2 provided byMGI
Primary source	MGI:MGI:2442746
See related	Ensembl:ENSMUSG00000038178
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	7630402D21Rik, BC042513, Lat4
Expression	Broad expression in duodenum adult (RPKM 79.3), small intestine adult (RPKM 65.9) and 19 other tissuesSee more
Orthologs	human all

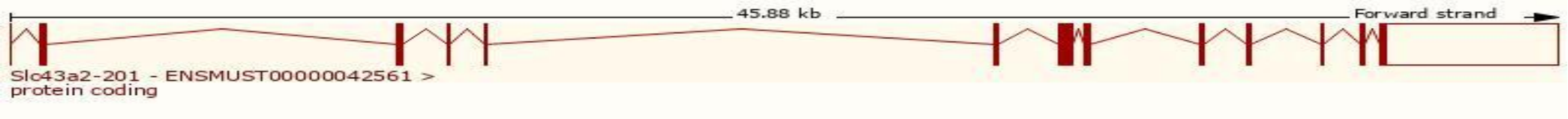
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

The gene has 11 transcripts,all transcripts are shown below:

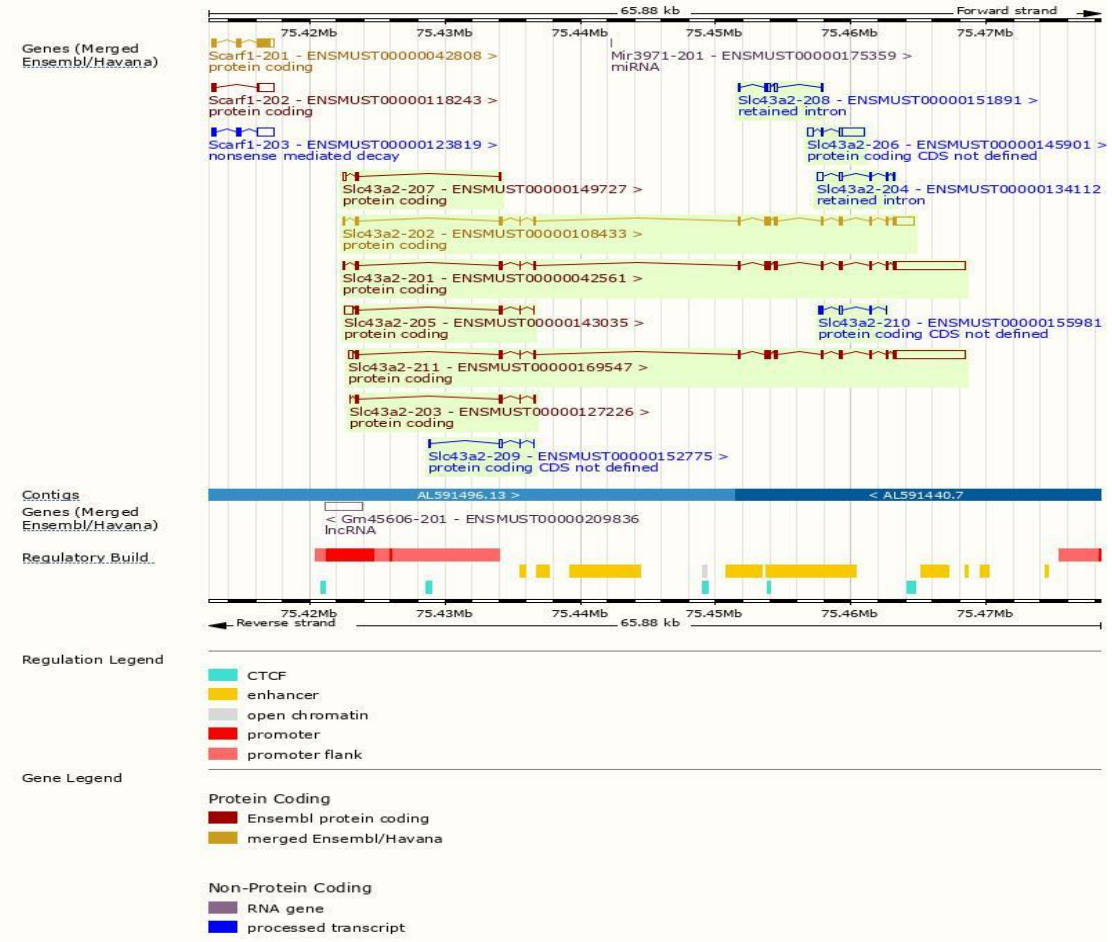
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Slc43a2-211	ENSMUST00000169547.9	7145	568aa	Protein coding	CCDS25051		TSL:1 , GENCODE basic , APPRIS P1 ,
Slc43a2-201	ENSMUST00000042561.14	6891	568aa	Protein coding	CCDS25051		TSL:1 , GENCODE basic , APPRIS P1 ,
Slc43a2-202	ENSMUST00000108433.8	3236	568aa	Protein coding	CCDS25051		TSL:1 , APPRIS P1 ,
Slc43a2-205	ENSMUST00000143035.8	1161	149aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Slc43a2-203	ENSMUST00000127226.3	613	167aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Slc43a2-207	ENSMUST00000149727.8	496	108aa	Protein coding	-		CDS 3' incomplete , TSL:2 ,
Slc43a2-206	ENSMUST00000145901.8	2292	No protein	Processed transcript	-		TSL:1 ,
Slc43a2-210	ENSMUST00000155981.2	407	No protein	Processed transcript	-		TSL:3 ,
Slc43a2-209	ENSMUST00000152775.2	372	No protein	Processed transcript	-		TSL:5 ,
Slc43a2-204	ENSMUST00000134112.8	841	No protein	Retained intron	-		TSL:1 ,
Slc43a2-208	ENSMUST00000151891.2	744	No protein	Retained intron	-		TSL:3 ,

The strategy is based on the design of *Slc43a2*-201 transcript, the transcription is shown below:



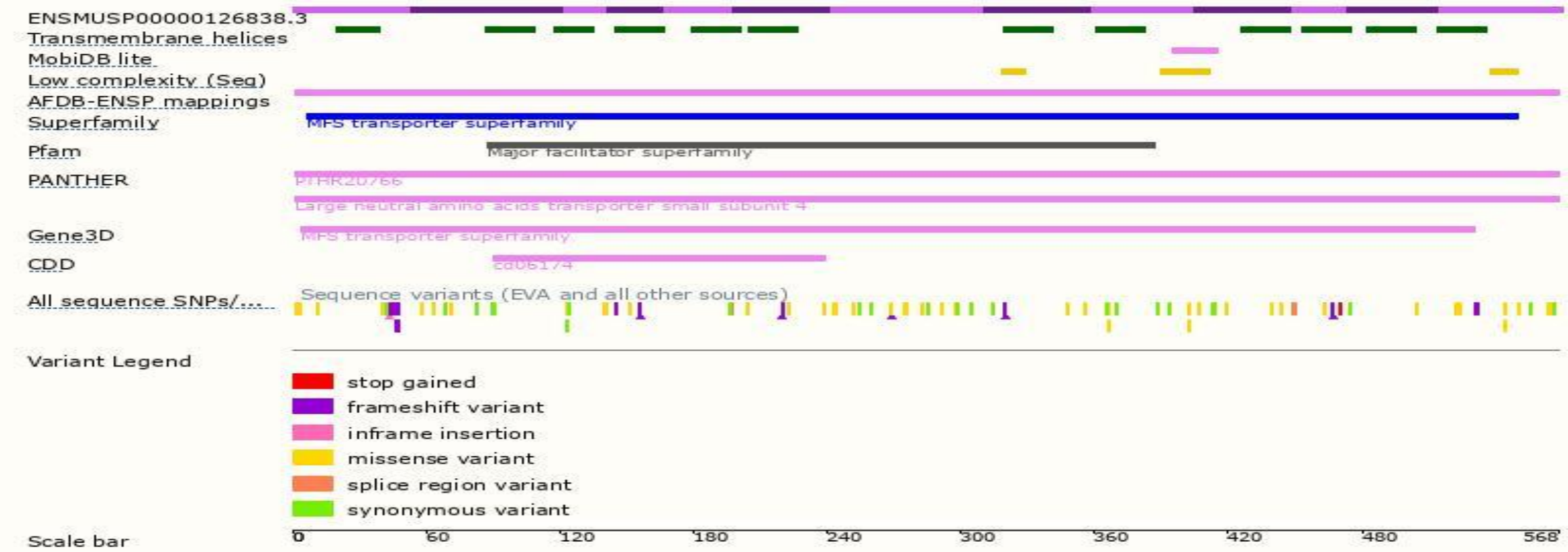
Source: https://www.ensembl.org

Genomic Information



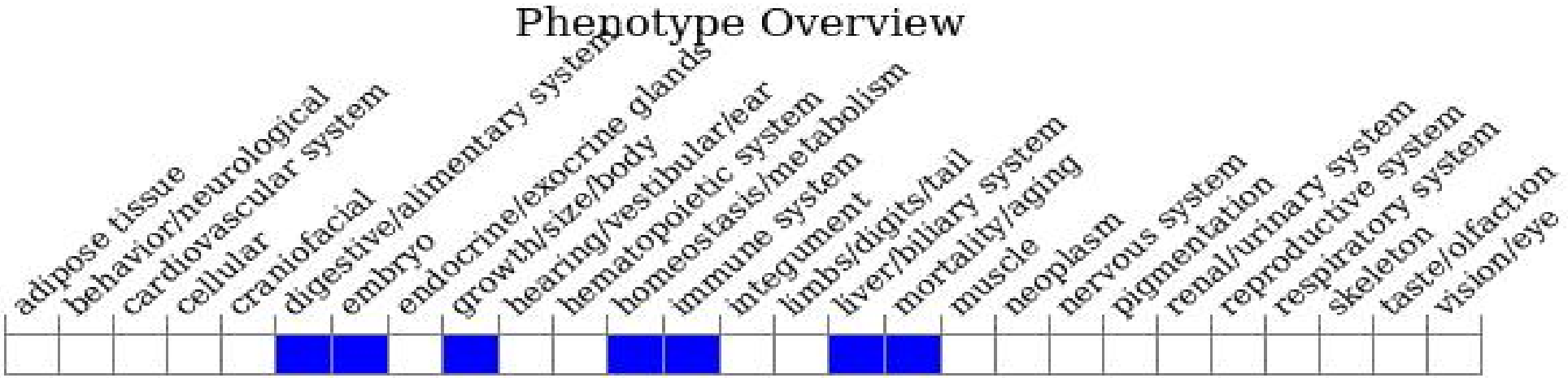
Source: : https://www.ensembl.org

Protein Information



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

Mouse Phenotype Information (MGI)



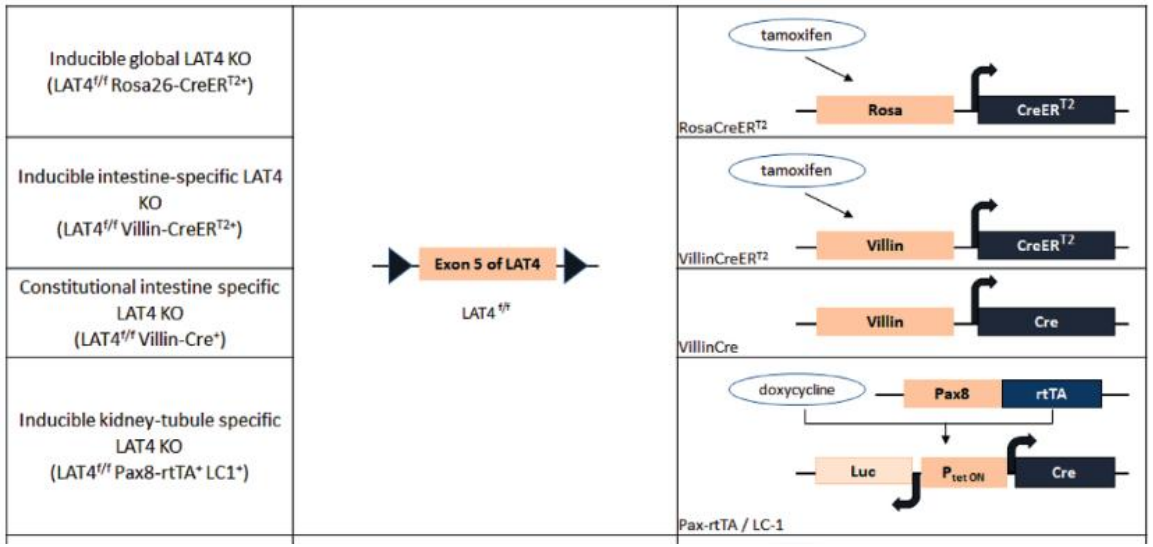
- Mice homozygous for a knock-out allele display fetal growth retardation, abnormal placental amino acid transport, slow postnatal weight gain, malnutrition and postnatal lethality, likely as a result of impaired intestinal amino acid absorption.

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

Important Information

- Transcript *Slc43a2-207* may not be affected.
- *Slc43a2* 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



Animals

Generation of floxed LAT4 mice. Lat4 (Slc43a2) floxed mice, *LAT4*$^{f/f}$ were produced by PolyGene Transgenetics (Rümlang, Zürich, Switzerland). The targeting vector containing exon 5 of the *Slc43a2* gene floxed with LoxP sites and a FRT flanked neomycin cassette (allowing for positive selection of clones) was transfected into C57Bl/6N-derived embryonic stem cells by electroporation. Positive clones containing the correct homologous recombination were further identified by PCR analysis and injected into blastocysts from grey C57Bl/6 mice, which were then transferred into CD-1 foster mice. Resulting chimeric mice were mated with grey Flp-deleter mice (C57Bl/6N derived) to remove the neomycin cassette.

Rajendran A, et al., Tissue-specific deletion of mouse basolateral uniporter LAT4 (Slc43a2) reveals its crucial role in small intestine and kidney amino acid transport. J Physiol. 2020 Nov;598(22):5109-5132