DreERT2-P2A-EGFP-T2A-Slc12a1
Cas9-KI Strategy

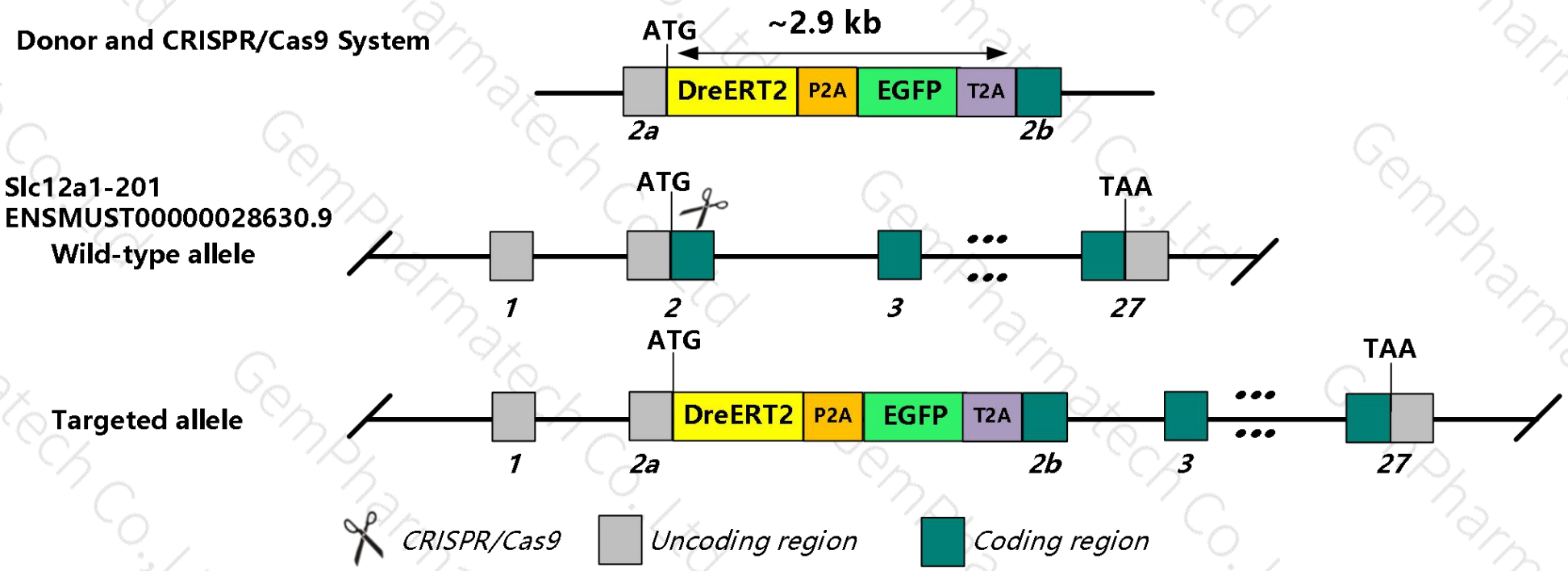
Designer:	**Yanhua Shen**
Reviwer	**Jia Yu**
Design Date:	**2021-11-05**

Project Overview

Project Name	***DreERT2-P2A-EGFP-T2A-Slc12a1***
Project type	**Cas9-KI**
Strain background	**C57BL/6JGpt**

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Slc12a1* gene. The schematic diagram is as follows:



Technical routes

- The *Slc12a1* gene has 4 transcripts. According to structure of *Slc12a1* gene, *Slc12a1-201*(ENSMUST00000028630.9) is selected for presentation of the recommended strategy.

- *Slc12a1-201* gene has 27 exons, with the ATG start codon in exon2 and TAA stop codon in exon27.

- We make *DreERT2-P2A-EGFP-T2A-Slc12a1* knockin mice via CRISPR/Cas9 system. CRISPR/Cas9 system and donor will be co-injected into zygotes. Cas9 endonuclease cleavage near start codon(ATG) of exon2 of *Slc12a1* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in *DreERT2-P2A-EGFP-T2A* after start coding(ATG) of *Slc12a1* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the existing MGI data, mice homozygous for disruptions in this gene do not survive to weaning and suffer from various metabolic abnormalities related to kidney function. Mice homozygous for an ENU-induced allele exhibit kidney disease, impaired urinary excretion of metabolism products, polyuria, and kidney alterations.
- The P2A/T2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of 2A expressing gene carries the 2A-translated polypeptide.
- Insertion of *DreERT2-P2A-EGFP-T2A* may affect the regulation of the 5' end of the *Slc12a1* gene.
- There may be 1 to 2 amino acid synonymous mutation in exon2 of *Slc12a1* gene in this strategy.
- The *Slc12a1* gene is located on the Chr2. If the knockin mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene after the gene coding region may affect the expression of endogenous and foreign genes. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Gene information (NCBI)

集萃药康 GemPharmatech

Slc12a1 solute carrier family 12, member 1 [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 20495, updated on 21-Oct-2021

Summary

Official Symbol	Slc12a1 provided by MGI
Official Full Name	solute carrier family 12, member 1 provided by MGI
Primary source	MGI:MGI:103150
See related	Ensembl:ENSMUSG00000027202
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	Nkcc2; mBSC1; urehr3; AI788571; D630042G03Rik
Expression	Restricted expression toward kidney adult (RPKM 70.5) See more
Orthologs	human all

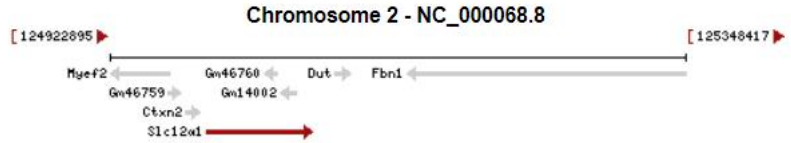
NEW **Try the new Gene table**
Try the new Transcript table

Genomic context

Location: 2 F1; 2 61.23 cM

See Slc12a1 in Genome Data Viewer

Exon count: 29

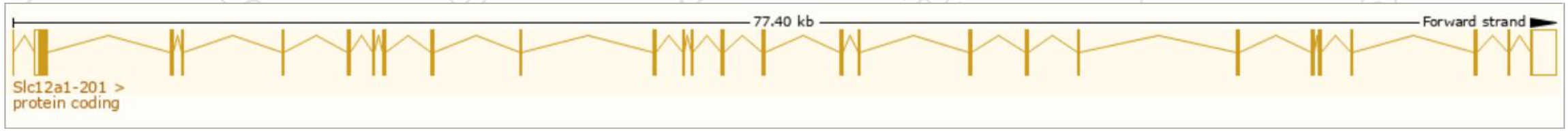


Transcript information （Ensembl）

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

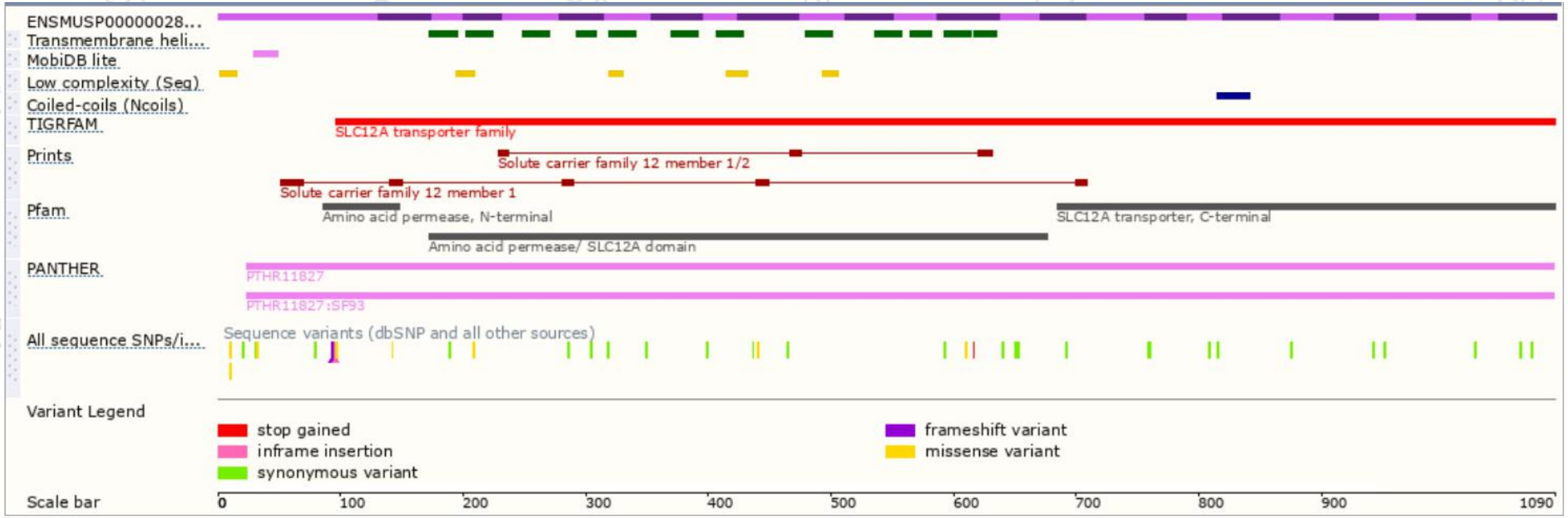
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Slc12a1-201	ENSMUST00000028630.9	4645	1090aa	Protein coding	CCDS50693	A2AQ50	GENCODE basic APPRIS P5 TSL:1
Slc12a1-202	ENSMUST00000110494.9	4740	1090aa	Protein coding	-	A2AQ52	GENCODE basic APPRIS ALT2 TSL:5
Slc12a1-203	ENSMUST00000110495.3	4642	1090aa	Protein coding	CCDS50694	A2AQ51	GENCODE basic APPRIS ALT2 TSL:5
Slc12a1-204	ENSMUST00000147095.2	1876	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Slc12a1-201* transcript,the transcription is shown below

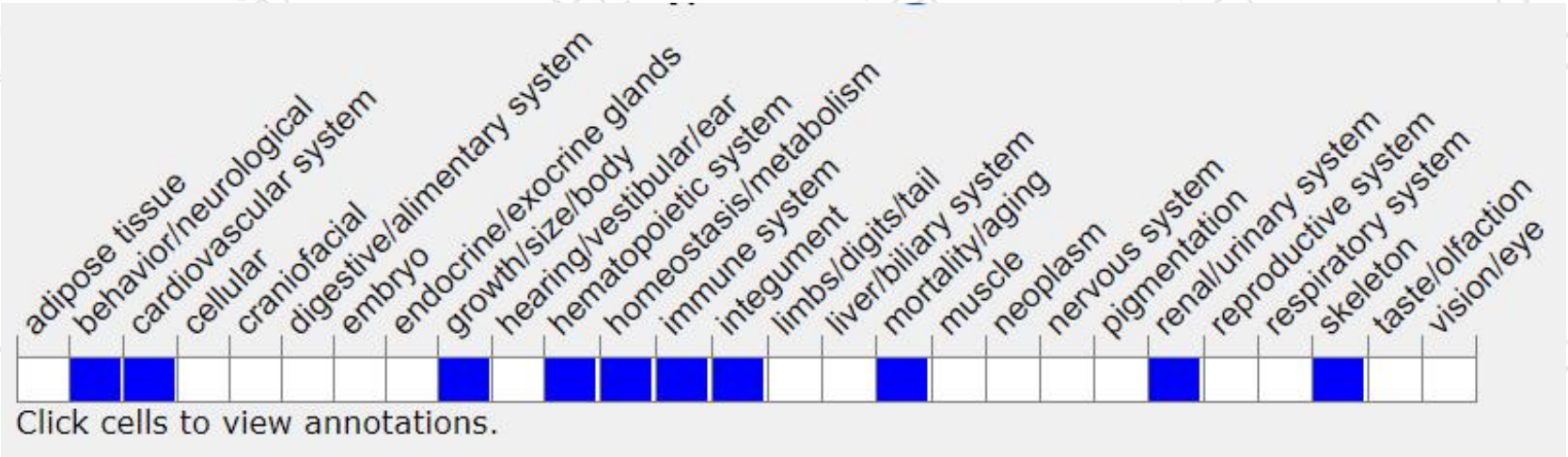


Genomic location distribution

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/marker/MGI:103150) .

Mice homozygous for disruptions in this gene do not survive to weaning and suffer from various metabolic abnormalities related to kidney function. Mice homozygous for an ENU-induced allele exhibit kidney disease, impaired urinary excretion of metabolism products, polyuria, and kidney alterations.

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

Tel: 025-5864 1534

集萃药康生物科技

GemPharmatech Co.,Ltd