Slc12a2-T197A&T201A&T206A Mouse Model Strategy

-CRISPR/Cas9 technology

Designer: Daohua Xu

Reviewer: Jia Yu

Design Date: 2020-12-8

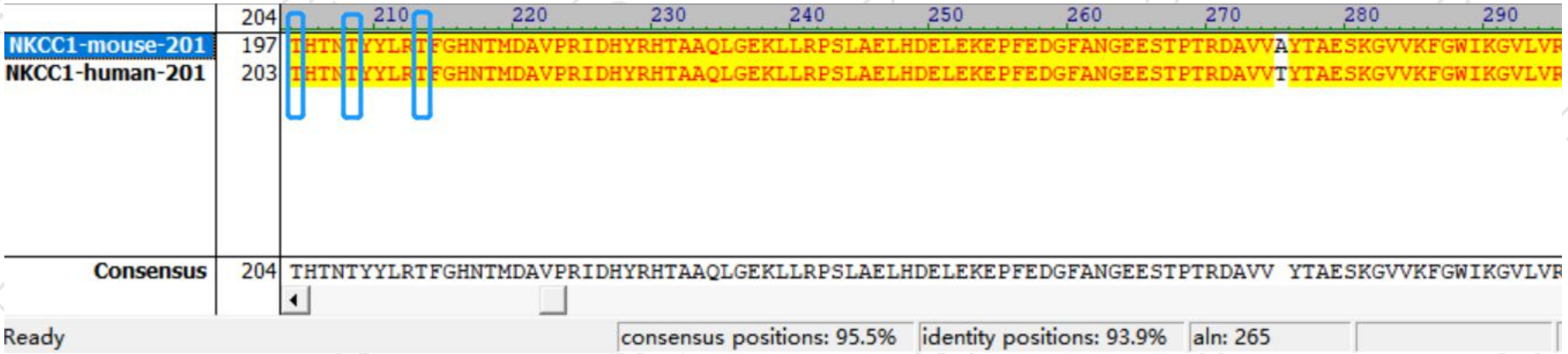
Project Overview

Project Name	**Slc12a2-T197A&T201A&T206A**
Project type	**cas9-ki(PM)**
Strain background	**C57BL/6JGpt**

Technical Description

- The mouse *Slc12a2* gene has 2 transcripts.
- This project produced *Slc12a2-T197A&T201A&T206A* point mutation on exon1 of the transcript of *Slc12a2-201*(ENSMUST00000115366.2). The 197th,201th and 206th amino acid will be mutated from T to A.
- In this project, *Slc12a2* 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.

Analysis of Homology

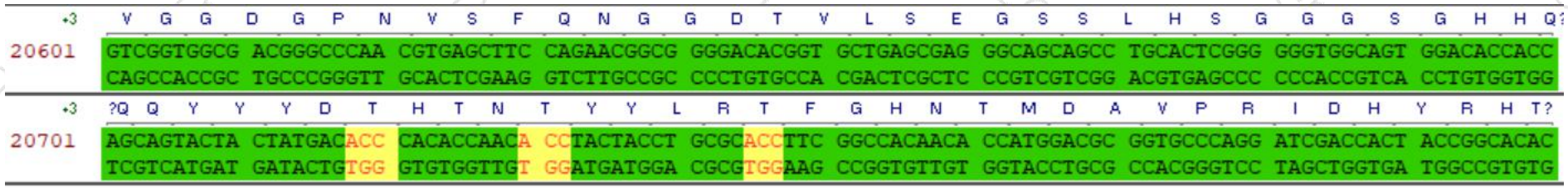


Identity positions：93.9%

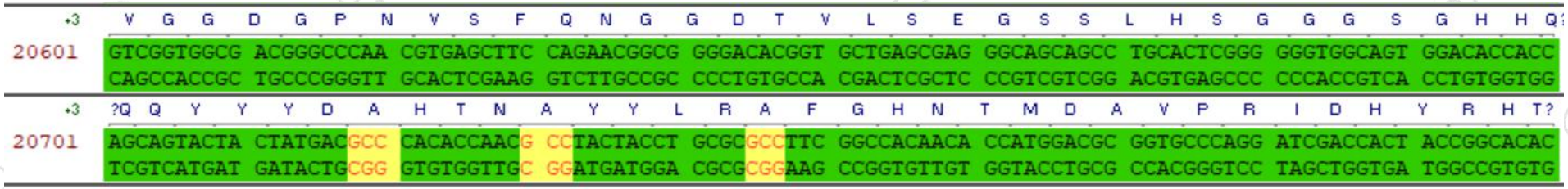
Mutation Site

集萃药康 GemPharmatech

Before mutation



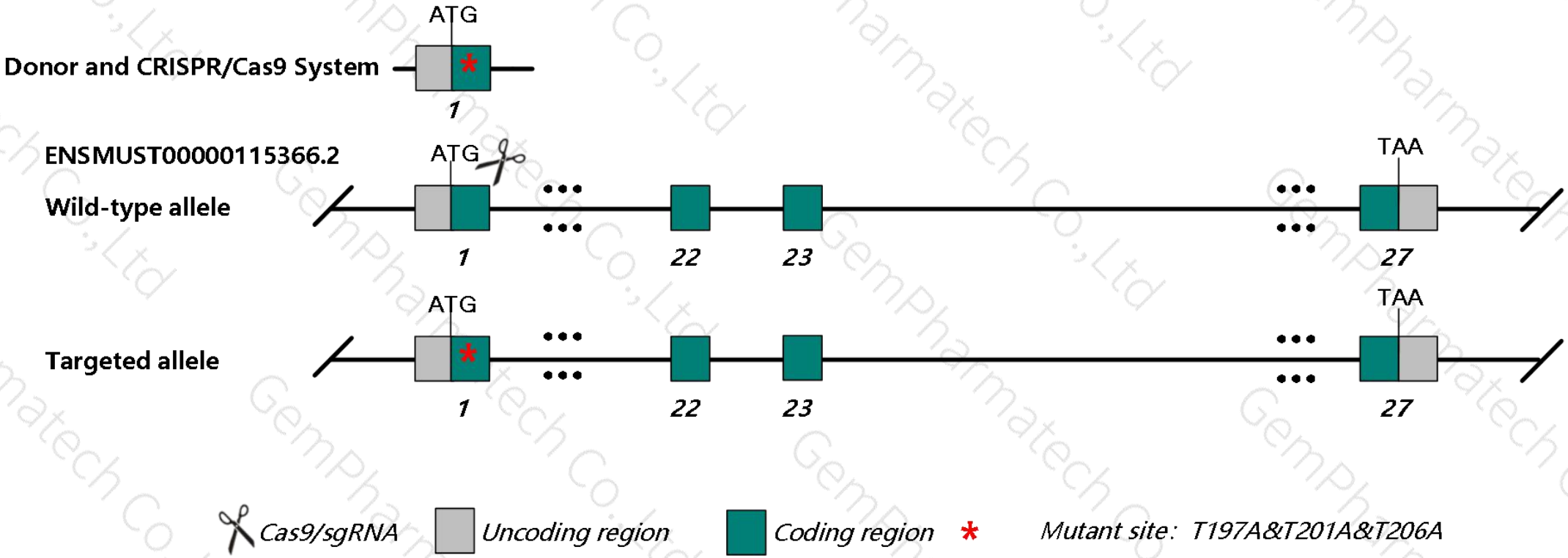
After mutation



The green region is exon1 of *Slc12a2-201,* and the yellow region represents the p.T197A&T201A&T206A mutation site.

Strategy

This model uses CRISPR/Cas9 technology to edit the *Slc12a2* gene and the schematic diagram is as follow:



Notice

- According to the data of MGI,homozygous mutants show variably severe deafness, head-shaking, circling, reduced endolymph secretion, male sterility, growth retardation, hypotension, reduced salivation, delayed ductal outgrowth of mammary epithelium and increased periweaning mortality.
- Two to four synonymous mutations of amino acids would be intronduced on exon1 of *Slc12a2*.
- Mouse *Slc12a2* gene is located on Chr18. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr18, 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)

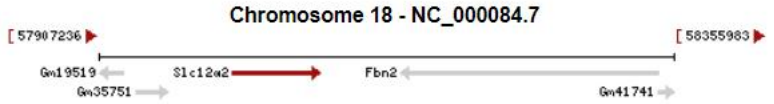
集萃药康 GemPharmatech

Slc12a2 solute carrier family 12, member 2 [*Mus musculus* (house mouse)]

Gene ID: 20496, updated on 22-Nov-2020

Summary

Official Symbol	Slc12a2 provided by MGI
Official Full Name	solute carrier family 12, member 2 provided by MGI
Primary source	MGI:MGI:101924
See related	Ensembl:ENSMUSG00000024597
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	sy; Nkcc; mBSC; Nkcc1; mBSC2; sy-ns; 9330166H04R; 9330166H04Rik
Expression	Broad expression in mammary gland adult (RPKM 43.3), colon adult (RPKM 19.3) and 20 other tissues See more
Orthologs	human all

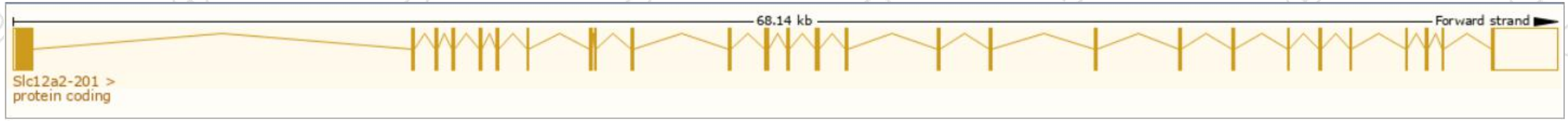


Transcript information （Ensembl）

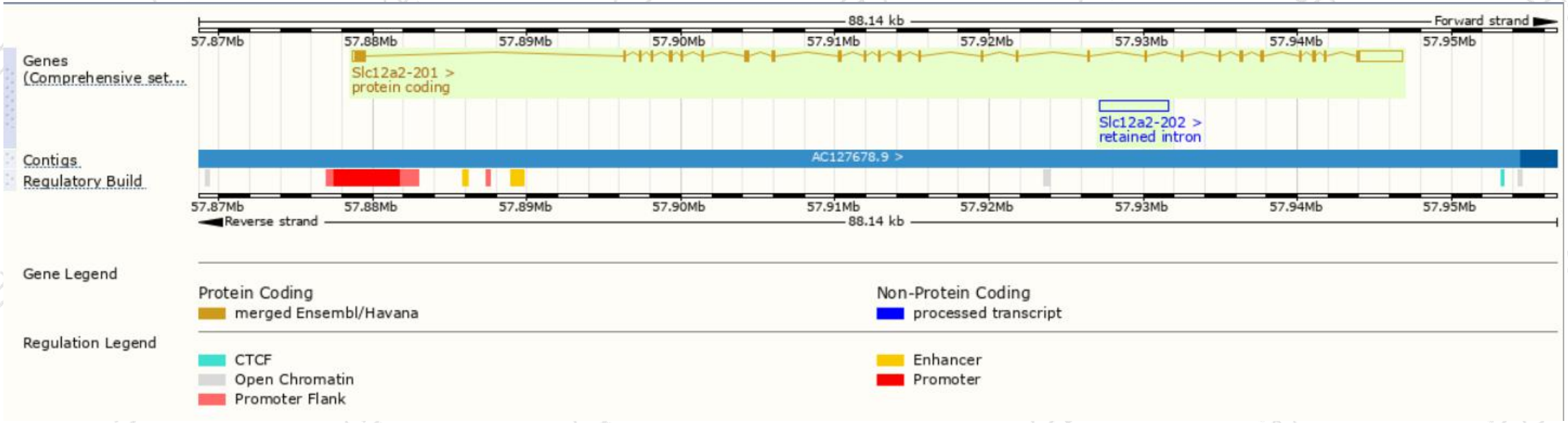
The gene has 2 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Slc12a2-201	ENSMUST00000115366.2	6520	1206aa	Protein coding	CCDS37826	E9QM38	TSL:1 GENCODE basic APPRIS P1
Slc12a2-202	ENSMUST00000236673.1	4480	No protein	Retained intron	-	-	-

The strategy is based on the design of *Slc12a2-201* transcript, the transcription is shown below:



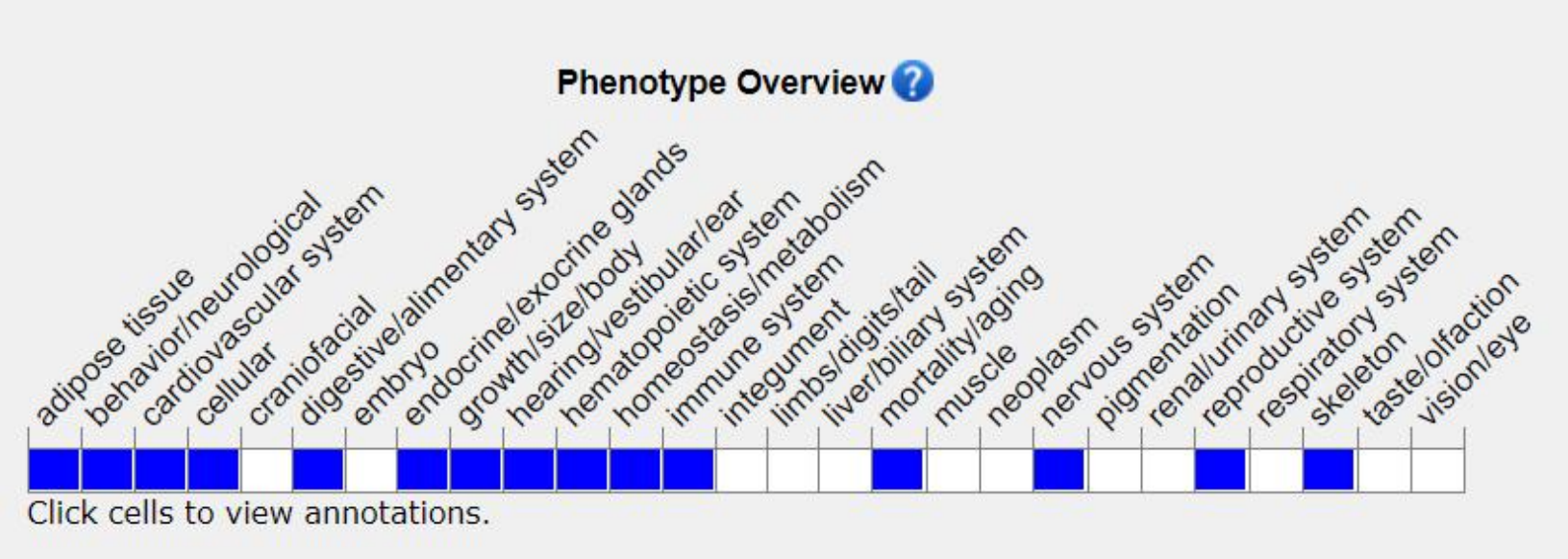
Genomic location distribution



Protein domain

Mouse phenotype description(MGI)

URL link is as follows:
http://www.informatics.jax.org/marker/MGI:101924



Homozygous mutants show variably severe deafness, head-shaking, circling, reduced endolymph secretion, male sterility, growth retardation, hypotension, reduced salivation, delayed ductal outgrowth of mammary epithelium and increased periweaning mortality.

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

集萃药康生物科技
GemPharmatech Co.,Ltd