GemPharmatech™

Slc1a3 Cas9-CKO Strategy

Designer: Daohua Xu

Reviewer:Jinling Wang

Design Date: 2024-3-26

Overview

Target Gene Name

- Slc1a3

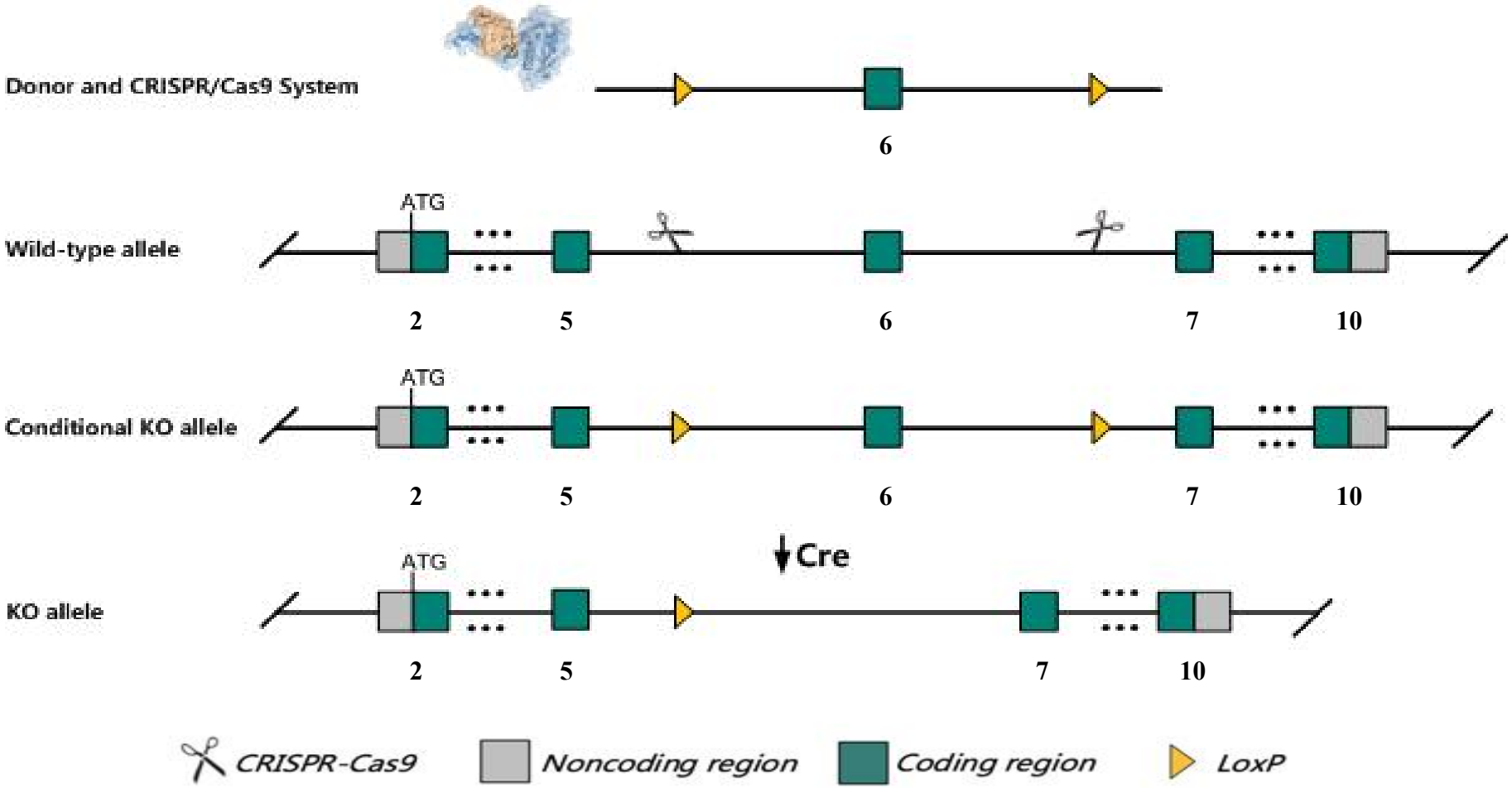
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy

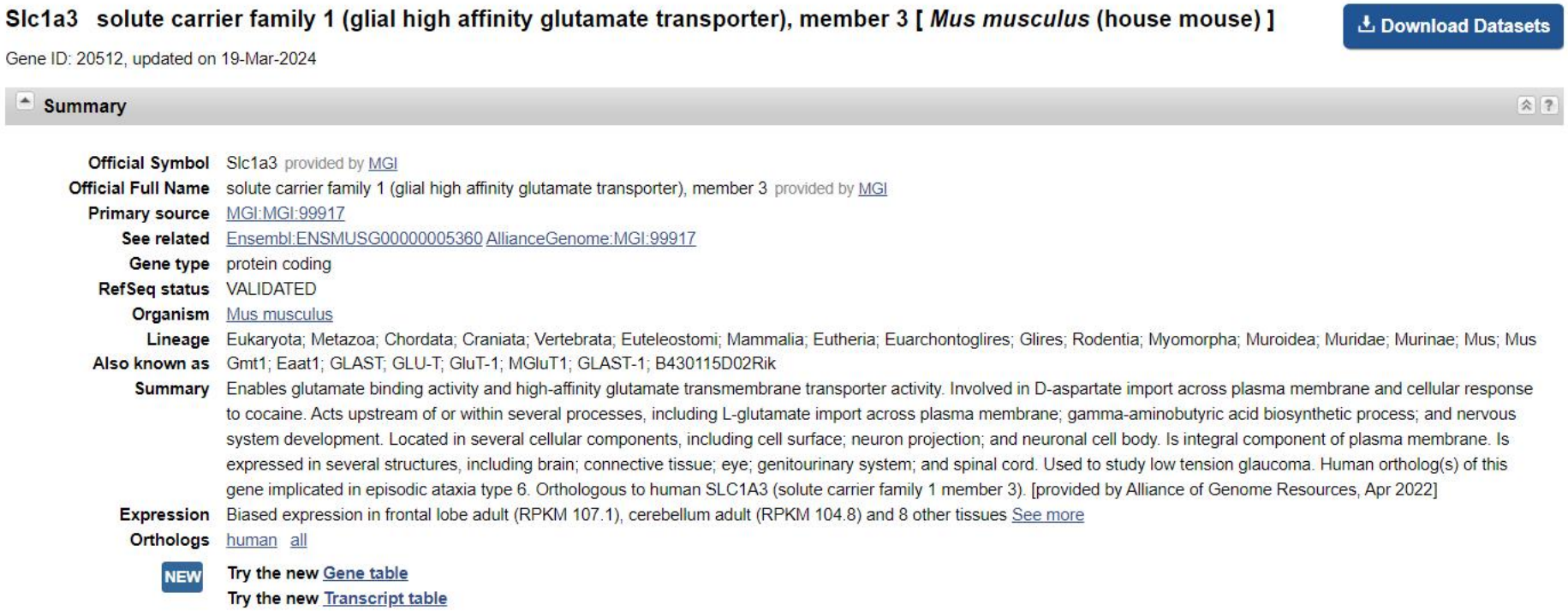


Schematic representation of CRISPR-Cas9 engineering used to edit the *Slc1a3* gene.

Technical Information

- The *Slc1a3* gene has 8 transcripts. According to the structure of *Slc1a3* gene, exon6 of *Slc1a3*-201 (ENSMUST00000005493.14) transcript is recommended as the knockout region. The region contains 293bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Slc1a3* 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



Slc1a3 solute carrier family 1 (glial high affinity glutamate transporter), member 3 [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 20512, updated on 19-Mar-2024

Summary

Official Symbol	Slc1a3 provided by MGI
Official Full Name	solute carrier family 1 (glial high affinity glutamate transporter), member 3 provided by MGI
Primary source	MGI:MGI:99917
See related	Ensembl:ENSMUSG00000005360 AllianceGenome:MGI:99917
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	Gmt1; Eaat1; GLAST; GLU-T; GluT-1; MGluT1; GLAST-1; B430115D02Rik
Summary	Enables glutamate binding activity and high-affinity glutamate transmembrane transporter activity. Involved in D-aspartate import across plasma membrane and cellular response to cocaine. Acts upstream of or within several processes, including L-glutamate import across plasma membrane; gamma-aminobutyric acid biosynthetic process; and nervous system development. Located in several cellular components, including cell surface; neuron projection; and neuronal cell body. Is integral component of plasma membrane. Is expressed in several structures, including brain; connective tissue; eye; genitourinary system; and spinal cord. Used to study low tension glaucoma. Human ortholog(s) of this gene implicated in episodic ataxia type 6. Orthologous to human SLC1A3 (solute carrier family 1 member 3). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Biased expression in frontal lobe adult (RPKM 107.1), cerebellum adult (RPKM 104.8) and 8 other tissues See more
Orthologs	human all

NEW Try the new Gene table

Try the new Transcript table

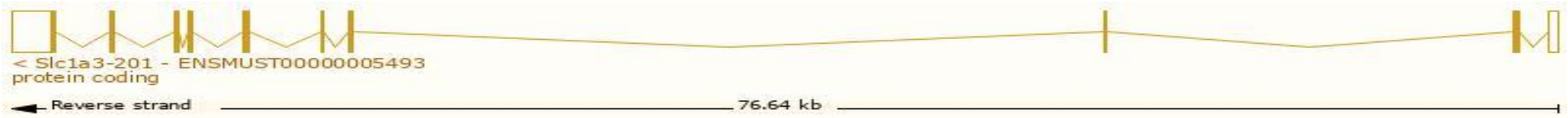
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

The gene has 8 transcripts,all transcripts are shown below:

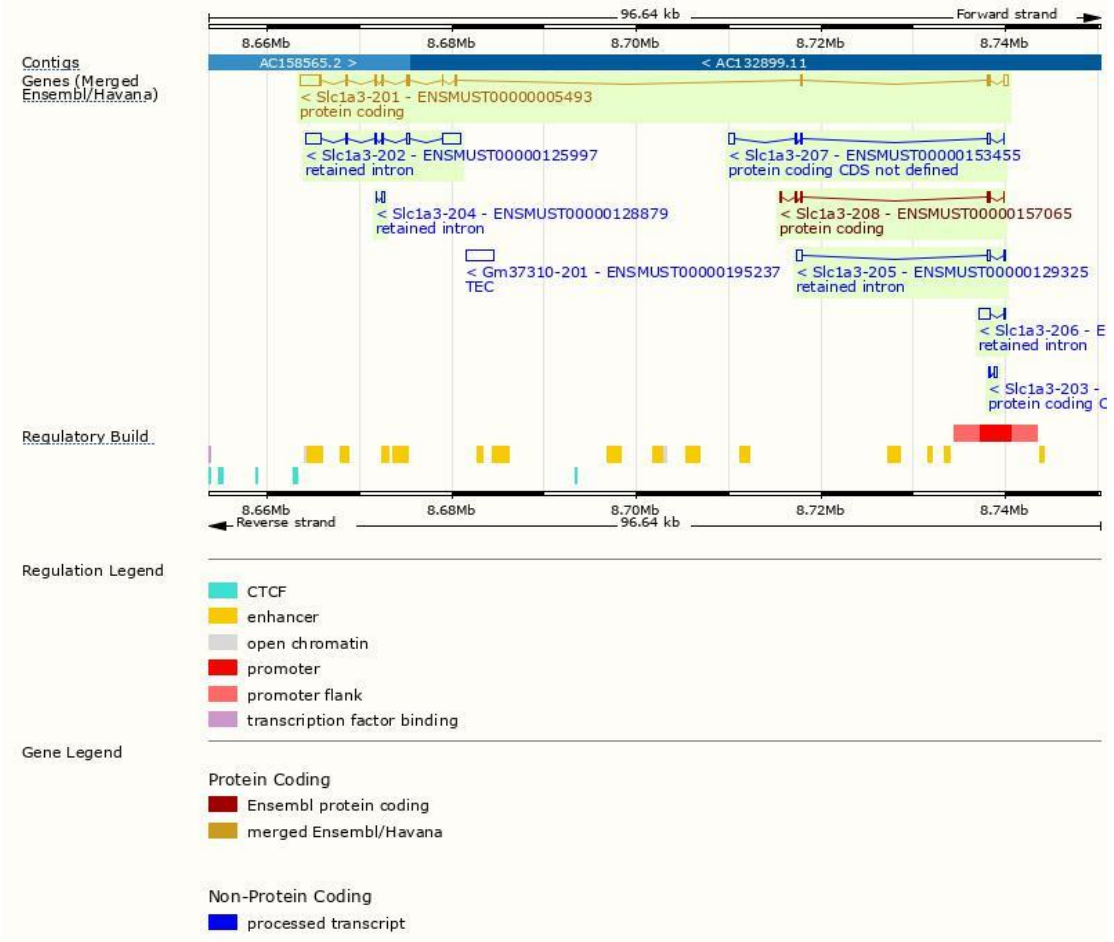
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000005493.14	Slc1a3-201	4163	543aa	Protein coding	CCDS27373	P56564 Q543U3	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1
ENSMUST00000157065.2	Slc1a3-208	728	129aa	Protein coding		D3YY51	GENCODE basic TSL:1
ENSMUST00000153455.8	Slc1a3-207	1252	No protein	Protein coding CDS not defined		-	TSL:1
ENSMUST00000126747.2	Slc1a3-203	402	No protein	Protein coding CDS not defined		-	TSL:5
ENSMUST00000125997.2	Slc1a3-202	4435	No protein	Retained intron		-	TSL:2
ENSMUST00000133309.2	Slc1a3-206	1373	No protein	Retained intron		-	TSL:1
ENSMUST00000129325.2	Slc1a3-205	964	No protein	Retained intron		-	TSL:2
ENSMUST00000128879.2	Slc1a3-204	469	No protein	Retained intron		-	TSL:2

The strategy is based on the design of *Slc1a3*-201 transcript, the transcription is shown below:



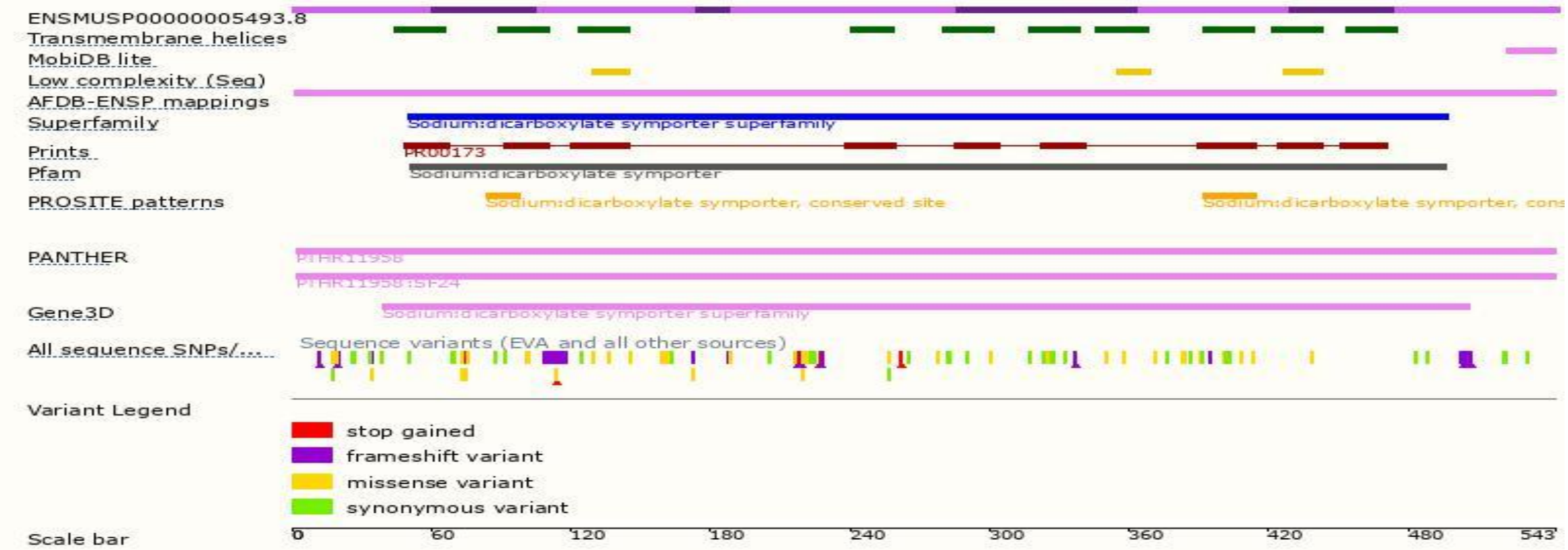
Source: https://www.ensembl.org

Genomic Information



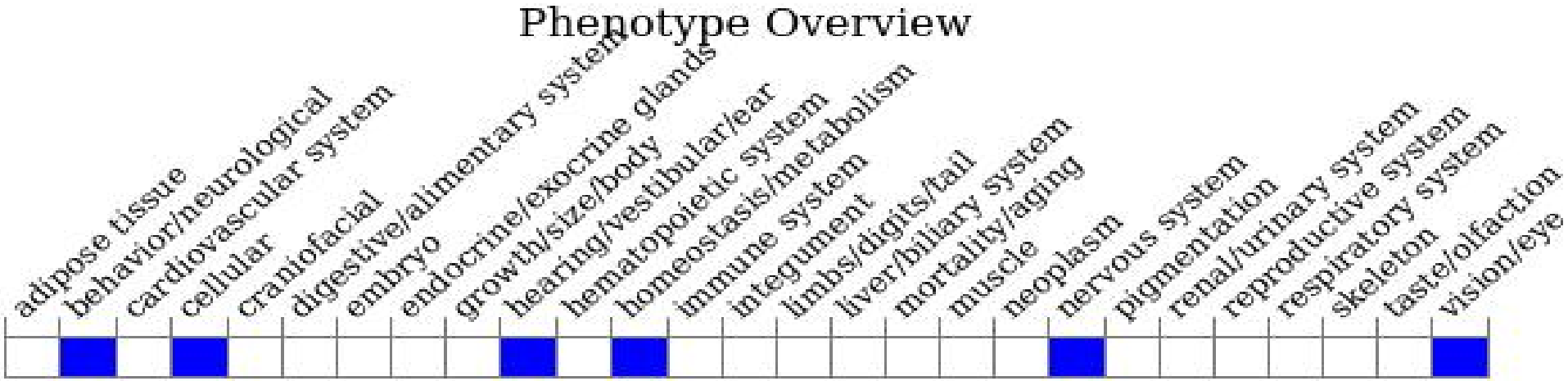
Source: : https://www.ensembl.org

Protein Information



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

Mouse Phenotype Information (MGI)



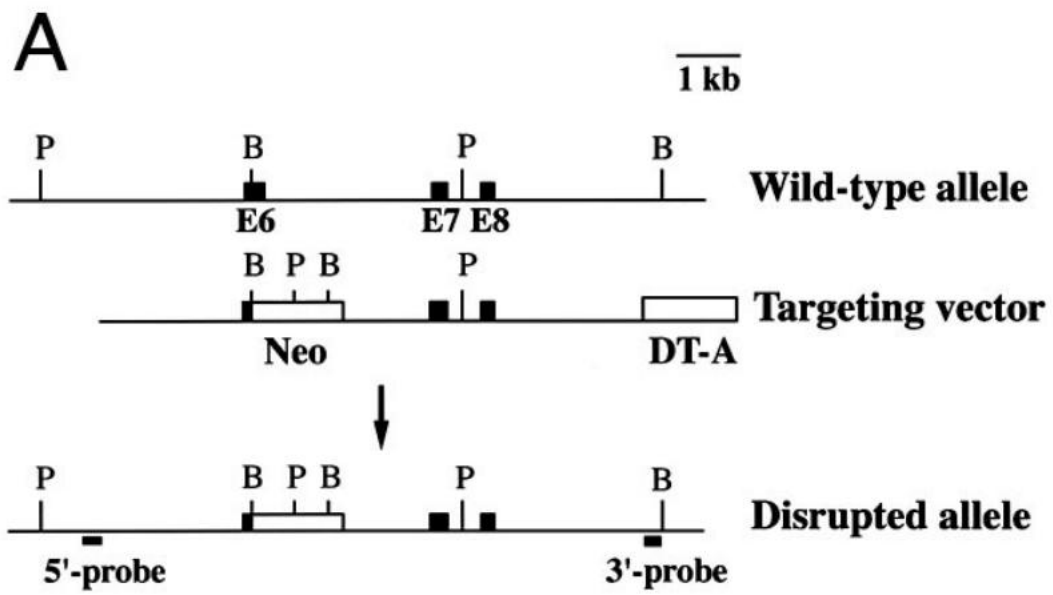
- Mice homozygous for disruptions in this gene display no abnormalities with respect to appearance or survival but do display functional abnormalities related to the central nervous system.

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

Important Information

- According to MGI information,mice homozygous for disruptions in this gene display no abnormalities with respect to appearance or survival but do display functional abnormalities related to the central nervous system.
- The effect of this strategy on the transcript of *Slc1a3*-208 is unknown.
- *Slc1a3* 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



The mouse GLAST gene was isolated from a mouse genomic library prepared from 129/SvJ mice DNA (Stratagene, La Jolla, CA, USA) by hybridization with the full length mouse GLAST cDNA (a mixture of the 748-bp *Eco*RI–*Bam*HI fragment and the 1044-bp *Bam*HI–*Sac*I fragment) used as probe (Tanaka, 1993a; Hagiwara *et al*., 1996). The targeting vector consisted of the 9.0-kb genomic sequence in which the 1.5-kb *Bam*HI–*Sma*I fragment encoding a part of the transmembrane region of GLAST was replaced with the 1.6-kb neomycin gene derived from pGK2Neo (Yagi *et al*., 1993). A diphtheria toxin A fragment gene derived from pMC1DT-3 (Yagi *et al*., 1993) was attached to the 3′ end of the GLAST-neomycin fragment for negative selection. E14 embryonic stem (ES) cells were transfected with *Not*I-digested targeting vector by electroporation and selected with G418. ES cell lines with targeted disruption of the GLAST gene were identified by Southern blot analysis and the targeted clone was obtained with a frequency of 1/512. Injection of ES cells into

Watase K, Hashimoto K, Kano M, Yamada K, Watanabe M, Inoue Y, Okuyama S, Sakagawa T, Ogawa S, Kawashima N, Hori S, Takimoto M, Wada K, Tanaka K. Motor discoordination and increased susceptibility to cerebellar injury in GLAST mutant mice. Eur J Neurosci. 1998 Mar;10(3):976-88. doi: 10.1046/j.1460-9568.1998.00108.x. PMID: 9753165.