

***Slc12a6-T991E&T1048E* Mouse Model Strategy**

-CRISPR/Cas9 technology

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Reviewer: Jia Yu

Design Date: 2021-10-26

Project Overview

Project Name

Slc12a6-T991E&T1048E

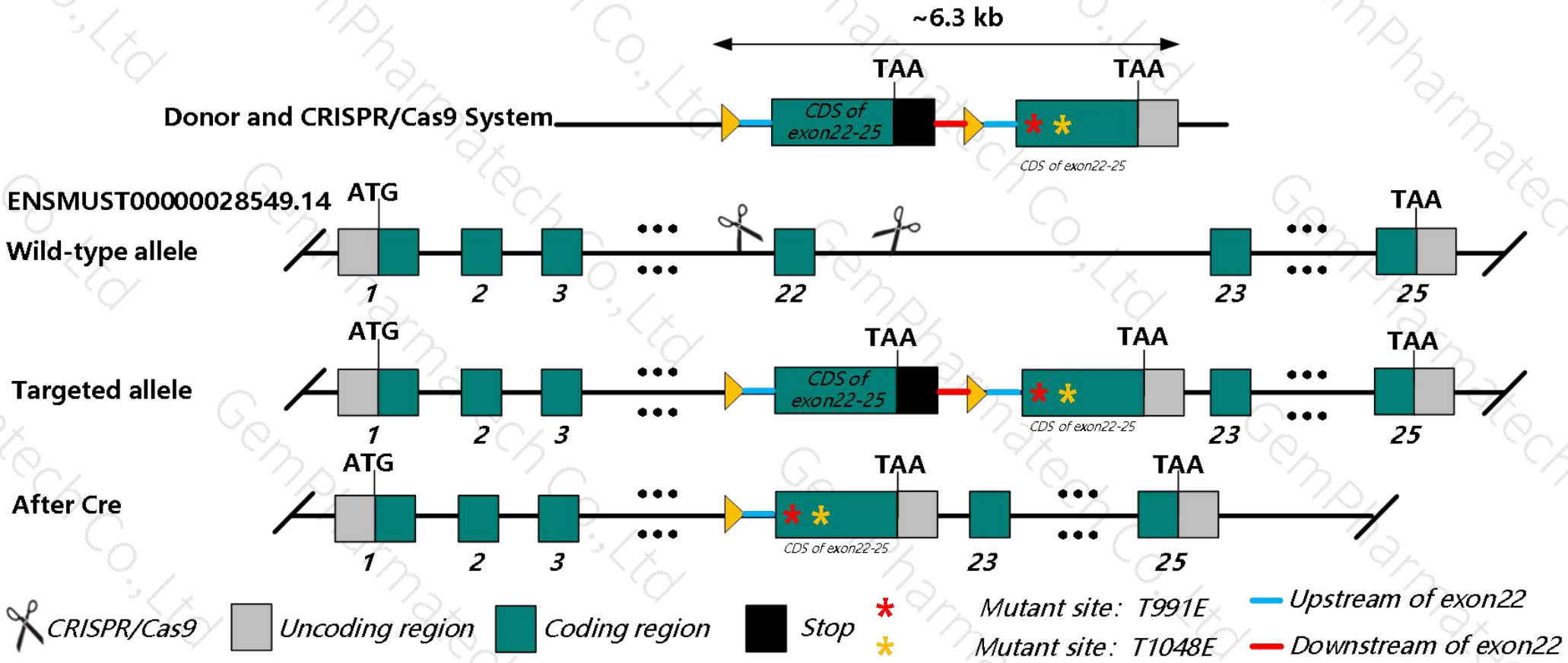
Project type

Cas9-KI(LSL)

Strain background

C57BL/6JGpt

This model uses CRISPR/Cas9 technology to edit the *Slc12a6* gene and the schematic diagram is as follow:



Technical Description

- The mouse *Slc12a6* gene has 8 transcripts.
- This project produced *Slc12a6-T991E&T1048E* point mutation on exon22&exon23 of the transcript of *Slc12a6-201*(ENSMUST00000028549.13). The 991th and 1048th amino acid will be mutated from T to E.
- In this project, *Slc12a6* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: CRISPR/Cas9 system 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.

- According to the data of MGI, homozygotes for targeted null mutations exhibit locomotor deficits, progressive neurodegeneration, slow progressive deafness and failure to breed.
- Transcript *Slc12a6-203* may not be affected.
- Mouse *Slc12a6* gene is located on Chr2. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr2, 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.

集萃药康
GemPharmatech

	-2																	H	D	S	D	I	S	A	Y	T	Y	E	R	T	L	?				
101601	CAACAATGTG TGGTTAACTC TTCCTTCTGC CTACTTTGTG TGCTGTCTGT CTGTGGCAGC ACGACAGTGA CATATCTGCC TATACATATG AGCGCACCGT GTTGTTACAC ACCAATTGAG AAGGAAGACG GATGAAACAC ACGACAGACA GACACCGTCG TGCTGTCACT GTATAGACGG ATATGTATAC TCGCGTGGGA																																			
	-2	%L	M	M	E	Q	R	S	Q	M	L	R	H	M	R	L	S	K	T	E	R	D	R	E												
101701	GATGATGGAG CAGAGGTCCC AGATGCTTCG GCATATGCGG CTGTCCAAAA CAGAGCGAGA CAGGGAGGTA AGACCTTGCT GAGTTCACCT GACCCTGTTC CTACTACCTC GTCTCCAGGG TCTACGAAGC CGTATACGCC GACAGGTTTT GTCTCGCTCT GTCCCTCAT TCTGGAACGA CTCAAAGTGA CTGGGACAAG																																			
101801	TCTGAGGCTG AGGCCTGTCA GTGATATAAA GACAGATACT TCCTCCTCCT GTTTCCTTAGT TACTCCAGTA GAACCTACCG TCTTCAGAAC ATTAAAGCAT AGACTCCGAC TCCGGACAGT CACTATATTT CTGTCTATGA AGGAGGAGGA CAAAGAATCA ATGAGGTCAT CTTGGATGGC AGAAGTCTTG TAAATTCGTA																																			
	+1																	A	Q	L	V	K	D	R	N	S	M	L	R	L	T	S	I	G	S	D
101901	GAGAAAAAGA ATAGTAAAAA TAACTGTGTT TGACTTTCTC CAGGACACAGC TGGTGAAAAGA TCGAAACTCA ATGCTACGCT TGACCAGCAT TGGCTCTGAT CTCTTTTTCT TATCATTTTT ATTGACACAA ACTGAAAGAG GTCGCTGTCG ACCACTTTCT AGCTTTGAGT TACGATGCGA ACTGGTCGTA ACCGAGACTA																																			
	+1	E	D	E	E	T	E	T	Y	Q	E	K	V	H	M	T	W	T	K	D	K	Y	M	A	S	R	G	Q	K	V	K	S	M	E	G	
102001	GAGGACGAAG AGACAGAAAC GTACCAGGAG AAGGTGCACA TGACTTGGAC CAAGGATAAA TACATGGCAT CCCGGGGGCA AAAGGTCAAG TCAATGGAAG CTCCTGCTTC TCTGTCTTTG CATGGTCTTC TTCCACGTGT ACTGAAACCTG GTTCTTATTT ATGTACCGTA GGGCCCCCGT TTTCCAGTTC AGTTACCTTG																																			

	#2	H	D	S	D	I	S	A	Y	T	Y	E	R	E	L?
101601	CAACAATGTG TGGTTAACTC TTCCTTCTGC CTACTTTGTG TGCTGTCTGT CTGTGCCAGC ACCGACAGTG CATATCTGCC TATACATATG AGCGC GAGCT GTTGTTACAC AC CAATTGAG AAGGAAGACG GATGAAACAC ACGACAGACA GACACCGTC G TGCTGTCACT GTATAGACGG ATATGTATAC TCGCG CTCGA														
+2	? L M M E Q R S Q M L R H M R L S K T E R D R E														
101701	GATGATGGAG CAGAGGTCCC AGATGCTTCG GCATATGCGG CTGTCCAAAA CAGAGCGAGA CAGGGAGGTA AGACCTTGCT GAGTTCACCT GACCCTGTTT CTACTACCTC GTCTCCAGGG TCTACGAAGC CGTATACGCC GACAGGTTTT GTCTCGCTCT GTCCCTCAT TCTGGAACGA CTCAAGTGA CTGGGACAAG														
101801	TCTGAGGCTG AGGCCTGTCA GTGATATAAA GACAGATACT TCCTCCTCCT GTTTCTTAGT TACTCCAGTA GAACCTACCG TCTTCAGAAC ATTAAAGCAT AGACTCCGAC TCCGGACAGT CACTATATTT CTGTCTATGA AGGAGGAGGA CAAAGAATCA ATGAGGTCAT CTTGGATGGC AGAAGTCTTG TAAATTCGTA														
+1	A Q L V K D R N S M L R L T S I G S D														
101901	GAGAAAAAGA ATAGTAAAAA TAAGTGTGTT TGACTTTTCTC CAGGACACAGC TGGTGAAAAGA TCGAAACTCA ATGCTACGCT TGACCAGCAT TGGCTCTGAT CTCTTTTTTCT TATCATTTTT ATTGACACAA ACTGAAAGAG GTC CGTGTGCG ACCACTTTCT AGCTTTGAGT TACGATGCGA ACTGGTCGTA ACCGAGACTA														
+1	E D E E T E T Y Q E K V H M E W T K D K Y M A S R G Q K V K S M E G														
102001	GAGGACGAAG AGACAGAAAC GTACCAGGAG AAGGTGCACA TGAGGTGGAC CAAGGATAAA TACATGGCAT CCCGGGGGCA AAAGGTCAAG TCAATGGAAG CTCCTGCTTC TCTGTCTTTG CATGGTCTC TTCCACGTGT ACCCTCACCTG GTTCTATATT ATGTACCGTA GGGCCCCCGT TTTCCAGTTC AGTTACCTTC														
+1	? G F Q D L L N M R														
102101	GATTCAGGA CCTACTTAAT ATGCGTCCGT AAGTGTGTGC ACTCTCAAAGC TTATCCTATG ATACAGTATC CTAGACAGTG GTACATATGT GACTGCCTGA CTAAGGTCTT GGATGAATTA TACGCAGGCA TTCACACACG TGAGAGTTCG AATAGGATAC TATGTCATAG GATCTGTCAC CATGTATACA CTGACGGACT														

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CDS of exon22-25

CDS of exon22-25

CACGACAGTGACATATCTGCCTATACATATGAGCGCACCCCTGATGATGGAGCAGAGGTCCCAGATGCTTCGGCATATGCGGC
TGTCCTAAAACAGAGCGAGACAGGGAGGCACAGCTGGTGAAAGATCGAACTCAATGCTACGCTTGACCAGCATTGGCTCT
GATGAGGACGAAGAGACAGAAACGTACCAGGAGAAGGTGCACATGACTTGGACCAAGGATAAATACATGGCATCCCGGGG
GCAAAAGGTCAAGTCAATGGAAGGATTCCAGGACCTACTTAATATGCGTCCGGACCAGTCCAACGTGAGACGGATGCATAC
AGCAGTGAAGCTCAATGAAGTTATAGTCAACAAGTCTCATGAAGCAAAGCTGGTTTTGTTGAATATGCCAGGACCACCCCG
GAACCCTGAAGGTGATGAAAACACTACATGGAATTTCTAGAAGTGCTCACTGAGGGATTAGAACGAGTCCTTCTTGTCCGGGG
TGGTGGCAGTGAGGTCATCACCATTACTCATAA

Mutant CDS of exon22-25

CACGACAGTGACATATCTGCCTATACATATGAGCGC**GAG**CTGATGATGGAGCAGAGGTCCCAGATGCTTCGGCATATGCGGC
TGTCCTAAAACAGAGCGAGACAGGGAGGCACAGCTGGTGAAAGATCGAACTCAATGCTACGCTTGACCAGCATTGGCTCT
GATGAGGACGAAGAGACAGAAACGTACCAGGAGAAGGTGCACATG**GAG**TGGACCAAGGATAAATACATGGCATCCCGGG
GGCAAAAGGTCAAGTCAATGGAAGGATTCCAGGACCTACTTAATATGCGTCCGGACCAGTCCAACGTGAGACGGATGCATA
CAGCAGTGAAGCTCAATGAAGTTATAGTCAACAAGTCTCATGAAGCAAAGCTGGTTTTGTTGAATATGCCAGGACCACCC
GGAACCCTGAAGGTGATGAAAACACTACATGGAATTTCTAGAAGTGCTCACTGAGGGATTAGAACGAGTCCTTCTTGTCCGGG
GTGGTGGCAGTGAGGTCATCACCATTACTCATAA

T991E&T1048E mutation sites in blue

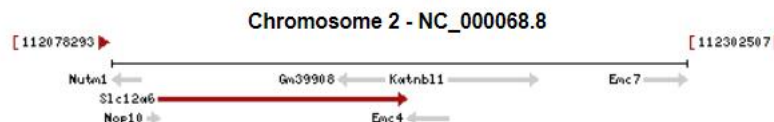
Gene name and location (NCBI)

Slc12a6 solute carrier family 12, member 6 [*Mus musculus* (house mouse)]

Gene ID: 107723, updated on 25-Sep-2020

Summary

Official Symbol	Slc12a6 provided by MGI
Official Full Name	solute carrier family 12, member 6 provided by MGI
Primary source	MGI:MG1:2135960
See related	Ensembl:ENSMUSG00000027130 Ensembl:ENSMUSG00000096764
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	ga; KCC3; gaxp; 9530023I19Rik
Expression	Ubiquitous expression in bladder adult (RPKM 21.4), thymus adult (RPKM 18.0) and 28 other tissues See more
Orthologs	human all

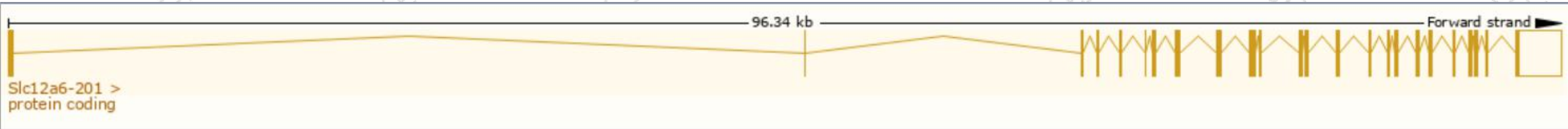


Transcript information (Ensembl)

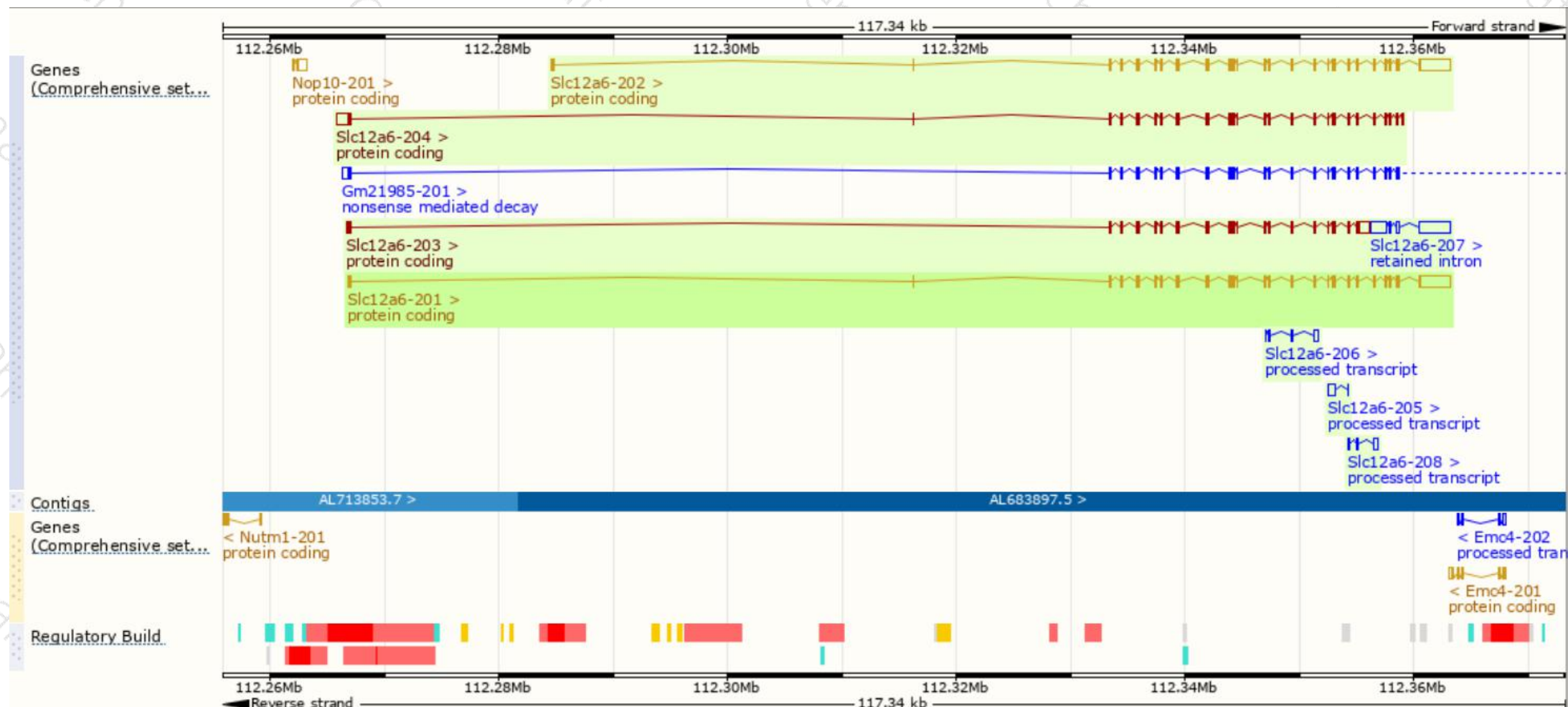
The gene has 8 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Slc12a6-201	ENSMUST00000028549.13	6105	1150aa	Protein coding	CCDS16551	Q924N4	TSL:1 GENCODE basic APPRIS P4
Slc12a6-202	ENSMUST00000053666.7	6060	1099aa	Protein coding	CCDS16552	Q924N4	TSL:1 GENCODE basic APPRIS ALT1
Slc12a6-204	ENSMUST00000110991.8	4556	1128aa	Protein coding	-	Q3V0N8	TSL:1 GENCODE basic
Slc12a6-203	ENSMUST00000110987.8	3722	946aa	Protein coding	-	A2AGJ9	TSL:1 GENCODE basic
Slc12a6-208	ENSMUST00000156470.1	766	No protein	Processed transcript	-	-	TSL:3
Slc12a6-206	ENSMUST00000133840.1	652	No protein	Processed transcript	-	-	TSL:3
Slc12a6-205	ENSMUST00000132752.1	600	No protein	Processed transcript	-	-	TSL:3
Slc12a6-207	ENSMUST00000137896.1	4464	No protein	Retained intron	-	-	TSL:2

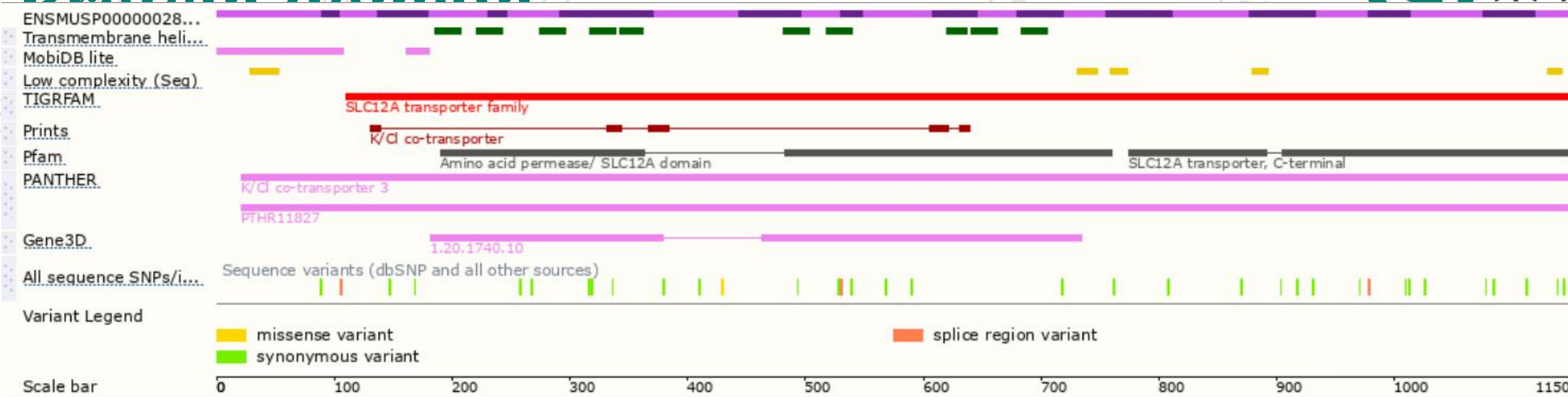
The strategy is based on the design of *Slc12a6-201* transcript, the transcription is shown below:



Genomic location distribution

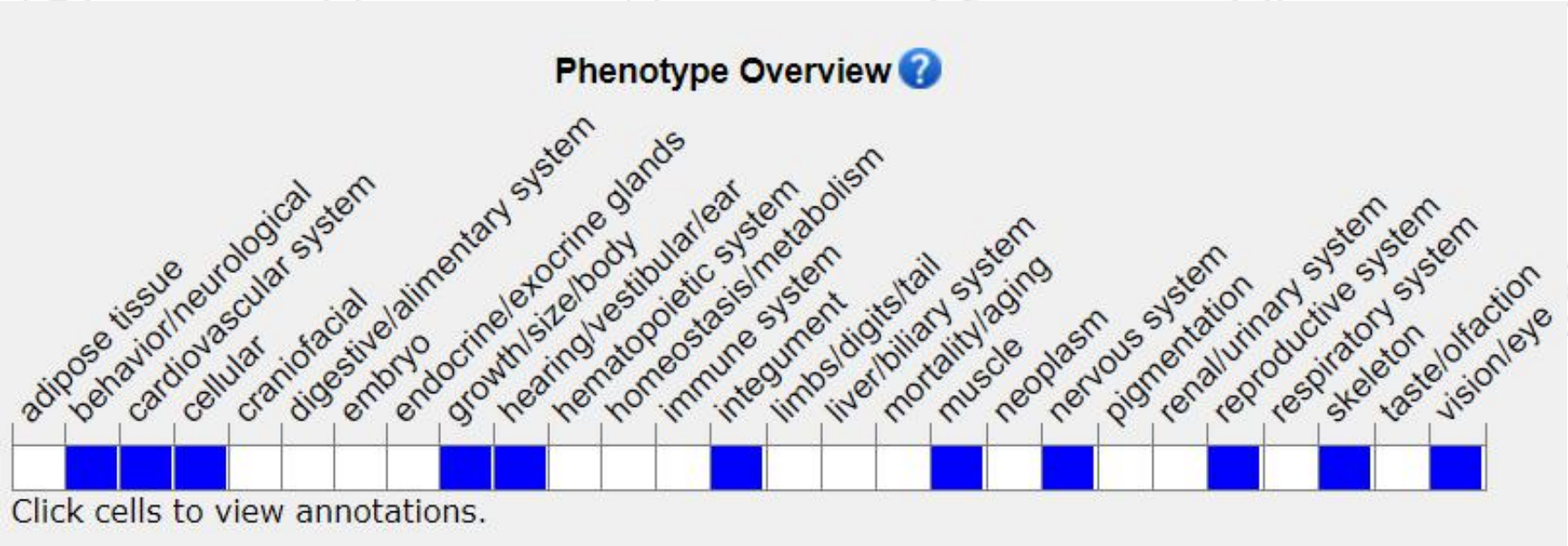


Protein domain



Mouse phenotype description(MGI)

URL link is as follows:
<http://www.informatics.jax.org/marker/MGI:2135960>



Homozygotes for targeted null mutations exhibit locomotor deficits, progressive neurodegeneration, slow progressive deafness and failure to breed.

Description of additional cycles

add item	cycle	cost(RMB)
<i>mSlc12a6</i> CDS exon22-25 order	0.5 month	779
<i>Mutant mSlc12a6</i> CDS exon22-25 order	0.5 month	779

The cycle of *mSlc12a6* CDS exon22-25 order and Mutant *mSlc12a6* CDS exon22-25 order will not be included in the contract cycle.

If the customer can provide CDS, the cost and cycle of this part can be canceled.

If you have any questions, please feel free to contact us.

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