Slc12a4-T926A&T983A Mouse Model Strategy

-CRISPR/Cas9 technology

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Reviewer: Xueting Zhang

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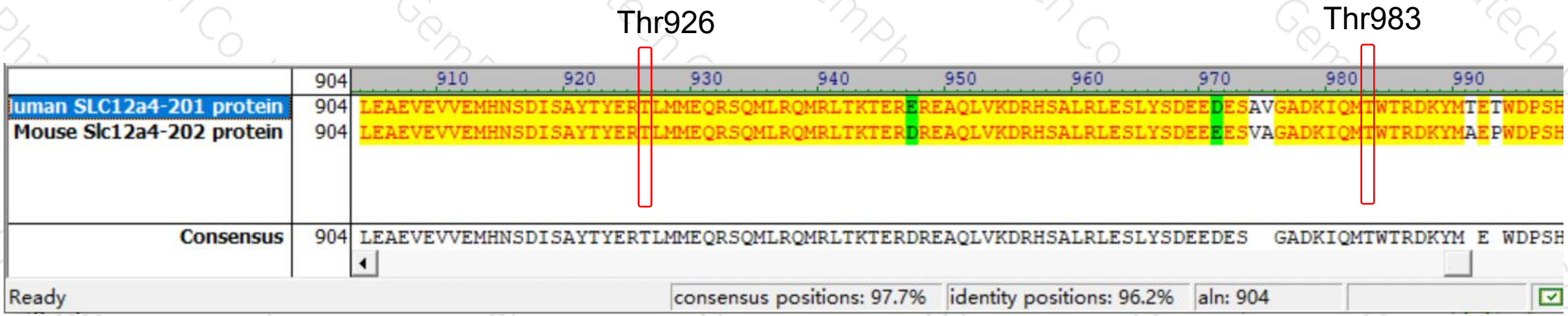
Project Overview

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.

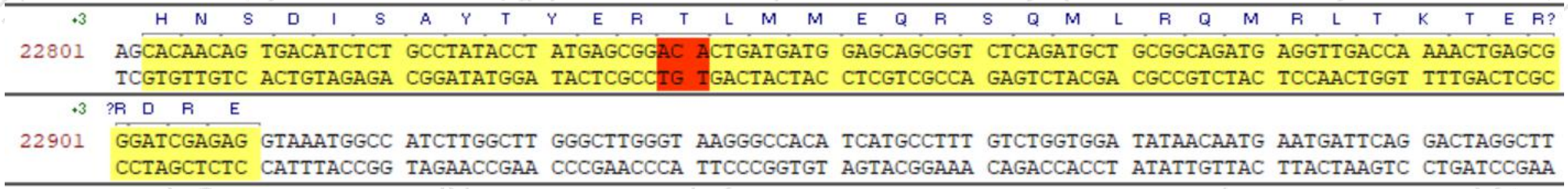
A comparison of the aa homology of human and mouse *Slc12a4* gene



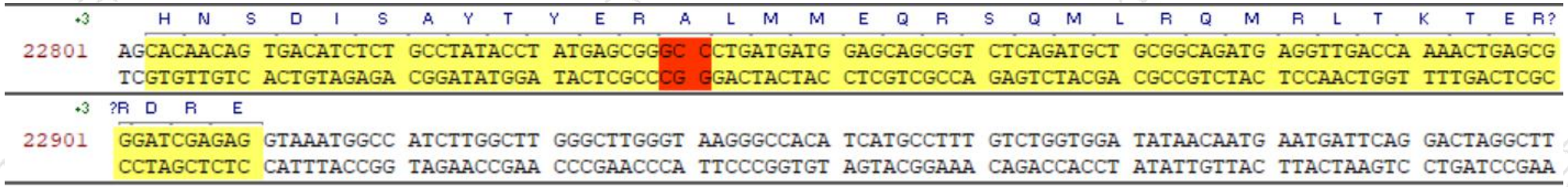
The926th and 983th amino acid(T) of human *SLC12A4* gene corresponds to the 926th and 983th amino acid(T) of mouse *Slc12a4* gene after comparing homology of mouse *Slc12a4* gene and human *SLC12A4* gene.

Mutation Site:T926A

Before mutation



After mutation



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

Mutation Site:T983A

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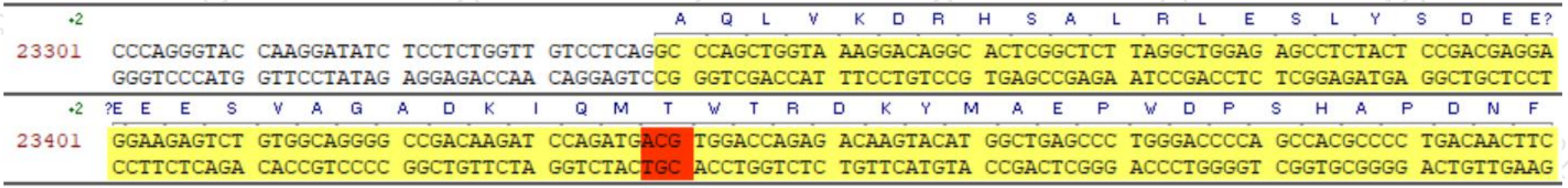
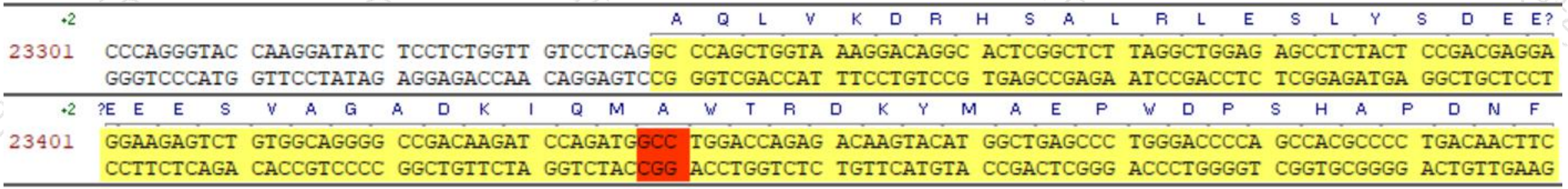
Before mutation



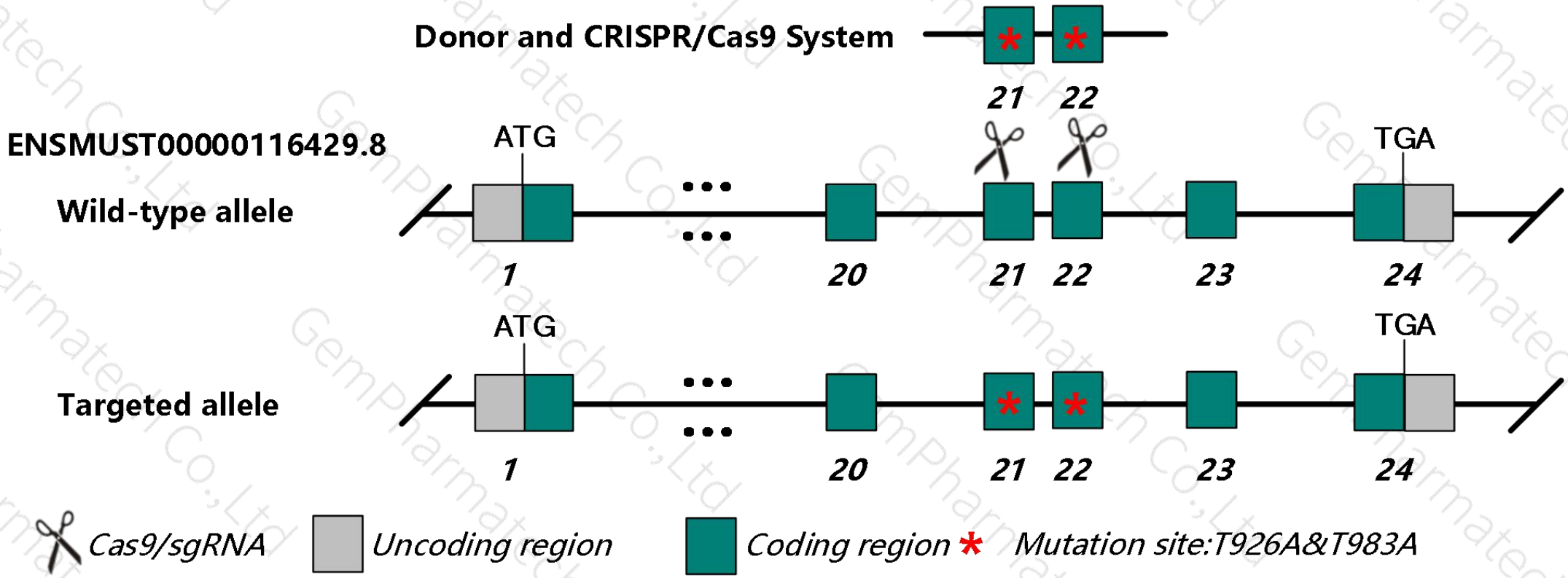
After mutation



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.
- 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.

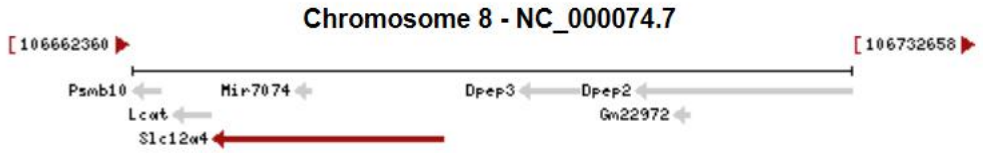
Gene name and location （NCBI)

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

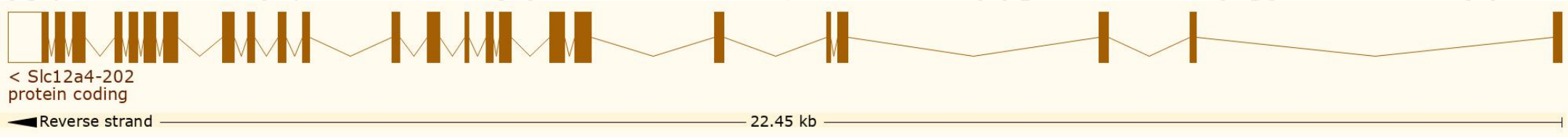


Transcript information （Ensembl）

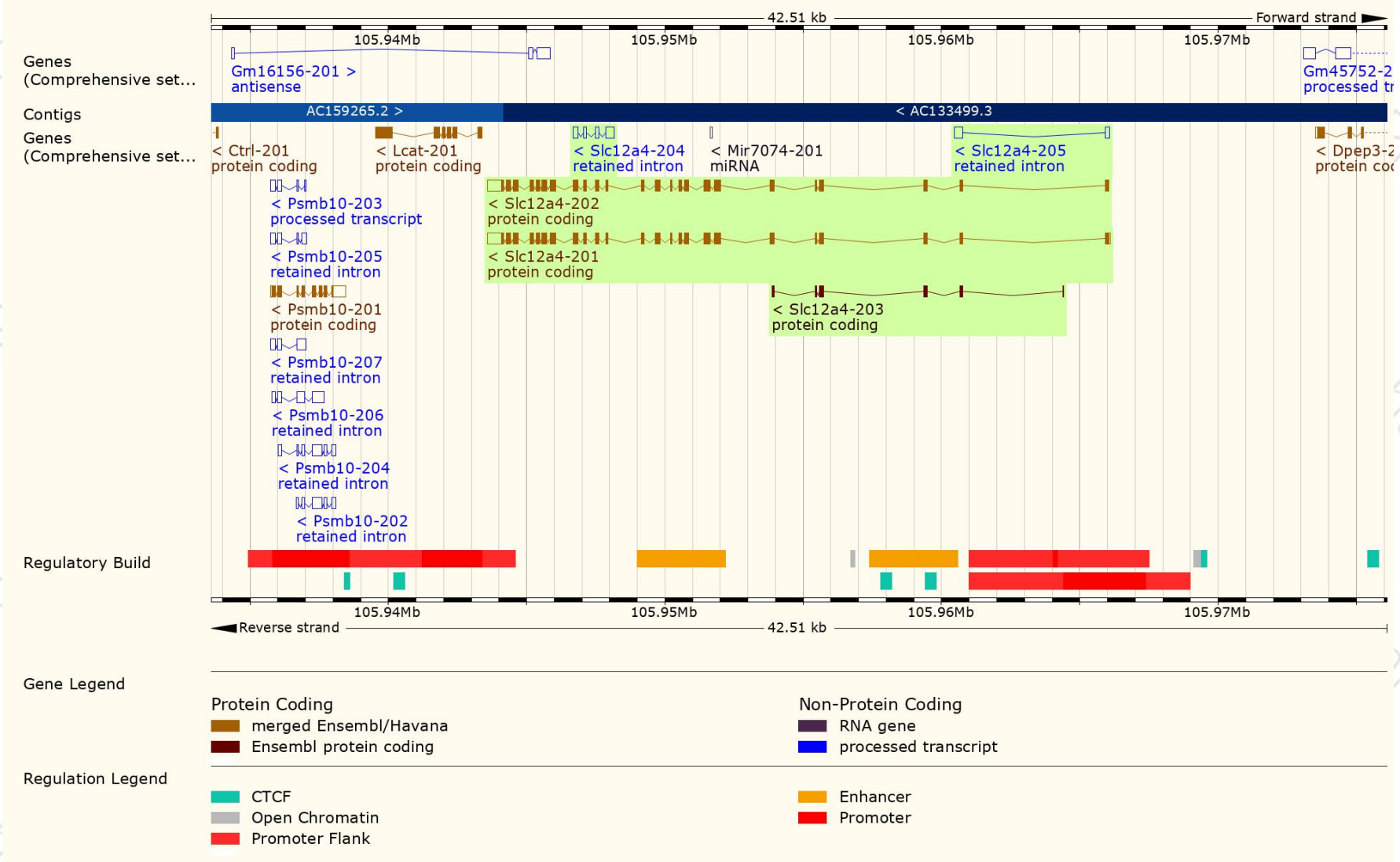
The gene has 5 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Slc12a4-201	ENSMUST00000034370.16	3811	1087aa	Protein coding	CCDS57642	F8WIJ0	TSL:1 GENCODE basic APPRIS ALT1
Slc12a4-202	ENSMUST00000116429.8	3755	1085aa	Protein coding	CCDS22623	Q3TWZ6 Q9JIS8	TSL:1 GENCODE basic APPRIS P3
Slc12a4-203	ENSMUST00000132231.1	512	171aa	Protein coding	-	F6TQE2	CDS 5' and 3' incomplete TSL:3
Slc12a4-204	ENSMUST00000141326.1	715	No protein	Retained intron	-	-	TSL:3
Slc12a4-205	ENSMUST00000143381.1	461	No protein	Retained intron	-	-	TSL:2

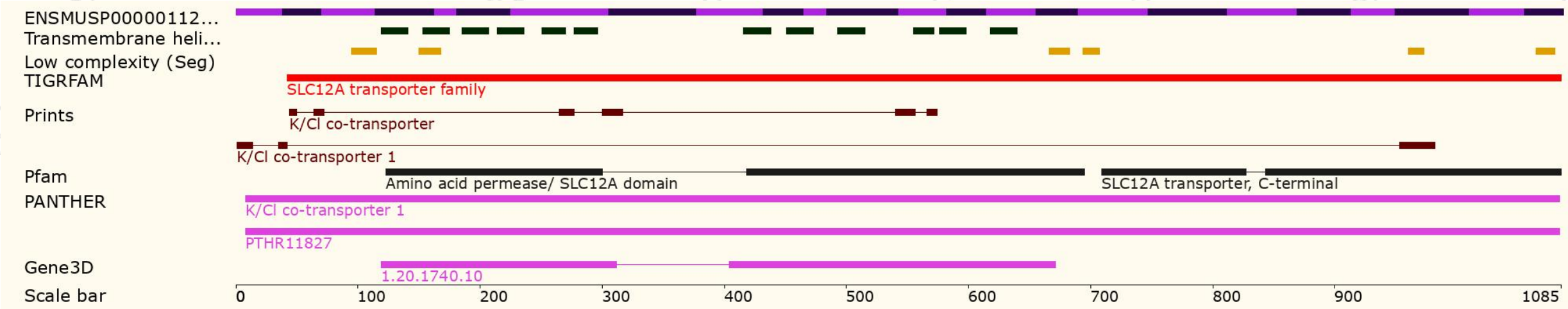
The strategy is based on the design of *Slc12a4-202* transcript, the transcription is shown below:



Genomic location distribution

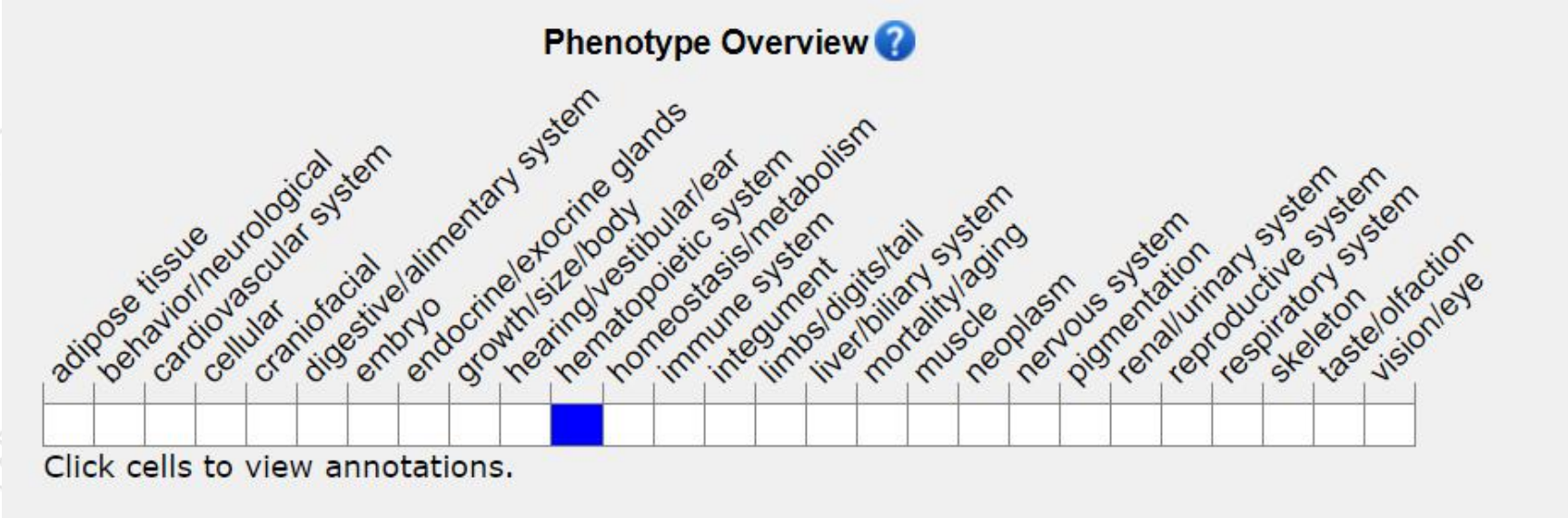


Protein domain



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.
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