

Slc6a5 Cas9-CKO Strategy

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Reviewer: Jia Yu

Project Overview



Project Name

Slc6a5

Project type

Cas9-CKO

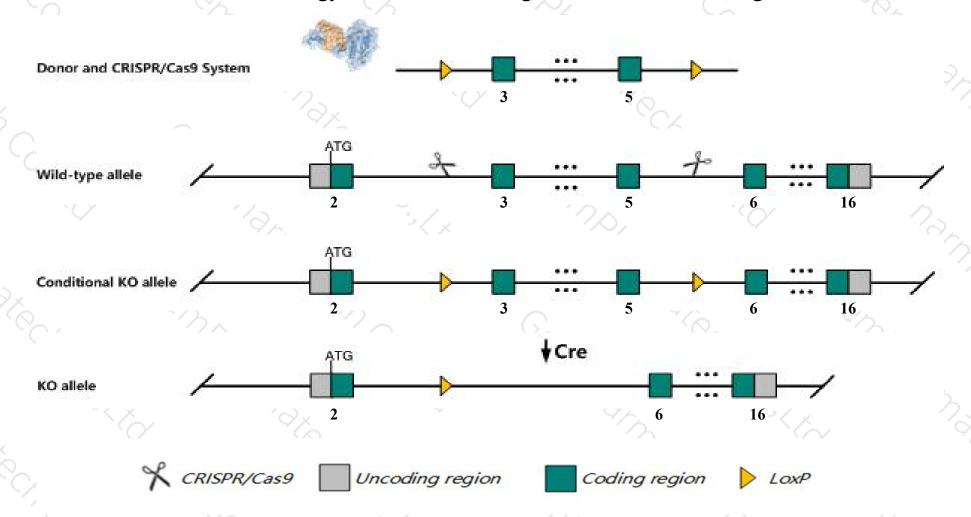
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Slc6a5 gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Slc6a5* gene has 4 transcripts. According to the structure of *Slc6a5* gene, exon3-exon5 of *Slc6a5-201*(ENSMUST00000056442.11) transcript is recommended as the knockout region. The region contains 445bp coding sequence.

 Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Slc6a5* 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, Homozygous mutant mice appear normal at birth but develop a complex neuromotor phenotype involving tremors, rigidity, and an impaired righting ability. Mutant mice die approximately 2 weeks after birth.
- > The *Slc6a5* 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)



SIc6a5 solute carrier family 6 (neurotransmitter transporter, glycine), member 5 [Mus musculus (house mouse)]

Gene ID: 104245, updated on 12-Aug-2019

Summary

↑ ?

Official Symbol Slc6a5 provided by MGI

Official Full Name solute carrier family 6 (neurotransmitter transporter, glycine), member 5 provided by MGI

Primary source MGI:MGI:105090

See related Ensembl: ENSMUSG00000039728

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 Glyt2; prestin

Expression Biased expression in cerebellum adult (RPKM 13.1), CNS E14 (RPKM 1.8) and 2 other tissues See more

Orthologs <u>human</u> all

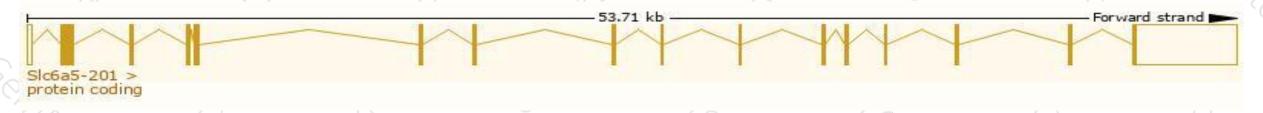
Transcript information (Ensembl)



The gene has 4 transcripts, all transcripts are shown below:

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|------------|--|--------------------------------------|--------------|-------------------------|-----------|------------|-------------------------------|--|
| Name | Transcript ID | bp | Protein | Biotype | ccds | UniProt | Flags | |
| SIc6a5-201 | ENSMUST00000056442.11 | 7065 | <u>791aa</u> | Protein coding | CCDS21308 | B2RQX9 | TSL:1 GENCODE basic APPRIS P1 | |
| SIc6a5-203 | ENSMUST00000207753.1 | 6966 | <u>791aa</u> | Protein coding | CCDS21308 | B2RQX9 | TSL:1 GENCODE basic APPRIS P1 | |
| SIc6a5-202 | ENSMUST00000107605.2 | 2467 | <u>791aa</u> | Protein coding | CCDS21308 | B2RQX9 | TSL:1 GENCODE basic APPRIS P1 | |
| SIc6a5-204 | ENSMUST00000209172.1 | 2409 | <u>181aa</u> | Nonsense mediated decay | - | A0A140LHI9 | TSL:1 | |
| 5 / 3 | | V 1900 0 | | ~ / / | | | | |

The strategy is based on the design of Slc6a5-201 transcript, The transcription is shown below



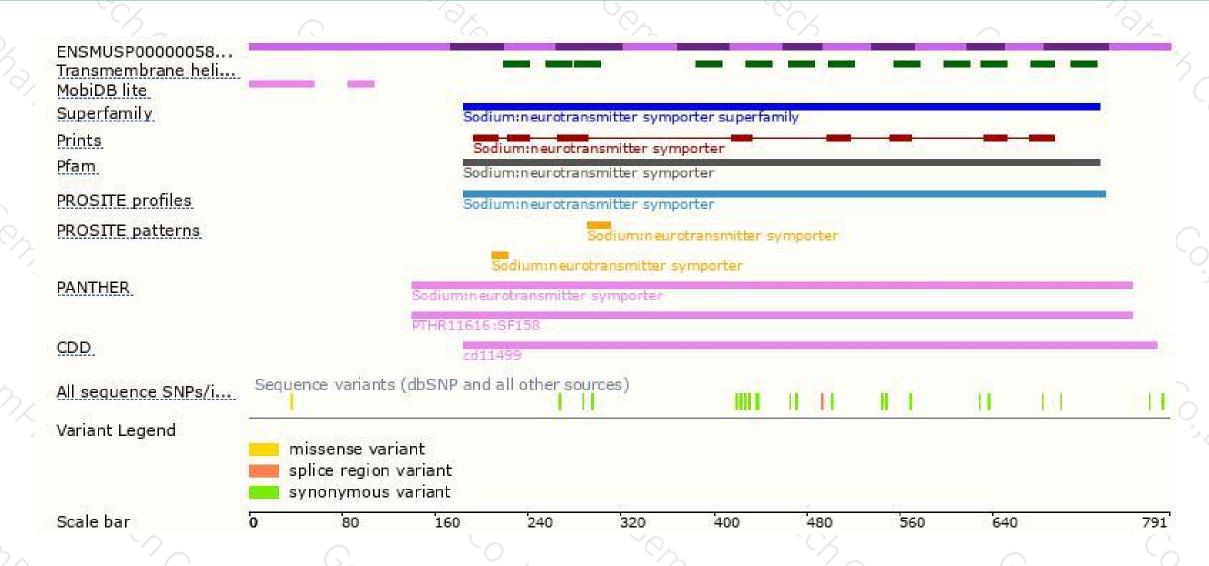
Genomic location distribution





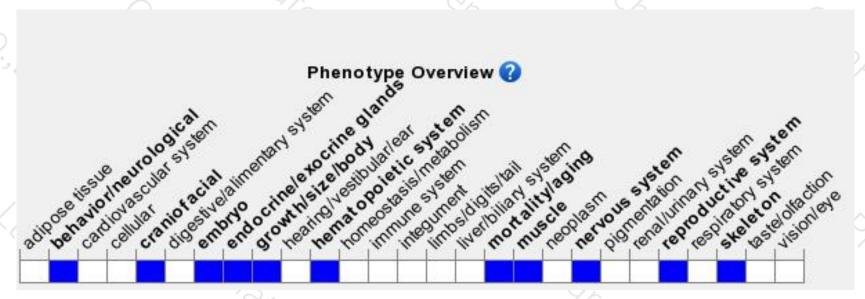
Protein domain





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, Homozygous mutant mice appear normal at birth but develop a complex neuromotor phenotype involving tremors, rigidity, and an impaired righting ability. Mutant mice die approximately 2 weeks after birth.



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





