

Slc1a5 Cas9-CKO Strategy Rohalanakoch Co.

Designer: Shilei Zhu

Project Overview



Project Name

Slc1a5

Project type

Cas9-CKO

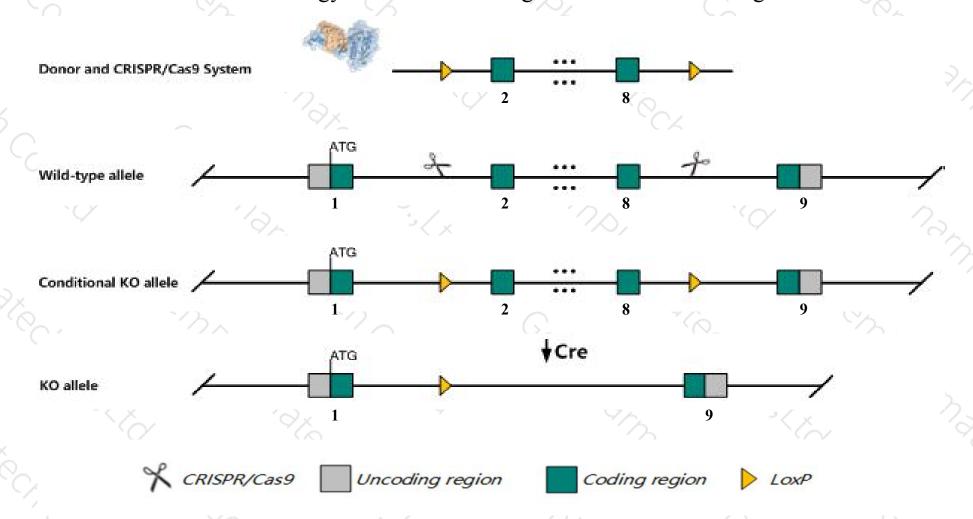
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Slc1a5* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Slc1a5* gene has 8 transcripts. According to the structure of *Slc1a5* gene, exon2-exon8 of *Slc1a5-201* (ENSMUST00000108496.8) transcript is recommended as the knockout region. The region contains 855bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Slc1a5* 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.
- 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.

Notice



- ➤ According to the existing MGI data, Mice homozygous for a knock-out allele exhibit reduced B cells, CD4+ memory T cells in older mice, Th1 and Th17 T cells, susceptibility to EAE and T cell uptake of glutamine and leucine.
- > The *Slc1a5* gene is located on the Chr7. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes 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 existing technological level.

Gene information (NCBI)



SIc1a5 solute carrier family 1 (neutral amino acid transporter), member 5 [Mus musculus (house mouse)]

Gene ID: 20514, updated on 31-Jan-2019

Summary

☆ ?

Official Symbol Slc1a5 provided by MGI

Official Full Name solute carrier family 1 (neutral amino acid transporter), member 5 provided by MGI

Primary source MGI:MGI:105305

See related Ensembl:ENSMUSG00000001918

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 AAAT, ASCT2, ATBO, M7V1, M7VS1, R16, RDRC, Slc1a7

Expression Broad expression in subcutaneous fat pad adult (RPKM 377.3), mammary gland adult (RPKM 328.6) and 17 other tissuesSee more

Orthologs <u>human</u> all

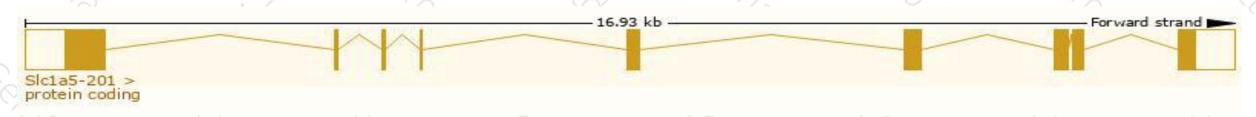
Transcript information (Ensembl)



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

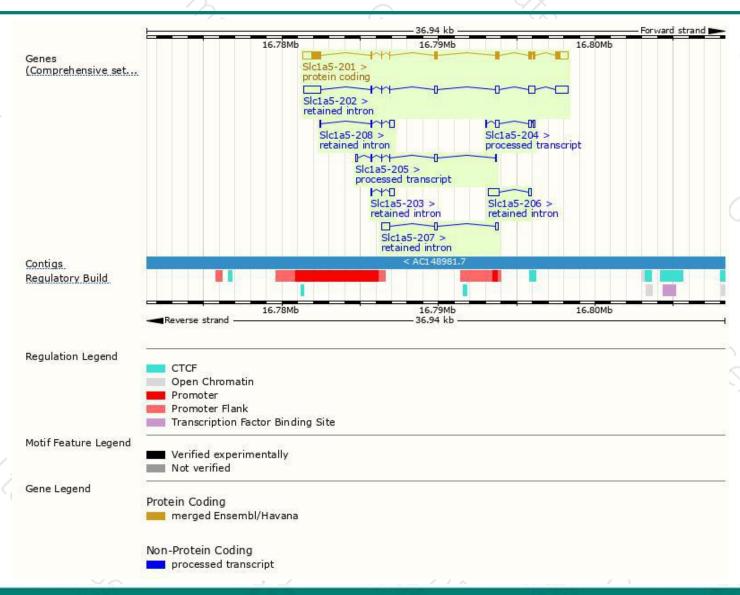
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
SIc1a5-201	ENSMUST00000108496.8	2764	<u>555aa</u>	Protein coding	CCDS39785	Q9ESU7	TSL:1 GENCODE basic APPRIS P1
SIc1a5-204	ENSMUST00000134407.1	613	No protein	Processed transcript	-	-	TSL:5
SIc1a5-205	ENSMUST00000135817.2	416	No protein	Processed transcript	ų.	-	TSL:2
SIc1a5-202	ENSMUST00000127401.7	2807	No protein	Retained intron	2	-	TSL:1
SIc1a5-206	ENSMUST00000141349.1	890	No protein	Retained intron	ā		TSL:2
SIc1a5-207	ENSMUST00000147814.1	853	No protein	Retained intron	-	-	TSL:3
SIc1a5-208	ENSMUST00000206444.1	475	No protein	Retained intron	ų.	ū.	TSL:2
SIc1a5-203	ENSMUST00000131664.1	396	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of Slc1a5-201 transcript, The transcription is shown below



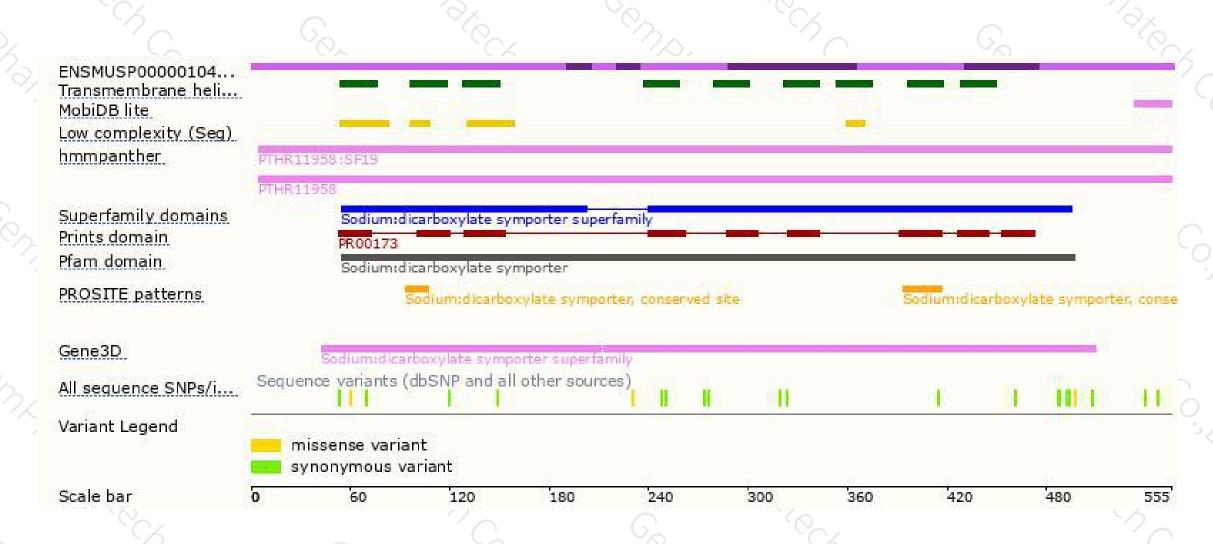
Genomic location distribution





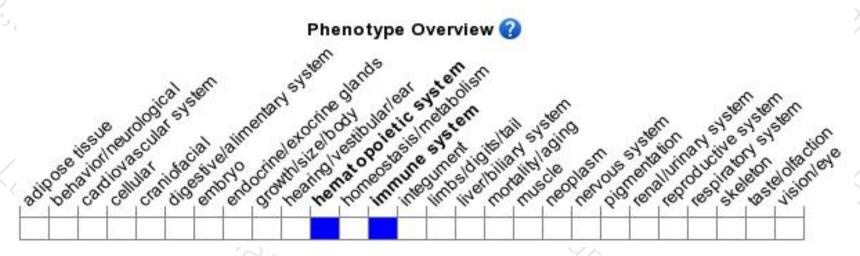
Protein domain





Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data, Mice homozygous for a knock-out allele exhibit reduced B cells, CD4+ memory T cells in older mice, Th1 and Th17 T cells, susceptibility to EAE and T cell uptake of glutamine and leucine.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





