# *Slc12a5* Cas9-KO Strategy

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**Reviewer: Jiayuan Yao**

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# Project Overview

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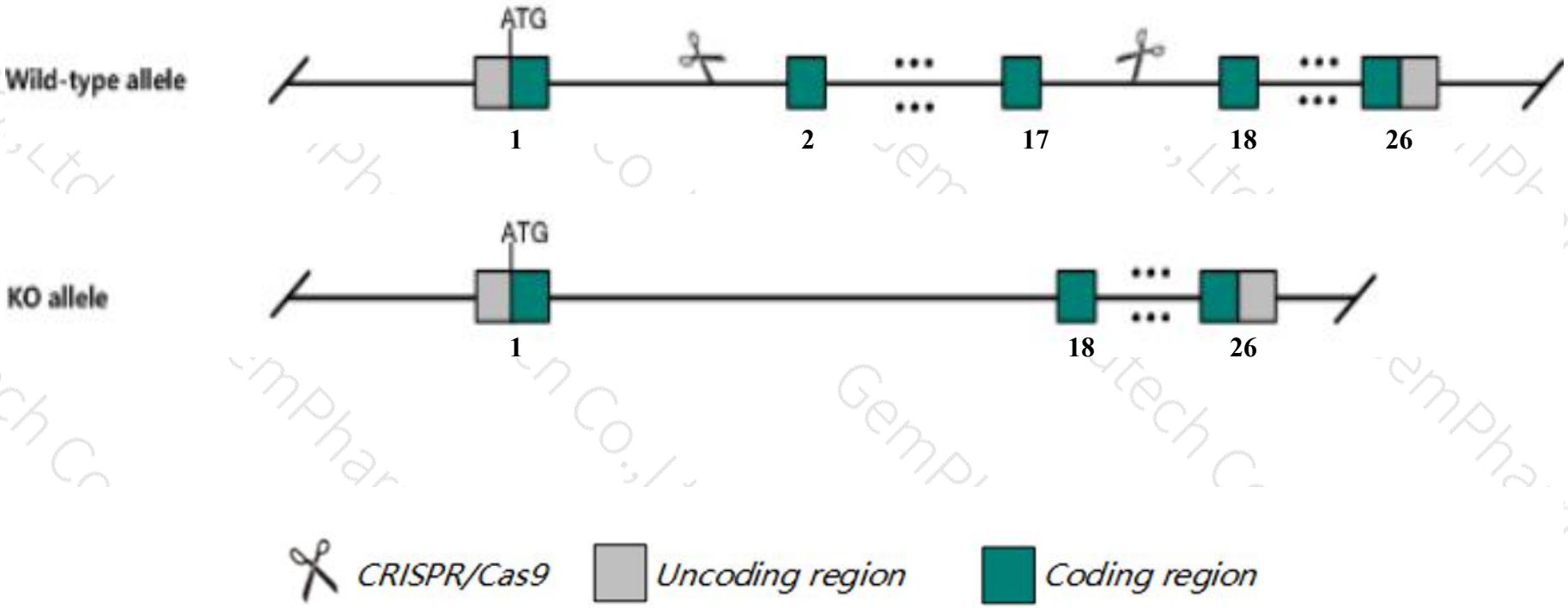
|---|---|
| **Project Name** | ***Slc12a5*** |
| **Project type** | **Cas9-KO** |
| **Strain background** | **C57BL/6JGpt** |

# Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Slc12a5* gene. The schematic diagram is as follows:



# Technical routes

➢ The *Slc12a5* gene has 10 transcripts. According to the structure of *Slc12a5* gene, exon2-exon17 of *Slc12a5-201*(ENSMUST00000099092.7) transcript is recommended as the knockout region.The region contains 2129bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Slc12a5* gene. The brief process is as follows: CRISPR/Cas9 system 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.

# Notice

➢ According to the existing MGI data,mice homozygous for disruptions in this gene die within a few minutes of birth of respiratory failure resulting from a motor nerve defect. Mice homozygous for a hypomorphic allele display postnatal lethality and tonic-clonic seizures.

➢ The *Slc12a5* gene is located on the Chr2. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

# Gene information（NCBI）

集萃药康 GemPharmatech

## Slc12a5 solute carrier family 12, member 5 [Mus musculus (house mouse)]

Gene ID: 57138, updated on 15-Mar-2020

### Summary

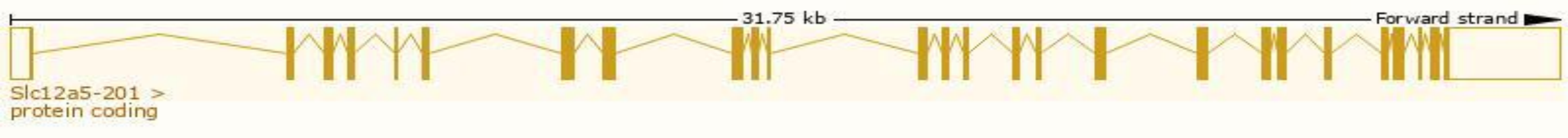
| | |
|---|---|
| **Official Symbol** | Slc12a5 provided by MGI |
| **Official Full Name** | solute carrier family 12, member 5 provided byMGI |
| **Primary source** | MGI:MGI:1862037 |
| **See related** | Ensembl:ENSMUSG00000017740 |
| **Gene type** | protein coding |
| **RefSeq status** | VALIDATED |
| **Organism** | Mus musculus |
| **Lineage** | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| **Also known as** | KCC2, mKIAA1176 |
| **Expression** | Biased expression in cerebellum adult (RPKM 71.6), cortex adult (RPKM 67.7) and 6 other tissuesSee more |
| **Orthologs** | human all |

# Transcript information（Ensembl）

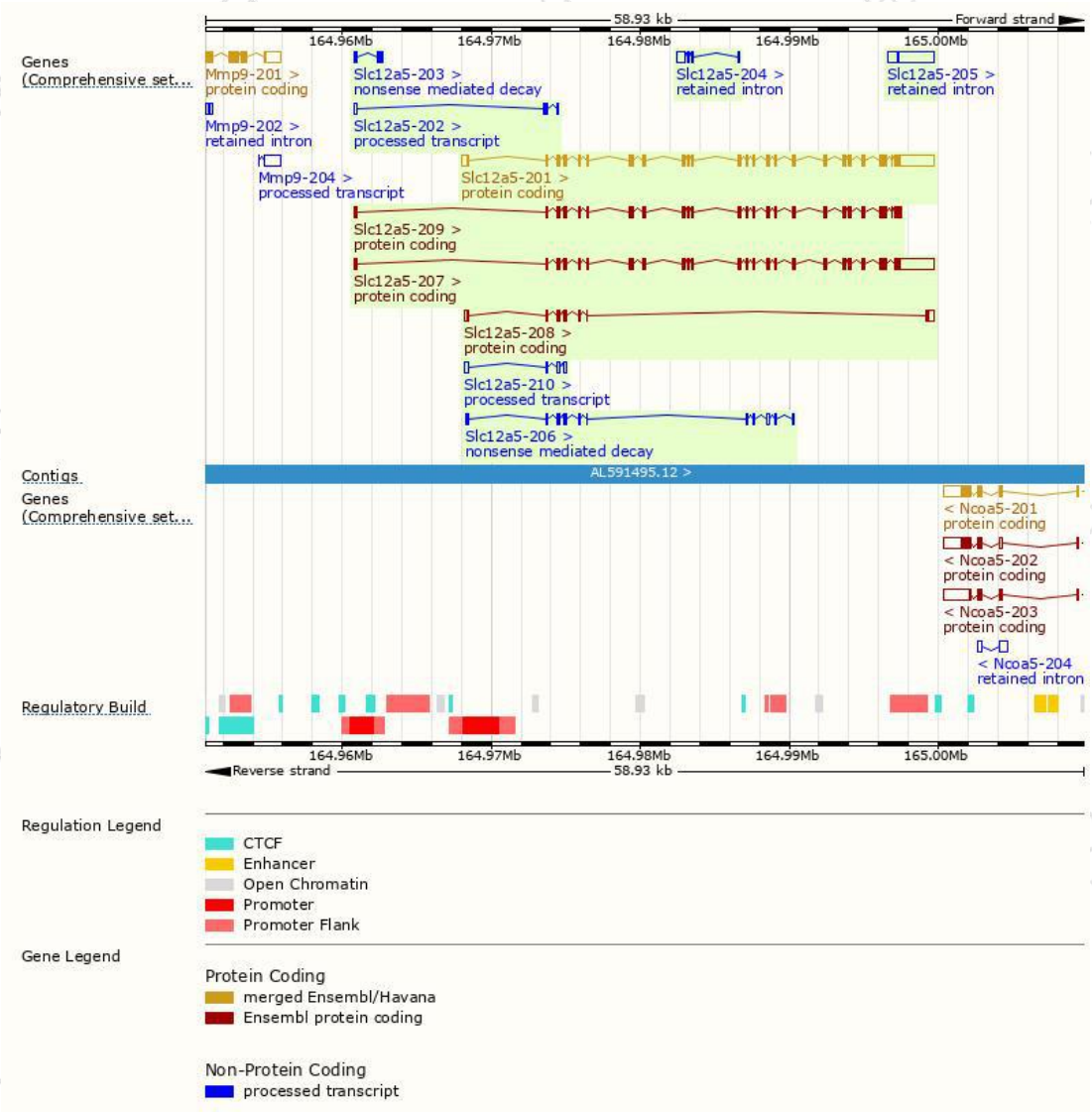
The gene has 10 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|---|---|---|---|---|---|---|---|
| **Slc12a5-201** | ENSMUST00000099092.7 | 6028 | 1115aa | Protein coding | CCDS38332 | A0A076FSX1 Q91V14 | TSL:1 GENCODE basic APPRIS P2 |
| **Slc12a5-207** | ENSMUST00000202223.3 | 5690 | 1133aa | Protein coding | - | A0A076FR46 | TSL:1 GENCODE basic |
| **Slc12a5-209** | ENSMUST00000202623.3 | 3574 | 1138aa | Protein coding | - | A0A076FRG6 Q91V14 | TSL:1 GENCODE basic APPRIS ALT2 |
| **Slc12a5-208** | ENSMUST00000202479.3 | 1202 | 231aa | Protein coding | - | A0A0J9YV84 | TSL:5 GENCODE basic |
| **Slc12a5-206** | ENSMUST00000202136.1 | 1106 | 186aa | Nonsense mediated decay | - | A0A0J9YU26 | TSL:5 |
| **Slc12a5-203** | ENSMUST00000125867.1 | 422 | 59aa | Nonsense mediated decay | - | A0A0J9YUW1 | TSL:3 |
| **Slc12a5-210** | ENSMUST00000208579.1 | 586 | No protein | Processed transcript | - | - | TSL:5 |
| **Slc12a5-202** | ENSMUST00000124372.5 | 465 | No protein | Processed transcript | - | - | TSL:2 |
| **Slc12a5-205** | ENSMUST00000137302.2 | 2996 | No protein | Retained intron | - | - | TSL:1 |
| **Slc12a5-204** | ENSMUST00000135918.1 | 744 | No protein | Retained intron | - | - | TSL:3 |

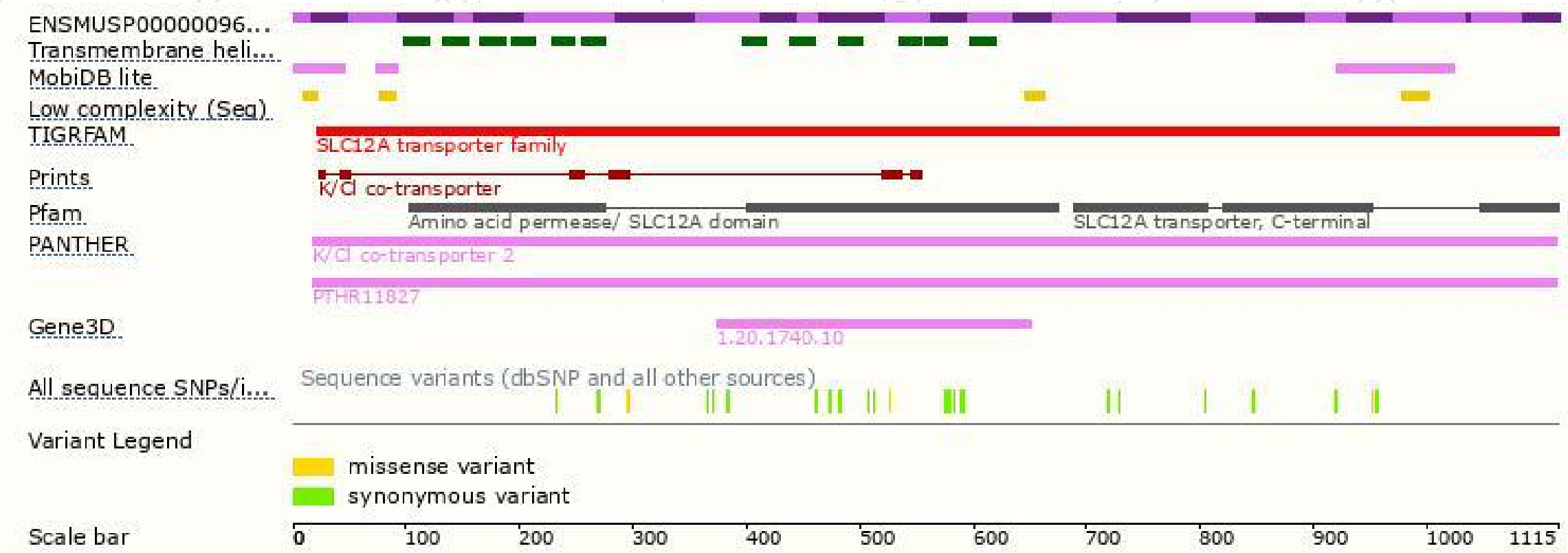
The strategy is based on the design of *Slc12a5-201* transcript,the transcription is shown below:



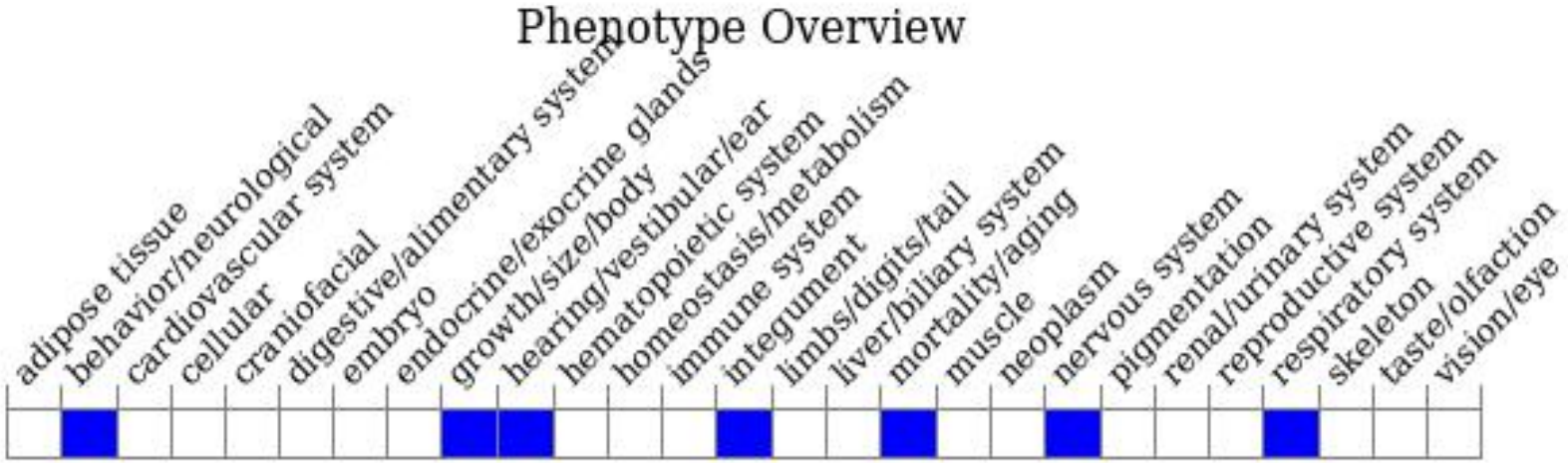
# Genomic location distribution



# 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 disruptions in this gene die within a few minutes of birth of respiratory failure resulting from a motor nerve defect. Mice homozygous for a hypomorphic allele display postnatal lethality and tonic-clonic seizures.

If you have any questions, you are welcome to inquire.

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