

# Slc28a1 Cas9-CKO Strategy

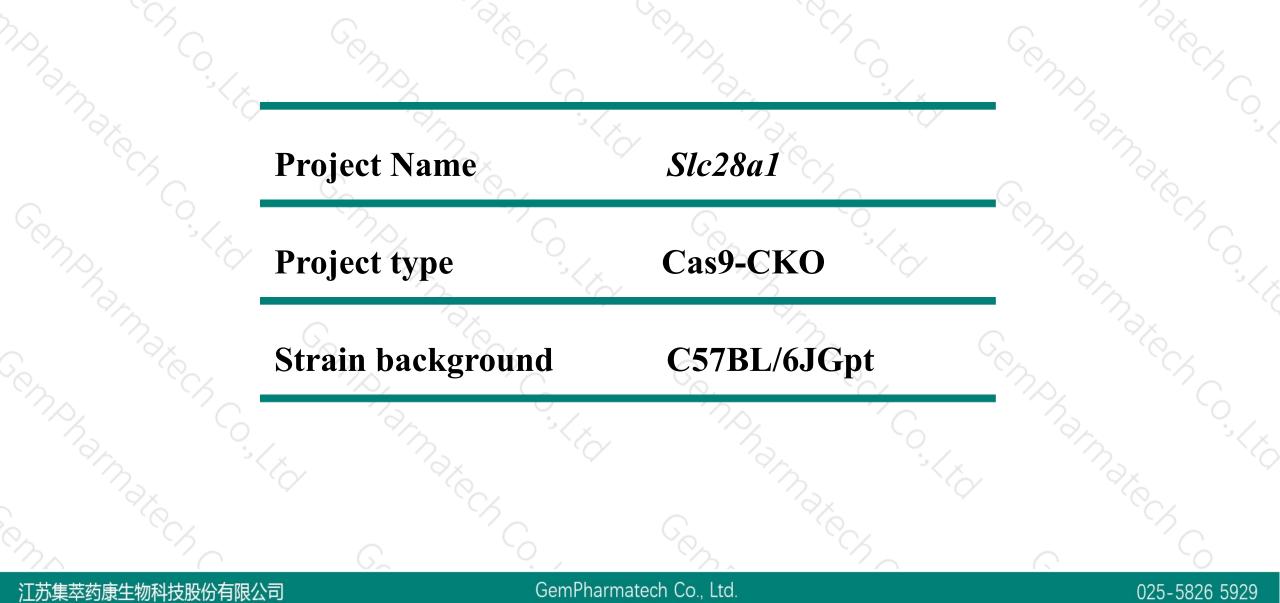
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**Reviewer: Xiaojing Li** 

Design Date: 2020-11-17

## **Project Overview**



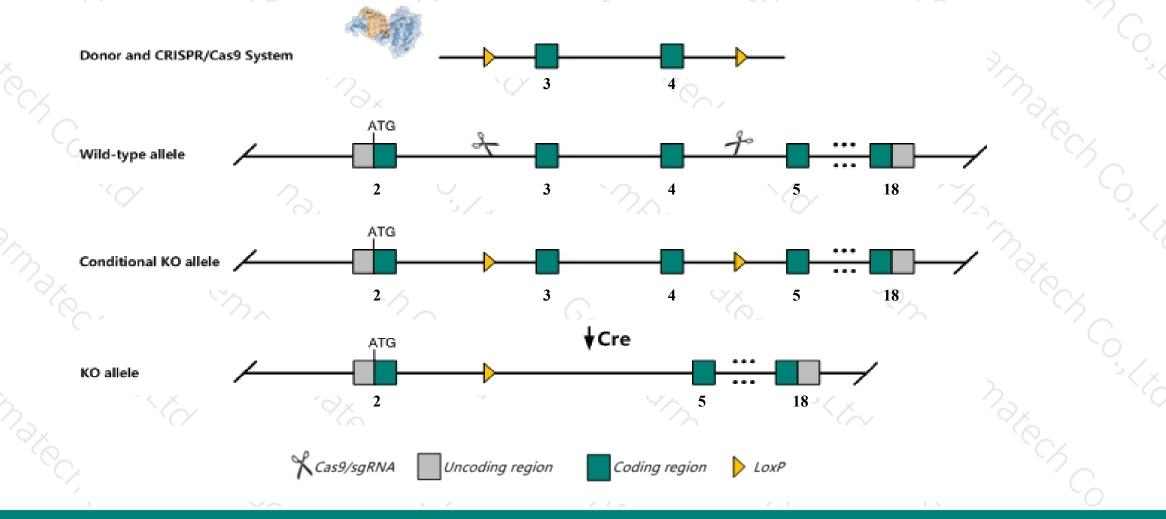


### **Conditional Knockout strategy**



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This model will use CRISPR/Cas9 technology to edit the *Slc28a1* gene. The schematic diagram is as follows:



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The Slc28a1 gene has 2 transcripts. According to the structure of Slc28a1 gene, exon3-exon4 of Slc28a1-202(ENSMUST00000119083.1) transcript is recommended as the knockout region. The region contains 181bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Slc28a1* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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.



- > The *Slc28a1* 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)**



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#### Slc28a1 solute carrier family 28 (sodium-coupled nucleoside transporter), member 1 [Mus musculus (house mouse)]

Gene ID: 434203, updated on 13-Mar-2020

#### Summary

| Official Symbol           | Sic28a1 provided by MGI   |
|---------------------------|---|
| <b>Official Full Name</b> | solute carrier family 28 (sodium-coupled nucleoside transporter), member 1 provided by MGI                                |
| Primary source            | MGI:MGI:3605073   |
| See related               | Ensembl:ENSMUSG0000025726   |
| 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             | Cnt1  |
| Expression                | Biased expression in duodenum adult (RPKM 39.3), small intestine adult (RPKM 22.2) and 3 other tissuesSee more            |
| Orthologs                 | human all   |

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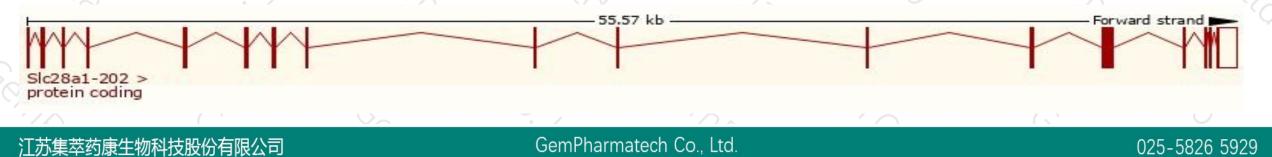
# **Transcript information (Ensembl)**



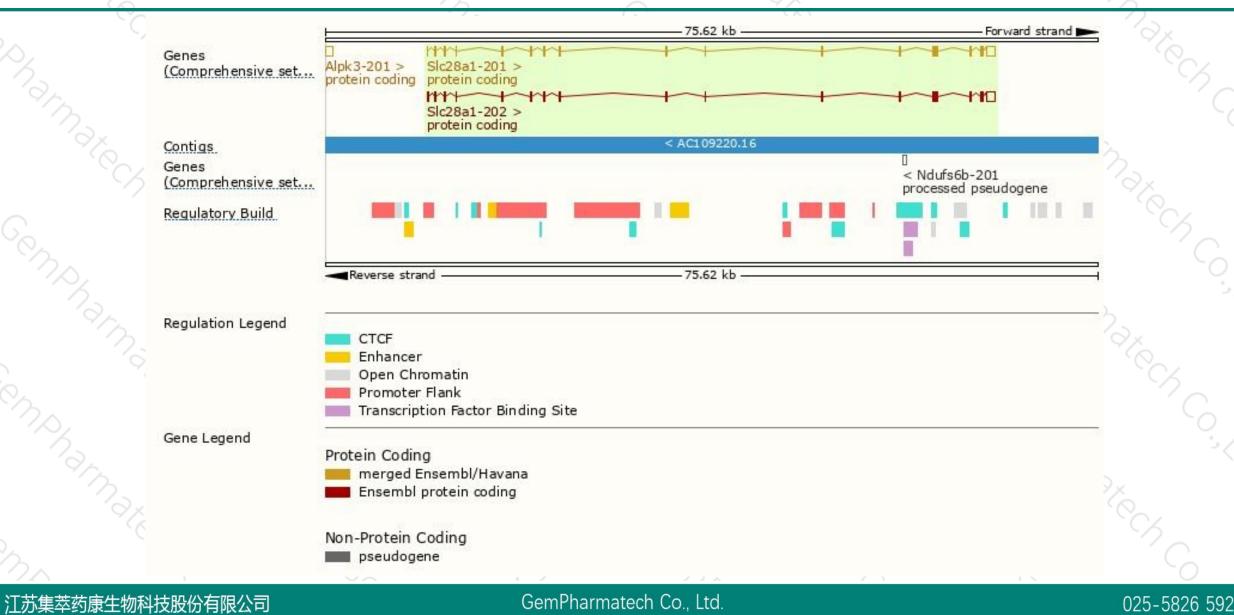
The gene has 2 transcripts, all transcripts are shown below:

|     | Name     | Transcript ID        | bp   | Protein      | Biotype        | CCDS      | UniProt | Flags                         |
|-----|----------|----------------------|------|--------------|----------------|-----------|---------|-------------------------------|
| slo | 28a1-202 | ENSMUST00000119083.1 | 2899 | <u>648aa</u> | Protein coding | CCDS21402 | E9PXX9  | TSL:5 GENCODE basic APPRIS P1 |
| slo | 28a1-201 | ENSMUST0000026820.10 | 2846 | <u>648aa</u> | Protein coding | CCDS21402 | E9PXX9  | TSL:1 GENCODE basic APPRIS P1 |

The strategy is based on the design of *Slc28a1-202* transcript, the transcription is shown below:



#### **Genomic location distribution**

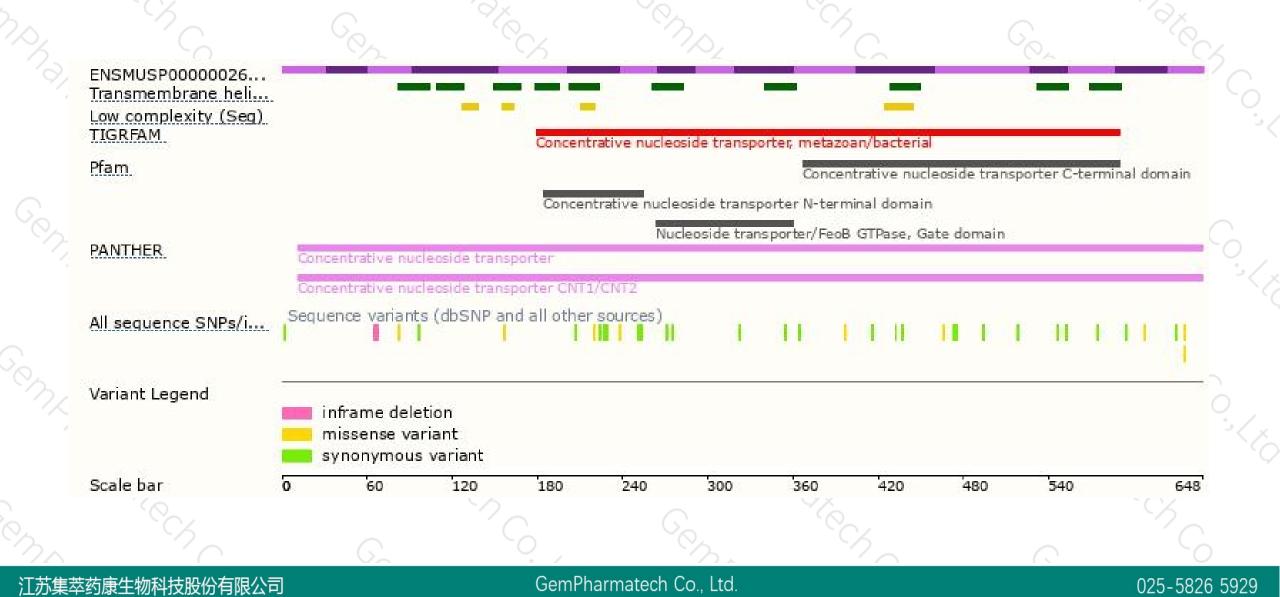


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#### **Protein domain**







If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



