Slc12a6 Cas9-KO Strategy

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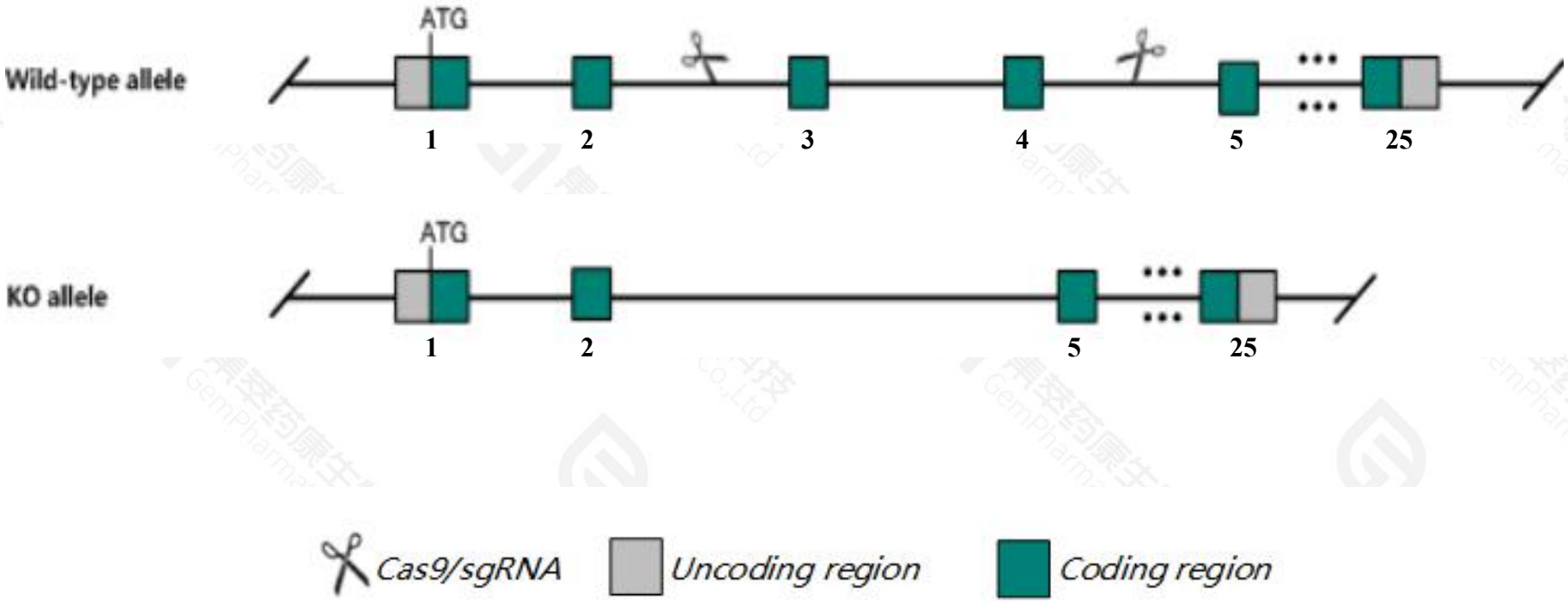
Design Date: 2021-6-10

Project Overview

Project Name	***Slc12a6***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Slc12a6* gene. The schematic diagram is as follows:



Technical routes

➢ The *Slc12a6* gene has 8 transcripts. According to the structure of *Slc12a6* gene, exon3-exon4 of *Slc12a6-201*(ENSMUST00000028549.14) transcript is recommended as the knockout region.The region contains 227bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Slc12a6* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

Notice

➢ The KO region contains functional region of the *Gm21985-201* gene.Knockout the region may affect the function of *Gm21985-201* gene

➢ According to the existing MGI data,homozygotes for targeted null mutations exhibit locomotor deficits, progressive neurodegeneration, slow progressive deafness and failure to breed.

➢ The *Slc12a6* gene is located on the Chr2. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.

➢ This strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes,all risk of the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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

Gene ID: 107723, updated on 18-Jan-2021

Summary

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

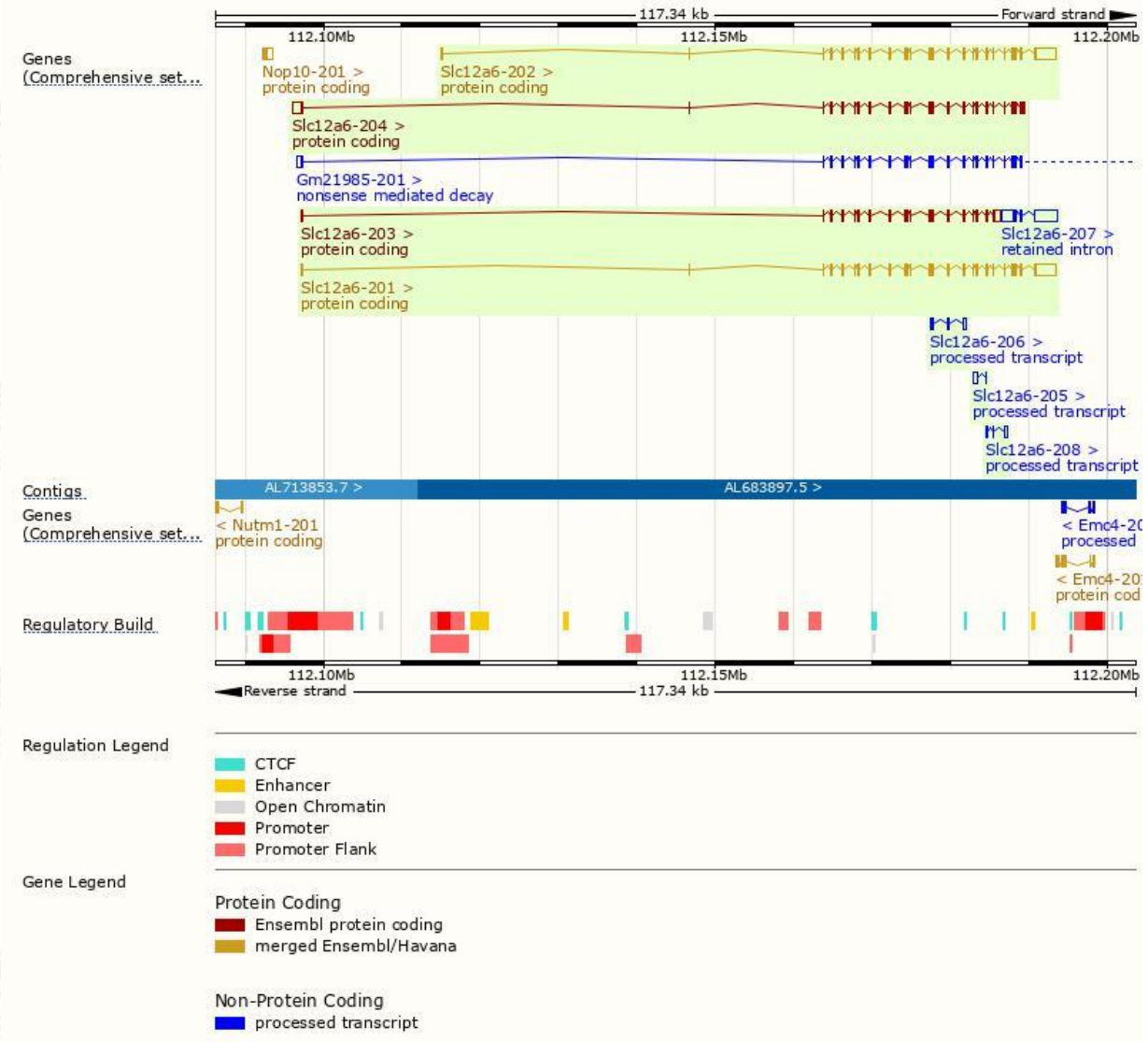
Transcript information（Ensembl）

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

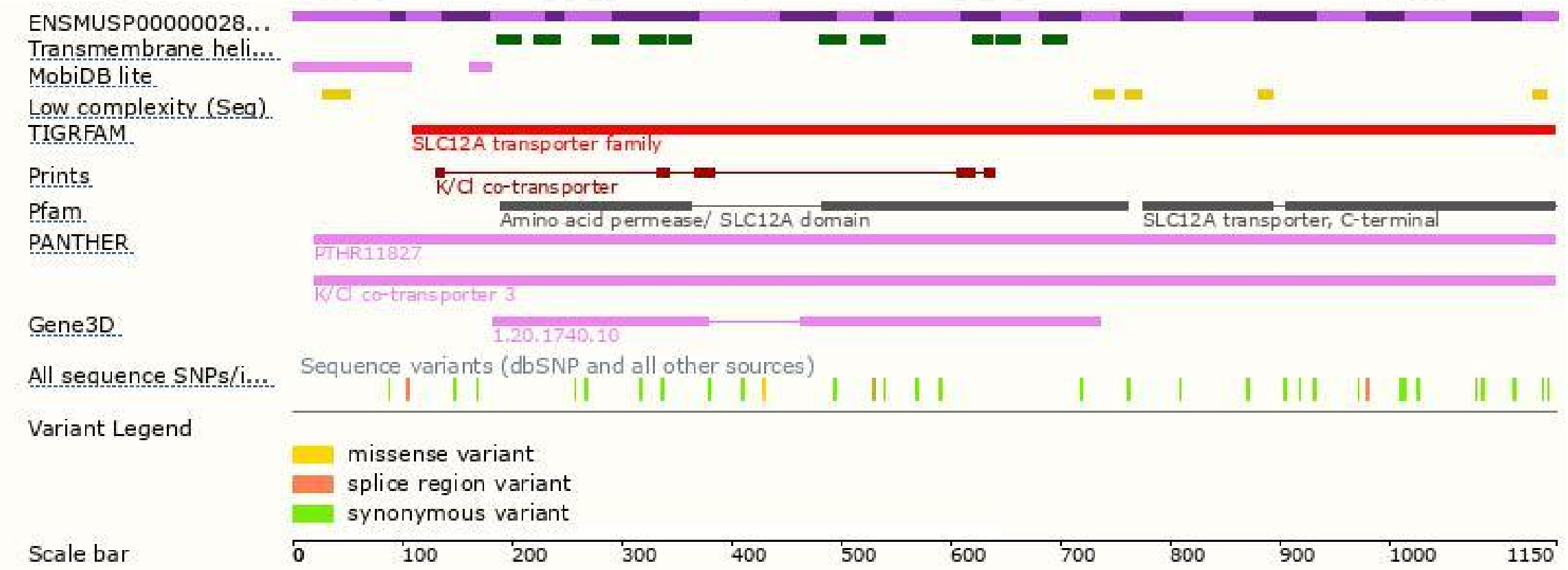
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Slc12a6-201	ENSMUST00000028549.14	6105	1150aa	Protein coding	CCDS16551		TSL:1 , GENCODE basic , APPRIS P4 ,
Slc12a6-202	ENSMUST00000053666.8	6060	1099aa	Protein coding	CCDS16552		TSL:1 , GENCODE basic , APPRIS ALT1 ,
Slc12a6-204	ENSMUST00000110991.9	4556	1128aa	Protein coding	-		TSL:1 , GENCODE basic ,
Slc12a6-203	ENSMUST00000110987.9	3722	946aa	Protein coding	-		TSL:1 , GENCODE basic ,
Slc12a6-208	ENSMUST00000156470.2	766	No protein	Processed transcript	-		TSL:3 ,
Slc12a6-206	ENSMUST00000133840.2	652	No protein	Processed transcript	-		TSL:3 ,
Slc12a6-205	ENSMUST00000132752.2	600	No protein	Processed transcript	-		TSL:3 ,
Slc12a6-207	ENSMUST00000137896.2	4464	No protein	Retained intron	-		TSL:2 ,

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

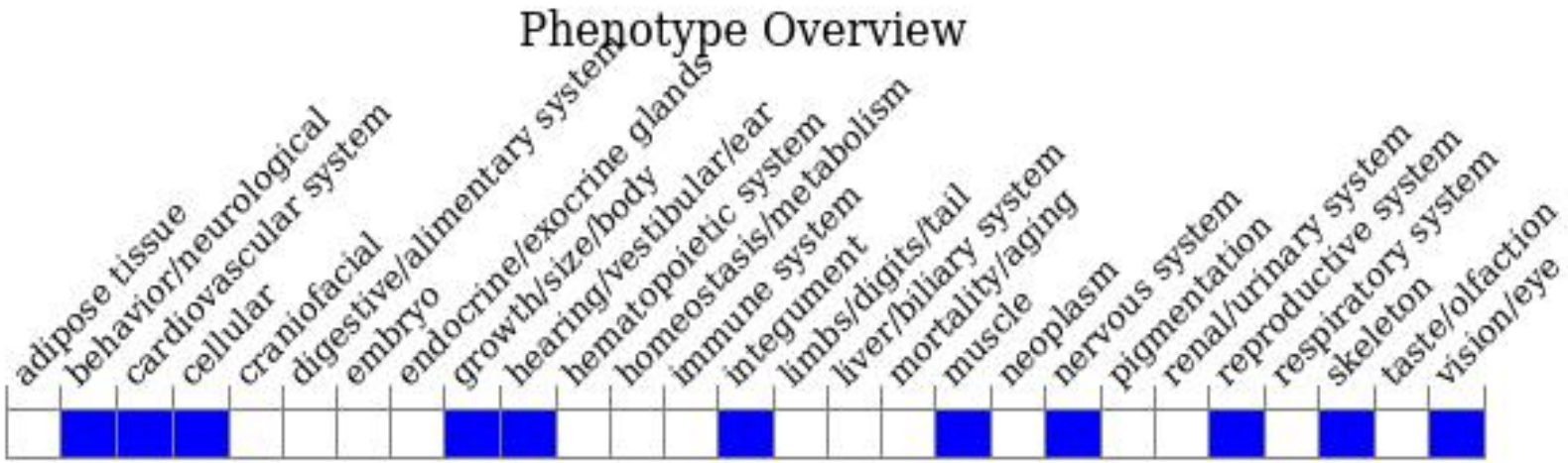
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data,homozygotes for targeted null mutations exhibit locomotor deficits, progressive neurodegeneration, slow progressive deafness and failure to breed.

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

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