Slc12a5 Cas9-CKO Strategy

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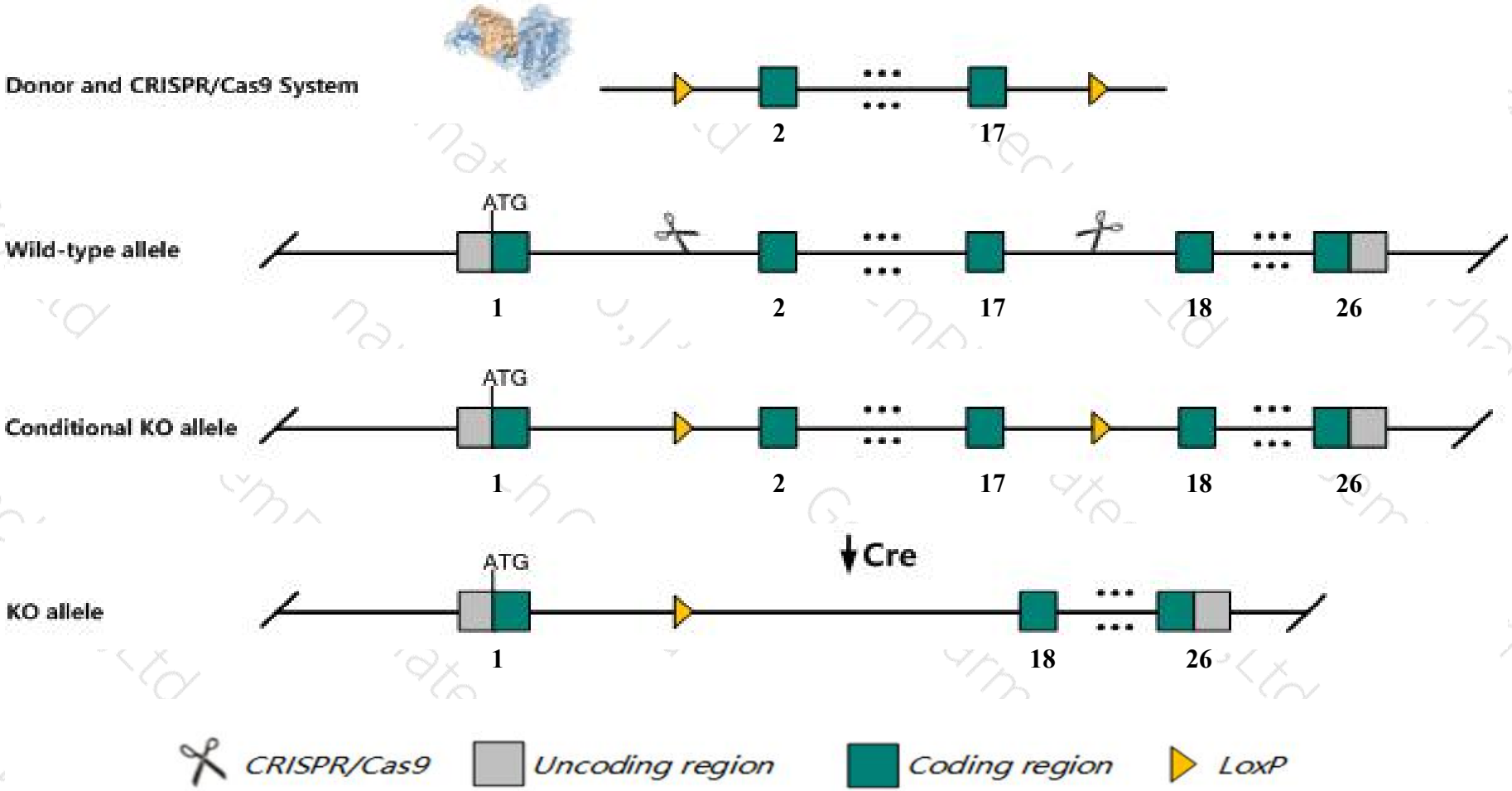
Project Overview

Project Name	***Slc12a5***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Slc12a5* gene. The schematic diagram is as follows:



Technical routes

➢ The *Slc12a5* gene has 10 transcripts. According to the structure of *Slc12a5* gene, exon2-exon17 of *Slc12a5-201*(ENSMUST00000099092.7) transcript is recommended as the knockout region.The region contains 2129bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Slc12a5* gene. The brief process is as follows:CRISPR/Cas9 system and Donor 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.

➢ The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Notice

➢ According to the existing MGI data,mice homozygous for disruptions in this gene die within a few minutes of birth of respiratory failure resulting from a motor nerve defect. Mice homozygous for a hypomorphic allele display postnatal lethality and tonic-clonic seizures.

➢ The *Slc12a5* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

集萃药康 GemPharmatech

Slc12a5 solute carrier family 12, member 5 [Mus musculus (house mouse)]

Gene ID: 57138, updated on 15-Mar-2020

Summary

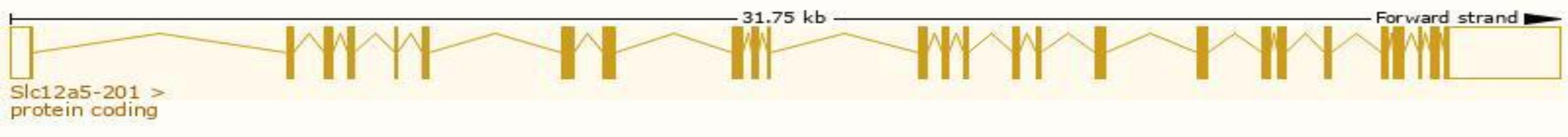
Official Symbol	Slc12a5 provided by MGI
Official Full Name	solute carrier family 12, member 5 provided byMGI
Primary source	MGI:MGI:1862037
See related	Ensembl:ENSMUSG00000017740
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	KCC2, mKIAA1176
Expression	Biased expression in cerebellum adult (RPKM 71.6), cortex adult (RPKM 67.7) and 6 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

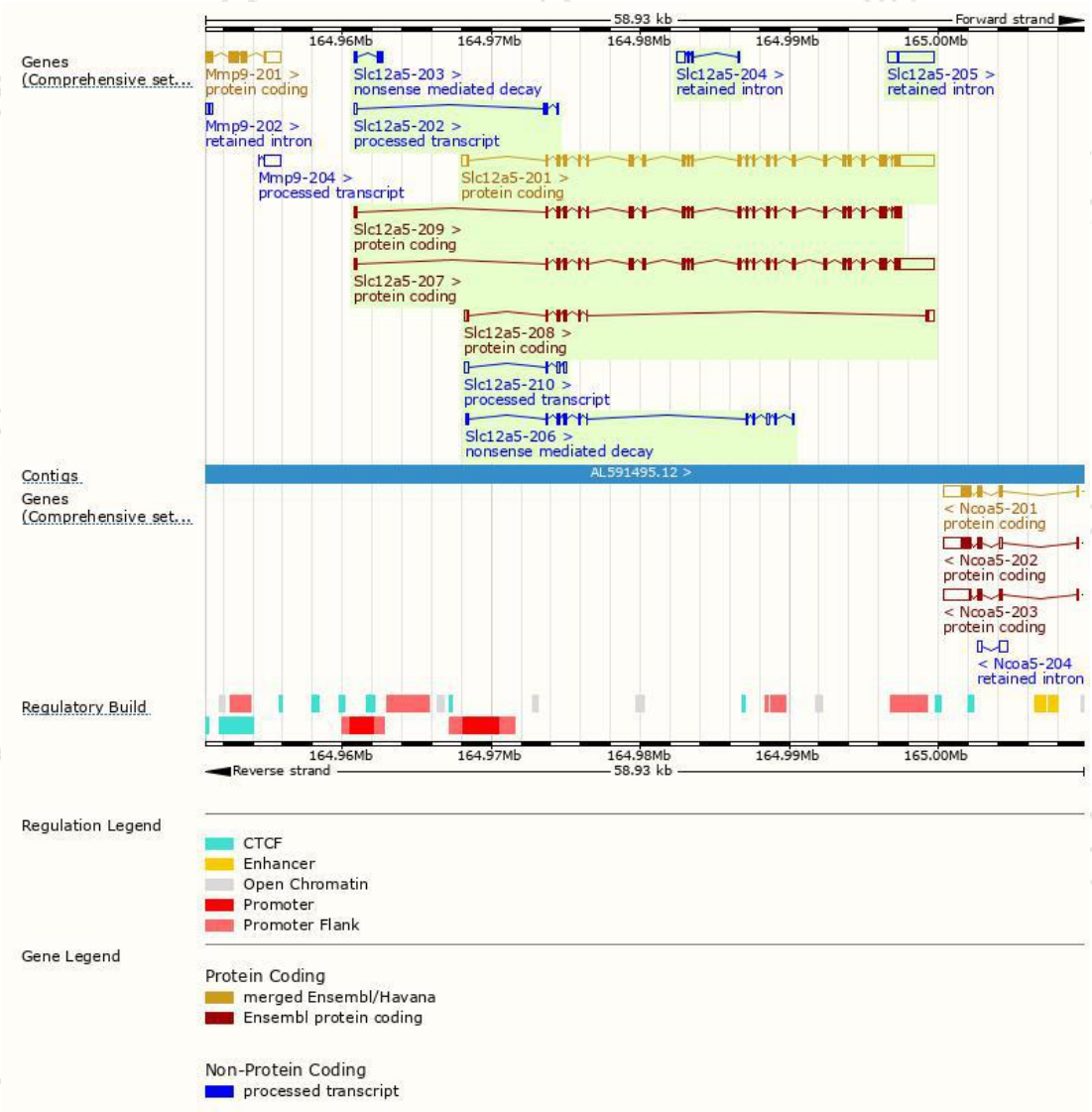
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Slc12a5-201	ENSMUST00000099092.7	6028	1115aa	Protein coding	CCDS38332	A0A076FSX1 Q91V14	TSL:1 GENCODE basic APPRIS P2
Slc12a5-207	ENSMUST00000202223.3	5690	1133aa	Protein coding	-	A0A076FR46	TSL:1 GENCODE basic
Slc12a5-209	ENSMUST00000202623.3	3574	1138aa	Protein coding	-	A0A076FRG6 Q91V14	TSL:1 GENCODE basic APPRIS ALT2
Slc12a5-208	ENSMUST00000202479.3	1202	231aa	Protein coding	-	A0A0J9YV84	TSL:5 GENCODE basic
Slc12a5-206	ENSMUST00000202136.1	1106	186aa	Nonsense mediated decay	-	A0A0J9YU26	TSL:5
Slc12a5-203	ENSMUST00000125867.1	422	59aa	Nonsense mediated decay	-	A0A0J9YUW1	TSL:3
Slc12a5-210	ENSMUST00000208579.1	586	No protein	Processed transcript	-	-	TSL:5
Slc12a5-202	ENSMUST00000124372.5	465	No protein	Processed transcript	-	-	TSL:2
Slc12a5-205	ENSMUST00000137302.2	2996	No protein	Retained intron	-	-	TSL:1
Slc12a5-204	ENSMUST00000135918.1	744	No protein	Retained intron	-	-	TSL:3

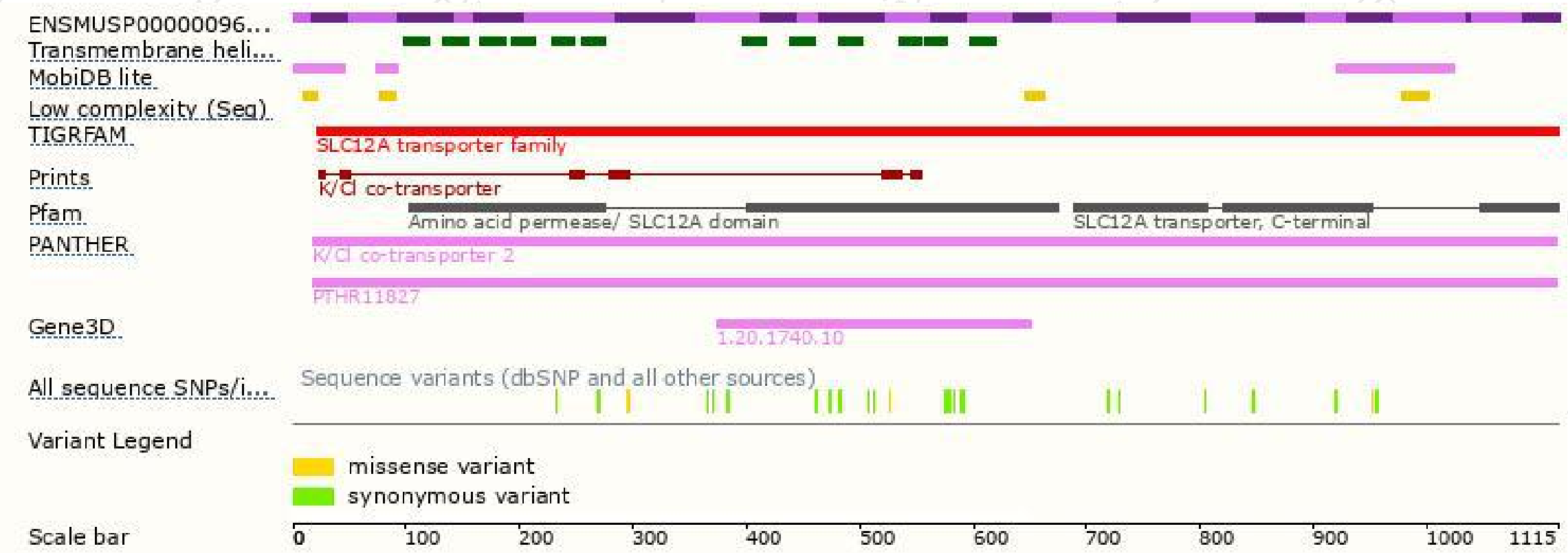
The strategy is based on the design of *Slc12a5-201* transcript,the transcription is shown below:



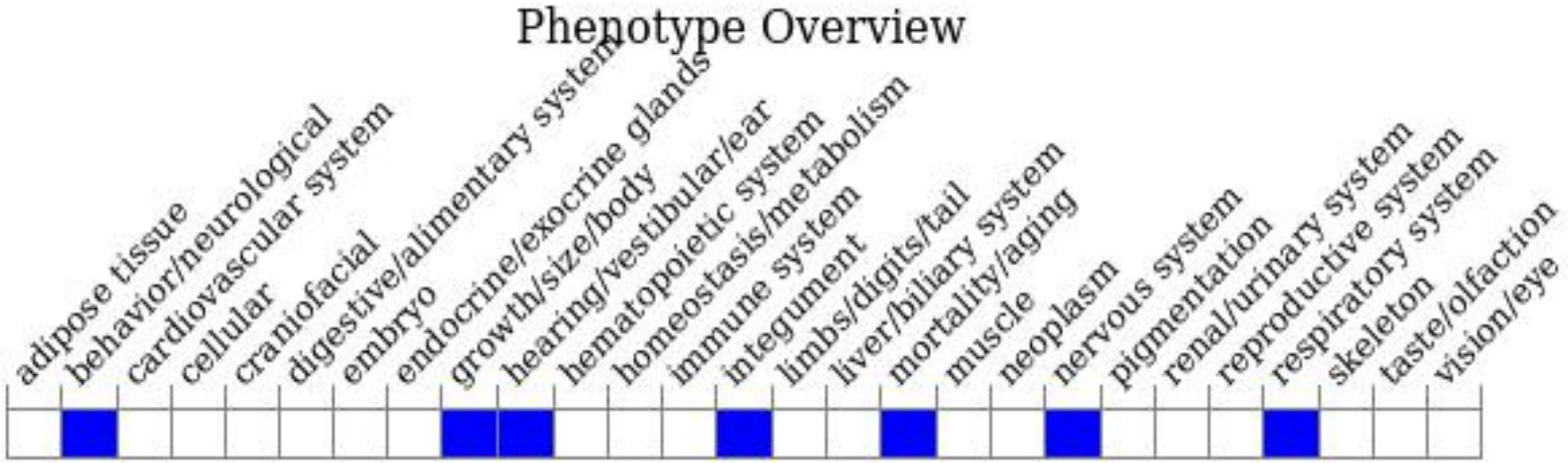
Genomic location distribution



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,mice homozygous for disruptions in this gene die within a few minutes of birth of respiratory failure resulting from a motor nerve defect. Mice homozygous for a hypomorphic allele display postnatal lethality and tonic-clonic seizures.

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

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