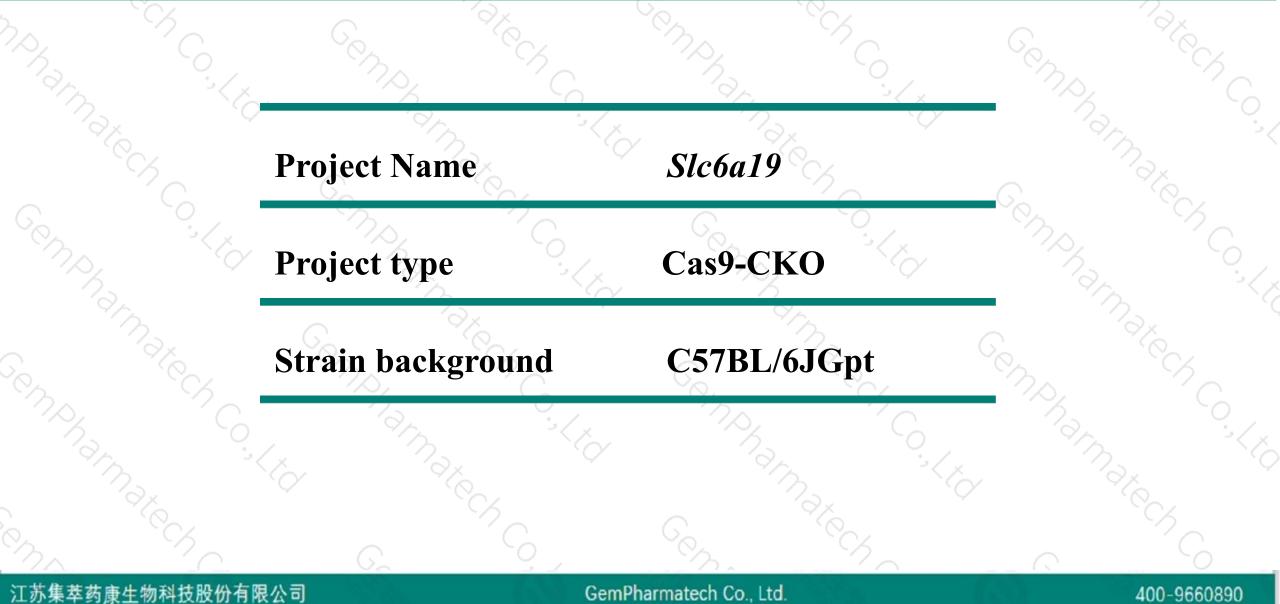


Slc6a19 Cas9-CKO Strategy

Designer:Xiaojing Li Reviewer:JiaYu Design Date:2020-2-11

Project Overview

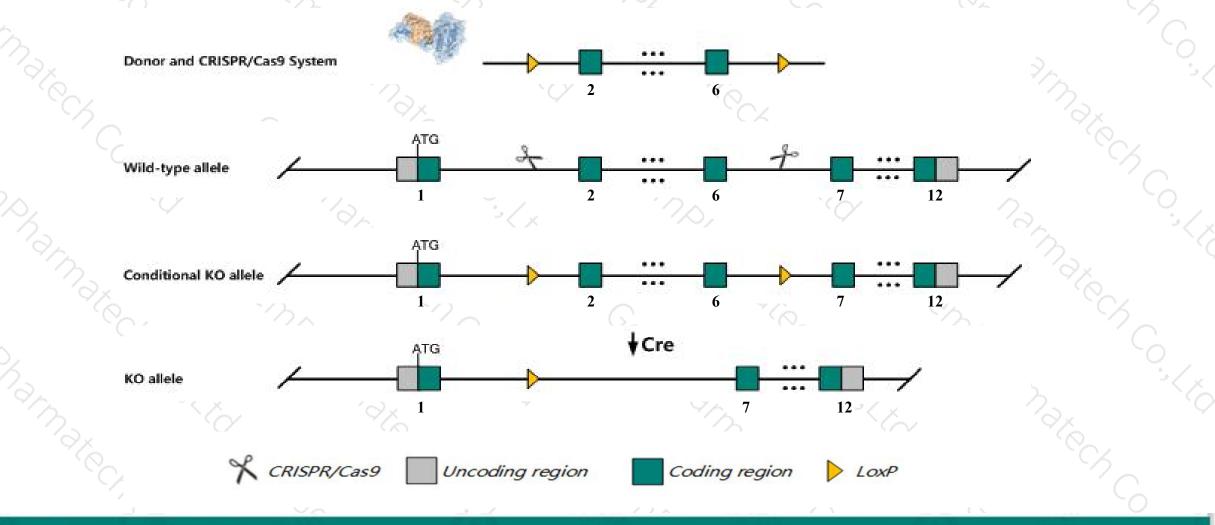




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Slc6a19* gene. The schematic diagram is as follows:



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The Slc6a19 gene has 7 transcripts. According to the structure of Slc6a19 gene, exon2-exon6 of Slc6a19-201 (ENSMUST00000022048.5) transcript is recommended as the knockout region. The region contains 685bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Slc6a19* 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.



- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit decreased body weight and impaired amino acid absorption and excretion.
- The Slc6a19 gene is located on the Chr13. 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)



SIc6a19 solute carrier family 6 (neurotransmitter transporter), member 19 [Mus musculus (house mouse)]

Gene ID: 74338, updated on 10-Oct-2019

Summary

Official Symbol Slc6a19 provided by MGI Official Full Name solute carrier family 6 (neurotransmitter transporter), member 19 provided by MGI Primary source MGI:MGI:1921588 See related Ensembl:ENSMUSG0000021565 Gene type protein coding RefSeq status VALIDATED Organism Mus musculus Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Lineage Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus Also known as B<0>AT1; 4632401C08Rik Expression Biased expression in kidney adult (RPKM 169.0), large intestine adult (RPKM 126.9) and 2 other tissues See more Orthologs human all

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The gene has 7 transcripts, all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|-------------|----------------------|------|--------------|-------------------------|-----------|---------------|-------------------------------|
| SIc6a19-201 | ENSMUST00000022048.5 | 3936 | <u>634aa</u> | Protein coding | CCDS26634 | Q3KN89 Q9D687 | TSL:1 GENCODE basic APPRIS P1 |
| SIc6a19-204 | ENSMUST00000124406.7 | 607 | <u>83aa</u> | Nonsense mediated decay | - | D6RJ80 | TSL:3 |
| SIc6a19-205 | ENSMUST00000132085.7 | 4192 | No protein | Retained intron | - | - | TSL:1 |
| SIc6a19-202 | ENSMUST00000120322.7 | 3592 | No protein | IncRNA | 4 | - | TSL:1 |
| SIc6a19-203 | ENSMUST00000123997.7 | 839 | No protein | IncRNA | 5 | ā | TSL:5 |
| SIc6a19-206 | ENSMUST00000139087.7 | 745 | No protein | IncRNA | - | - | TSL:3 |
| SIc6a19-207 | ENSMUST00000140878.1 | 360 | No protein | IncRNA | 2 | - | TSL:3 |

The strategy is based on the design of Slc6a19-201 transcript, The transcription is shown below

< Slc6a 19-201 protein coding

Reverse strand

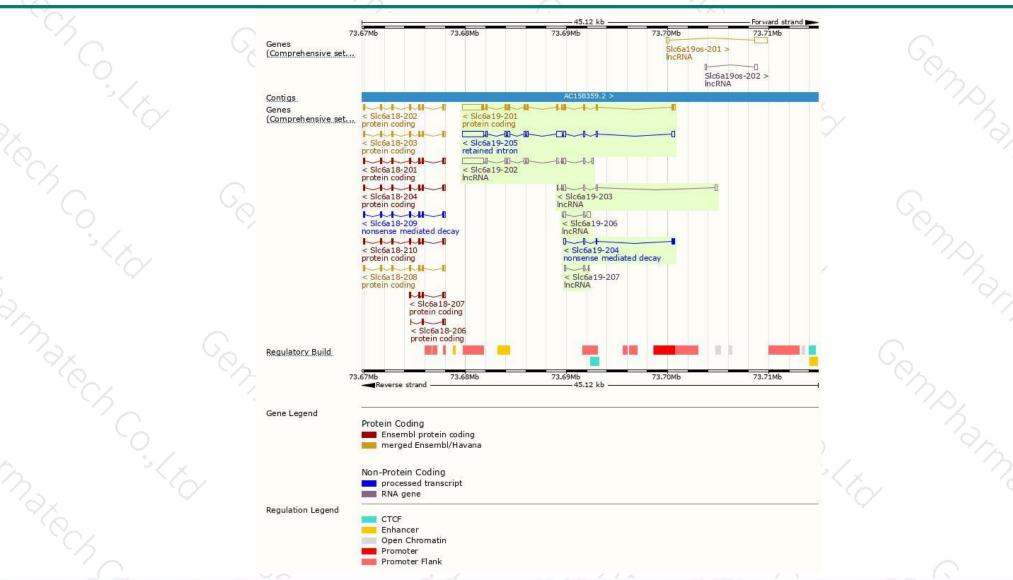
- 21.04 kb --

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Genomic location distribution





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Protein domain



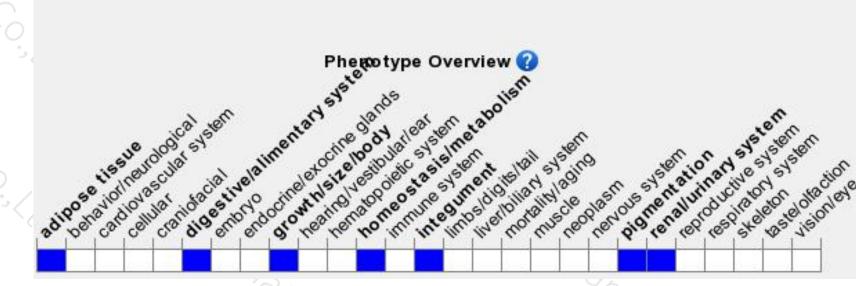
| | | ×0° | | 20, | | | | | | | |
|---|---|---|-----------------|---------------|--------|----|--------|-----|----------|--|--|
| ENSMUSP00000022 Transmembrane heli Low complexity (Seg) | / American | | - | _ | | | - | | 2 | | |
| Superfamily | Sodium:neurotr | ransmitter symporter | superfamily | 1921 | | | | | -++7 | | |
| Prints | | | amino acid SLC6 | 5 transporter | | | | | | | |
| Pfam | With the second s | transmitter symporte ansmitter symporter | | - | | | 2-01 V | | - | | |
| PROSITE profiles | Sodium:neurotr | ansmitter symporter | | | | | | | <u> </u> | | |
| PROSITE patterns | Sodiumine | aurotransmitter sympo | orter | | | | | | | | |
| PANTHER | Sodium:neurotrans | | | | | | | | | | |
| | PTHR11616;SF125 | | | | | | | | | | |
| All sequence SNPs/i | Sequence variants (| dbSNP and all othe | ir sources) | 111 | 10 | | i - | 1 | 16 | | |
| Variant Legend | missense variant synonymous variant | | | | | | | | | | |
| Scale bar | 0 60 | 120 180 | 240 | 300 | 360 42 | .0 | 480 | 540 | 634 | | |
| | | | | х О. | | | | | 50 | | |
| \sim | | | | | -7~ | | | | | | |

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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 decreased body weight and impaired amino acid absorption and excretion.



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



