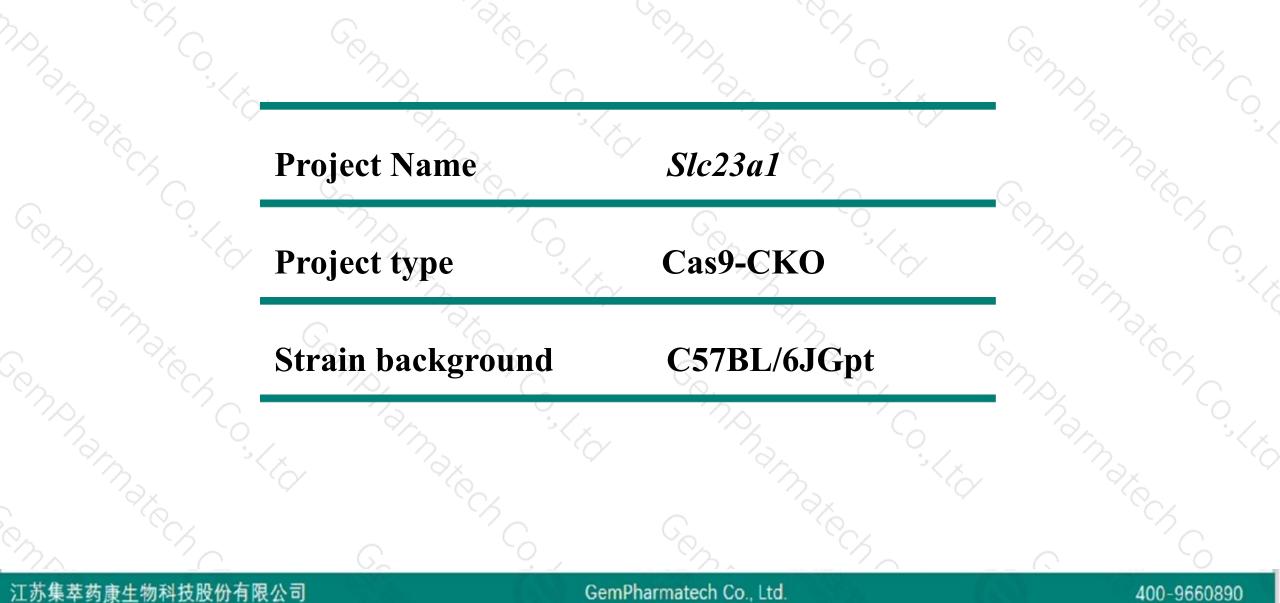


Slc23a1 Cas9-CKO Strategy

Designer:Xueting Zhang Reviewer:Yanhua Shen Date:2019-10-19

Project Overview



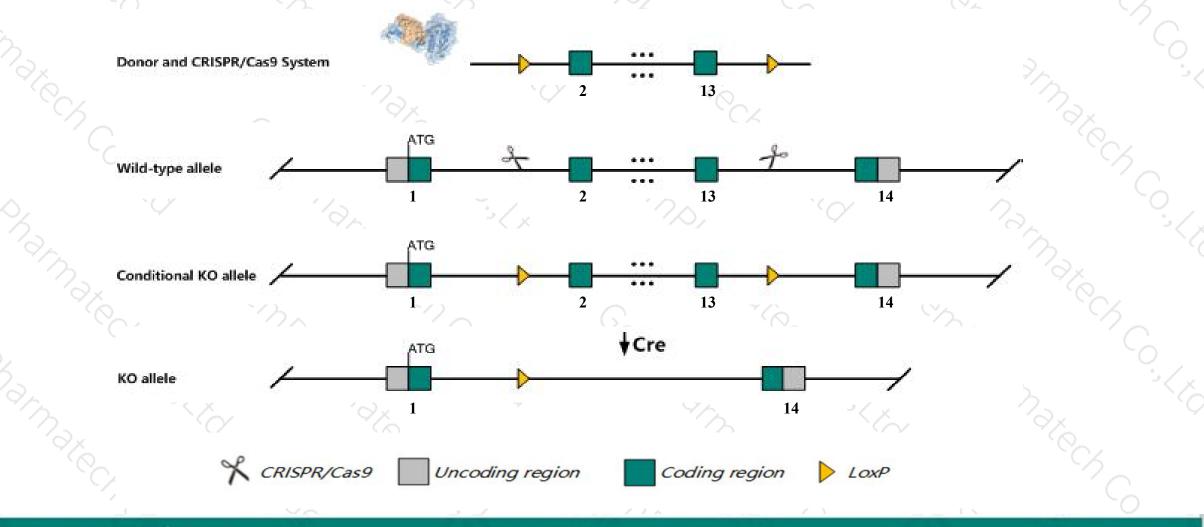


Conditional Knockout strategy



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This model will use CRISPR/Cas9 technology to edit the *Slc23a1* gene. The schematic diagram is as follows:



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The Slc23a1 gene has 4 transcripts. According to the structure of Slc23a1 gene, exon2-exon13 of Slc23a1-201 (ENSMUST00000025212.7) transcript is recommended as the knockout region. The region contains 1534bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Slc23a1* 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 abnormal ascorbate homeostasis and early postnatal lethality associated with lethargy and lack of gastric milk. Heterozygous mice of homozgous dams exhibit a similar phenotype.
- The Slc23a1 gene is located on the Chr18. 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.

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Gene information (NCBI)

SIc23a1 solute carrier family 23 (nucleobase transporters), member 1 [Mus musculus (house mouse)]

Gene ID: 20522, updated on 10-Oct-2019

Summary

| Official Symbol | SIc23a1 provided by MGI | |
|------------------------|--|-----|
| Official Full Name | solute carrier family 23 (nucleobase transporters), member 1 provided by MGI | |
| Primary source | <u>MGI:MGI:1341903</u> | |
| See related | Ensembl:ENSMUSG0000024354 | |
| 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 | SVCT1; YSPL3; Slc23a2; D18Ucla2 | |
| Expression | Biased expression in kidney adult (RPKM 78.2), liver adult (RPKM 28.3) and 6 other tissues See more | |
| Orthologs | human all | |
| | | |

Chr

18

Location

Genomic context

Location: 18 B2; 18 19.17 cM

Status

current

previous assembly

Assembly

GRCm38.p6 (GCF 000001635.26)

MGSCv37 (GCF 000001635.18)

See SIc23a1 in Genome Data Viewer

NC_000084.5 (35774258..35786881, complement) 18

NC_000084.6 (35604224..35629845, complement)

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\$?

Exon count: 19

108

Build 37.2

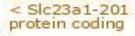
Annotation release



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

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|-------------|----------------------|------|--------------|-------------------------|-----------|---------------|-------------------------------|
| SIc23a1-201 | ENSMUST00000025212.7 | 3026 | <u>605aa</u> | Protein coding | CCDS29144 | <u>Q9Z2J0</u> | TSL:1 GENCODE basic APPRIS P1 |
| SIc23a1-204 | ENSMUST00000237305.1 | 1889 | <u>53aa</u> | Nonsense mediated decay | | D6RDS7 | |
| SIc23a1-203 | ENSMUST00000236196.1 | 565 | No protein | IncRNA | 120 | - | |
| SIc23a1-202 | ENSMUST00000235744.1 | 498 | No protein | IncRNA | 125 | 12 | |

The strategy is based on the design of Slc23a1-201 transcript, The transcription is shown below



Reverse strand

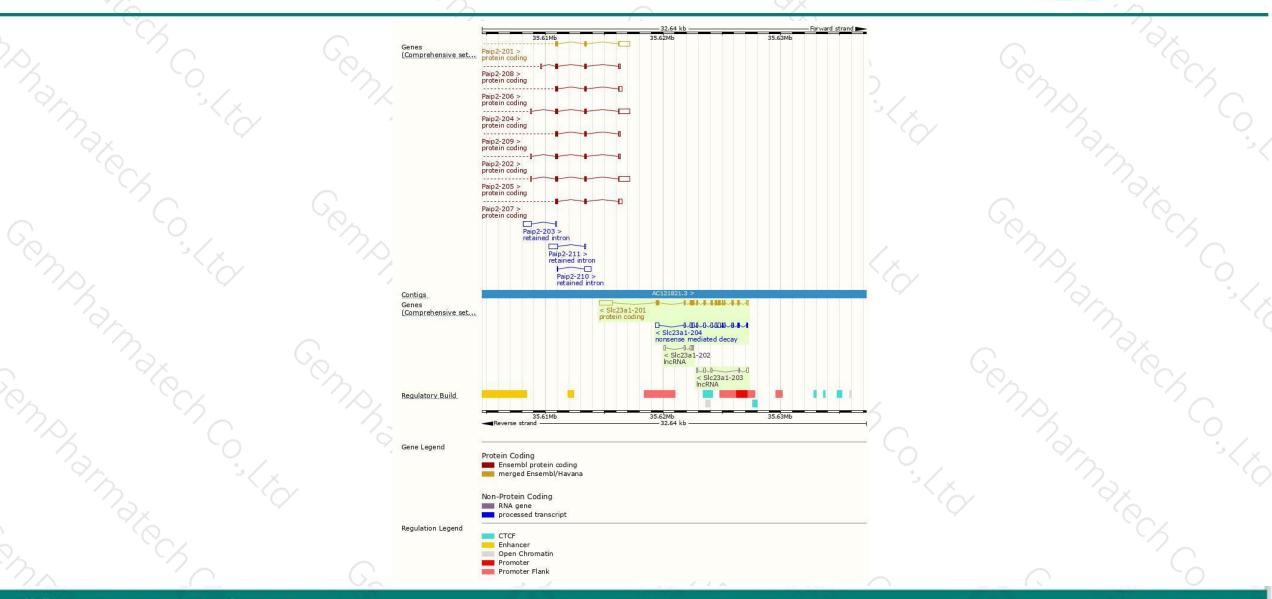
- 12.64 kb -

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Genomic location distribution



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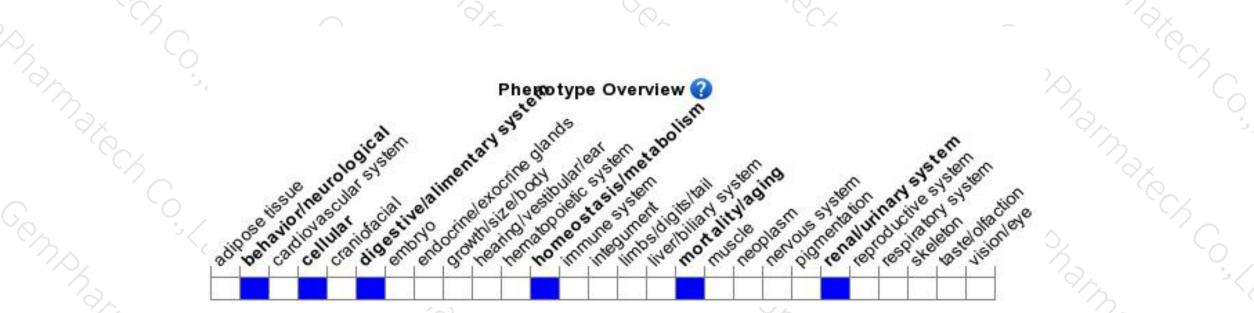
Protein domain



| - 'drj | s its | - ^s | | Co. | 1 | 275 | -0- | | | | $^{\sim}$ C |
|--------|--|---|------------------|----------------|-----------|----------------|--|-----|-----|--------|-------------|
| C | ENSMUSP00000025 Transmembrane heli MobiDB lite Low complexity (Seg) Pfam | Xanthin | e/uracil/vitamir | C permease | | | | | | | < |
| °C7 | PANTHER | Solute carrier fami | | | | | | | | | 6 |
| | All sequence SNPs/i Variant Legend | PTHR11119 Sequence variar | ts (dbSNP and | d all other so | urces) | \overline{V} | ίú | ſ | ŝ, | | |
| SCNX | vanant Legend | missense v splice regic synonymou | n variant | | | | | | | | 0 |
| | Scale bar | 0 60 | 120 | 180 | 240 | 300 | 360 | 420 | 480 | 540 6 | 05 |
| | 1 ar | $\langle \gamma \rangle$ | | | | - ^; | | | | 4 X CA | |
| | ~~~~ | | | 0 | G. | | [°] [°] [°] | | | | |
| 江苏集萃 | 药康生物科技股份有限公 | 公司 | | Gem | Pharmatec | h Co., Ltd. | | | | 400-9 | 660890 |

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 abnormal ascorbate homeostasis and early postnatal lethality associated with lethargy and lack of gastric milk. Heterozygous mice of homozgous dams exhibit a similar phenotype.

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



