# Slc12a4-T926A&T983A Mouse Model Strategy -CRISPR/Cas9 technology

Designer: Xiaojing Li

**Reviewer: Xueting Zhang** 

**Design Date: 2020-11-5** 

# **Project Overview**



**Project Name** 

Slc12a4-T926A&T983A

**Project type** 

cas9-ki(PM)

Strain background

C57BL/6JGpt

### **Technical Description**



- The mouse *Slc12a4* gene has 5 transcripts. The human *SLC12A4* gene has 19 transcripts.
- According to the structure of *Slc12a4* gene and requirements of customer,the 926th and 983th amino acid(T) of human *SLC12A4* gene corresponds to the 926th and 983th amino acid(T) of mouse *Slc12a4* gene after comparing homology of mouse *Slc12a4* gene and human *SLC12A4* gene. This project produced *Slc12a4*-T926A&T983A mutation on exon21&exon22 of the transcript of *Slc12a4*-202(ENSMUST00000116429.8,NM\_009195.3). The 926th and 983th amino acids will be mutated from T to A, and the corresponding codon will be mutated to GCC by the ACA and ACG.
- The mouse *Slc12a4-202* transcript contains 24 exons. The translation initiation site ATG is located at exon1, and the translation termination site TGA is located at exon24, encoding 1085aa.
- In this project, *Slc12a4* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: In vitro, sgRNA and donor vectors were constructed. Cas9, sgRNA and donor were injected into the fertilized eggs of C57BL/6JGpt mice for homologous recombination, and obtained positive F0 mice identified by PCR and sequencing analysis. The stable inheritable positive F1 mice model was obtained by mating F0 mice with C57BL/6JGpt mice.





3. 76		Con .	Thr9	26				Thr983	9X
	904	910	920	930 940	950	960	970	980	990
luman SLC12a4-201 protei Mouse Slc12a4-202 protei	*******		DISAYTYERTLMM DISAYTYERTLMM	MEQRSQMLRQMRLTKTE MEQRSQMLRQMRLTKTE	ER <mark>E</mark> REAQLVE ER <b>D</b> REAQLVE	TDRHSALRLESLYS TDRHSALRLESLYS	SDEE <mark>DES</mark> AV SDEE <mark>E</mark> ESVA		KYMTETWDPSH KYMAEPWDPSH
Consensu	s 904	LEAEVEVVEMHNS	DISAYTYERTLM	MEQRSQMLRQMRLTKTE	RDREAQLVE	MORHSALRLESLYS	SDEEDES	GADKIQMTWTRD	KYM E WDPSH
Ready		~	c	onsensus positions: 97	7.7% identi	ty positions: 96.29	6 aln: 904		

The 926th and 983th amino acid(T) of human *SLC12A4* gene corresponds to the 926th and 983th amino acid(T) of mouse *Slc12a4* gene after comparing homology of mouse *Slc12a4* gene and human *SLC12A4* gene.

### **Mutation Site: T926A**



#### **Before mutation**

+3	H	ı N	s	D	I	S	Α	Υ	Т	Υ	E	R	Т	L	M	М	E	Q	R	s	Q	м	L	R	Q	М	В	L	Т	K	Т	E R	?
22801	AGC	CAAC	AG	TGAC	ATCT	CT	GCC	TAT	ACCT	AT	GAG	CGGA	CZ	CTG	ATGA	ATG	GAG	CAG	CGGT	CT	CAG	ATGO	T	GCGG	CAG	ATG	AGG	TTG	ACCA	AA	ACTO	AGC	G
	TCG	GTT	STC	ACTG	TAGA	GA	CGG	ATA	TGGA	TA	CTC	GCCT	G I	GAC'	TACT	CAC	CTC	GTC	GCCA	GA	GTC:	TACG	A (	CGCC	GTC	TAC	TCC	AAC	TGGT	TT	TGAC	TCG	C
+3	?R D	R	Ε																														
22901	GGA:	CGA	GAG	GTAA	ATGG	CC	ATC:	TTG	GCTT	GG	GCT	TGGG	T A	AGG	GCCI	ACA	TCA	TGC	CTTT	GT	CTG	GTGG	A T	TATA	ACA	ATG	AAT	GAT	TCAG	GA	CTAG	GCT	T
	CCT	AGCT	CTC	CATT	TACC	GG	TAG	AAC	CGAA	CC	CGA	ACCC	AI	TCC	CGGI	GT	AGT	ACG	GAAA	CA	GAC	CACC	T	TATA	TGT	TAC	TTA	CTA	AGTO	CT	GATO	CGA	A

#### After mutation

											. 47											3 707									
+3	Н	N	s	D	1	S	Α	Υ	Т	Y I	E F	3 /	A L	M	M	Е	Q	R	s	Q	М	L	R	Q	М	R	L	Т	K	Т	E R
22801	AGCA	CAAC	AG '	TGAC.	ATCT	CT	GCCT	TATA	CCT	ATG	AGC	GGC	CCT	GATG	ATG	GAG	CAG	CGGT	CTC	AGI	TGC	T G	GGC	CAGA	ATG	AGG	TTG	ACCA	AA	ACT	SAGCO
	TCGT	GTTG:	TC .	ACTG	TAGA	GA	CGGI	TAT	GGA	TACT	rcg	CCCG	GGA	CTAC	TAC	CTC	GTC	GCCA	GAG	TCI	TACG	A C	CCC	STCI	CAC	TCC.	AAC	rggt	TT	TGA	CTCGC
+3	?R D	R	E																												
-3 22901	?R D			GTAA	ATGG	CC	ATC	TGG	CTT	GGGG	CTT	GGT	AAG	GGCC	ACA	TCA'	TGCC	CTTT	GTO	TGG	TGG	A TA	TAA	ACAZ	TG	AAT	GAT	CAG	GA	CTAC	GCTI
TE EVENE LI		CGAG	AG																									and the	10000		

The yellow region is exon 21 of Slc12a4-202, the red region is mutation site:c.ACA>GCC;p.T>A.

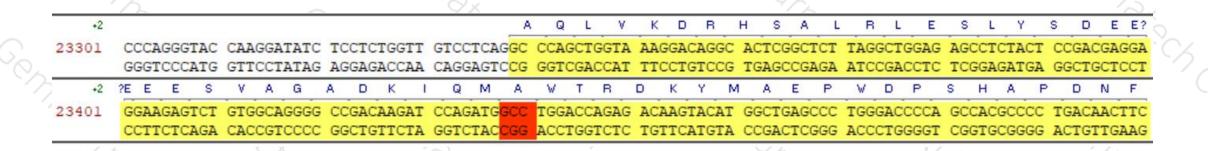
### **Mutation Site: T983A**



#### **Before mutation**

+2				<u> </u>										Α	Q	L	٧	K	D	R	Н	S	Α	L	R	L	Е	s	L	Y	5	D	Е	E?
23301	CC	CA	GGT	AC	CAA	GGA	TATC	TC	CTC	TGG	ТТ	GTCC	TCA	GGC	CCA	GCT(	GGTA	AA	GGA	CAGG	C I	ACTC	GGCI	CT	TAG	GCT	GGAG	AG	CCT	CTAC	T	CCGA	CGA	GGA
	GG	GT(	CCC	TG	GTT	CCT	ATAG	AG	GAG.	ACC	AA	CAGG	AGT	CCG	GGT	CGA	CCAT	TT	CCT	STCC	G 1	rgag	CCGI	GA	ATC	CGA	CCTC	TO	GGA	GAT	5A	GGCT	GCT	CCT
+2	?E	Е	E	s	٧	Α	G	Α	D	K	- 1	Q	М	Т	W	Т	R	D	K	Υ	М	Α	Е	Р	W	D	Р	s	Н	Α	Р	D	N	F
23401	GG	AA	GAGT	CT	GTG	GCA	GGGG	CC	GAC	AAG	AT	CCAG	ATG	ACG	TGG	ACC	AGAG	AC	AAGI	TACA	T	GCT	GAGO	CC	TGG	GAC	CCCI	GC	CAC	GCC	CC '	TGAC	AAC	TTC
	CC	TT	CTC	GA	CAC	CGT	cccc	GG	CTG	TTC	ra.	GGTC	TAC	TGC	ACC:	rgg:	CTC	TG	TTC	ATGT	A C	CCGA	CTCG	GG	ACC	CTG	GGGI	CG	GTG	CGGG	G .	ACTG	TTG.	AAG

#### After mutation

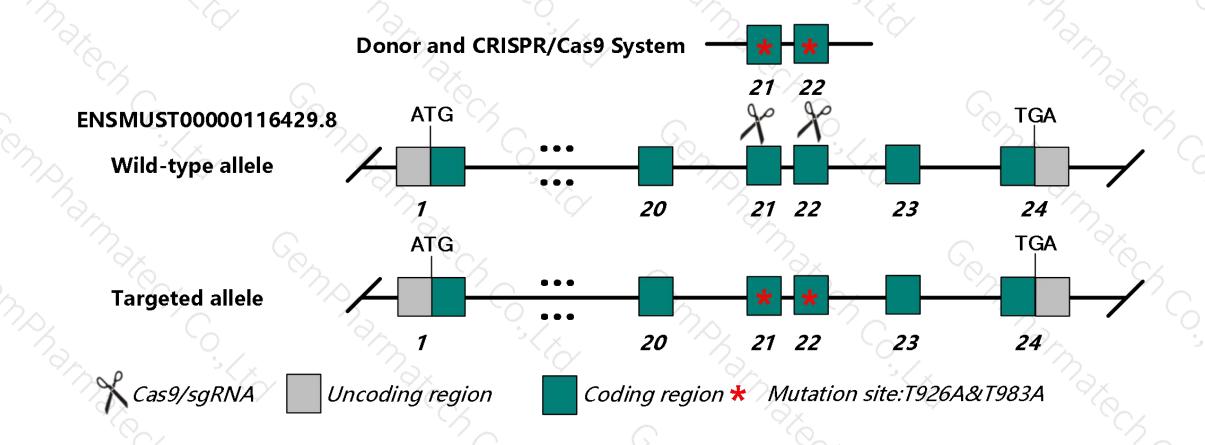


The yellow region is exon 22 of Slc12a4-202, the red region is mutation site:c.ACG>GCC;p.T>A.

# Strategy



This model uses CRISPR/Cas9 technology to edit the *Slc12a4* gene and the schematic diagram is as follow:



#### **Notice**



- According to the data of MGI, mice homozygous for a constitutively active mutation display microcytosis and hypochromic anemia.
- ➤ One or two synonymous mutations of amino acids will be intronduced on exon21 and exon22 of *Slc12a4*.
- ➤ The effect on the transcript-203 is unknown.
- > The mutation site is about 1.2kb away from the 5-terminal of *Lcat*-201, which may affect its 5-terminal regulatory function.
- $\triangleright$  The mutation site is overlapped with the sequence of the Gm16156 gene, the effect on the Gm16156 gene is unknown.
- ➤ Mouse *Slc12a4* gene is located on Chr8. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr8, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

# Gene name and location (NCBI)



#### Slc12a4 solute carrier family 12, member 4 [ Mus musculus (house mouse) ]

Gene ID: 20498, updated on 10-Oct-2020

#### Summary

**☆** 

Official Symbol Slc12a4 provided by MGI

Official Full Name solute carrier family 12, member 4 provided by MGI

Primary source MGI:MGI:1309465

See related Ensembl:ENSMUSG00000017765

Gene type protein coding
RefSeq status VALIDATED
Organism Mus musculus

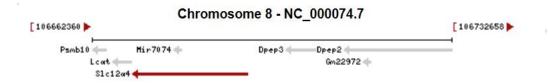
Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;

Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus

Also known as KCC1; RBCKCC1; AW546649

Expression Ubiquitous expression in ovary adult (RPKM 36.4), lung adult (RPKM 32.8) and 28 other tissues See more

Orthologs human all



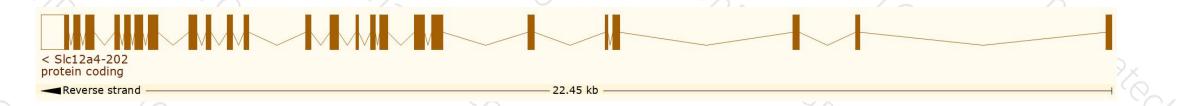
# Transcript information (Ensembl)



The gene has 5 transcripts, and all transcripts are shown below:

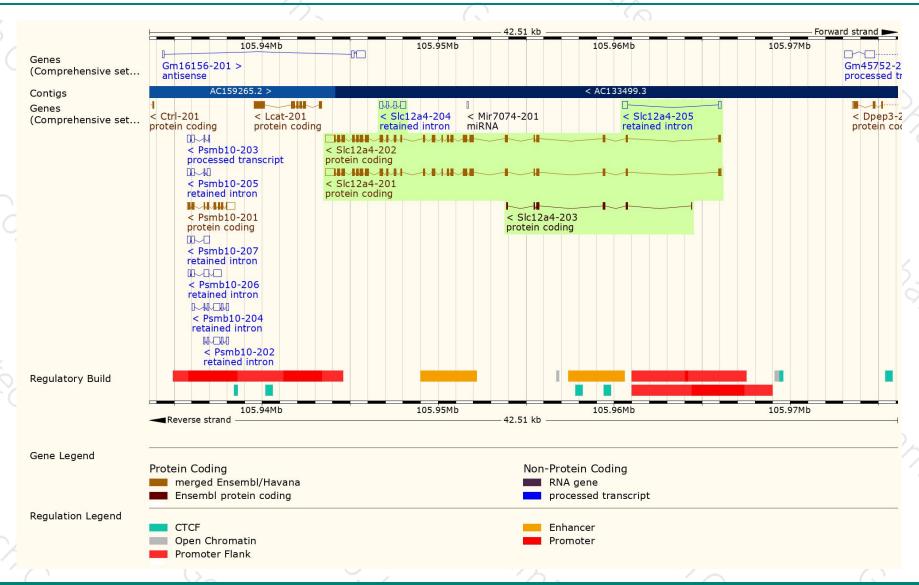
Name 🔺	Transcript ID	bp 🛊	Protein	Biotype 🍦	CCDS 🍦	UniProt	Flags
SIc12a4-201	ENSMUST00000034370.16	3811	1087aa	Protein coding	CCDS57642译	F8WIJ0r	TSL:1 GENCODE basic APPRIS ALT1
SIc12a4-202	ENSMUST00000116429.8	3755	<u>1085aa</u>	Protein coding	CCDS22623译	Q3TWZ6ॡ Q9JIS8ॡ	TSL:1 GENCODE basic APPRIS P3
SIc12a4-203	ENSMUST00000132231.1	512	<u>171aa</u>	Protein coding	-	F6TQE2₺	CDS 5' and 3' incomplete TSL:3
SIc12a4-204	ENSMUST00000141326.1	715	No protein	Retained intron		50)	TSL:3
SIc12a4-205	ENSMUST00000143381.1	461	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Slc12a4-202* transcript, the transcription is shown below:



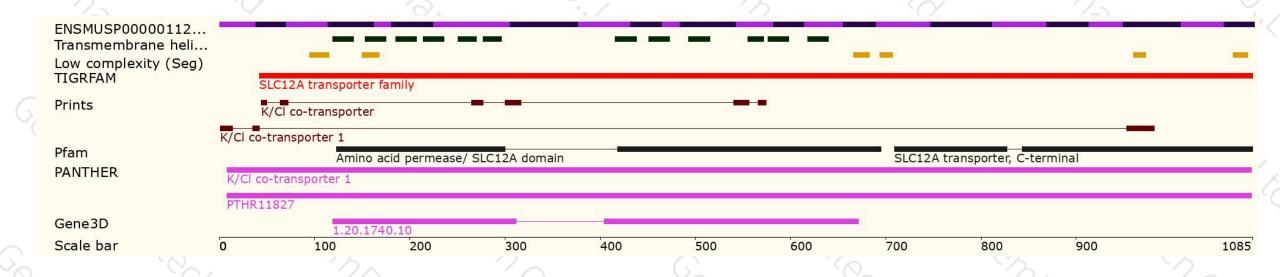
### Genomic location distribution





### Protein domain



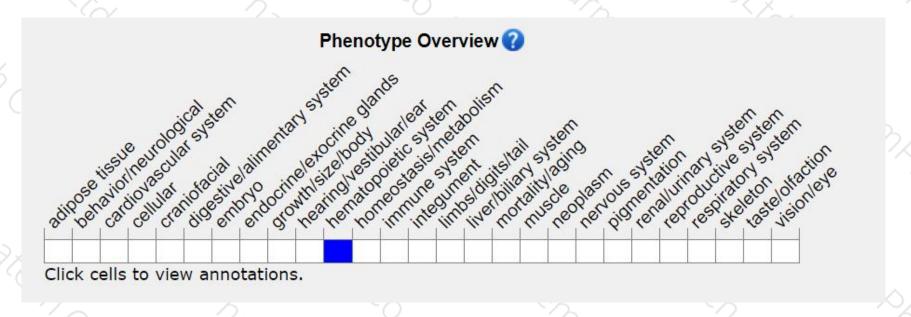


### Mouse phenotype description(MGI)



URL link is as follows:

http://www.informatics.jax.org/marker/MGI:1309465



Mice homozygous for a constitutively active mutation display microcytosis and hypochromic anemia.

If you have any questions, please feel free to contact us. Tel: 025-5864 1534





