Slc22a14 Cas9-KO Strategy

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Reviewer: Xiaojing Li

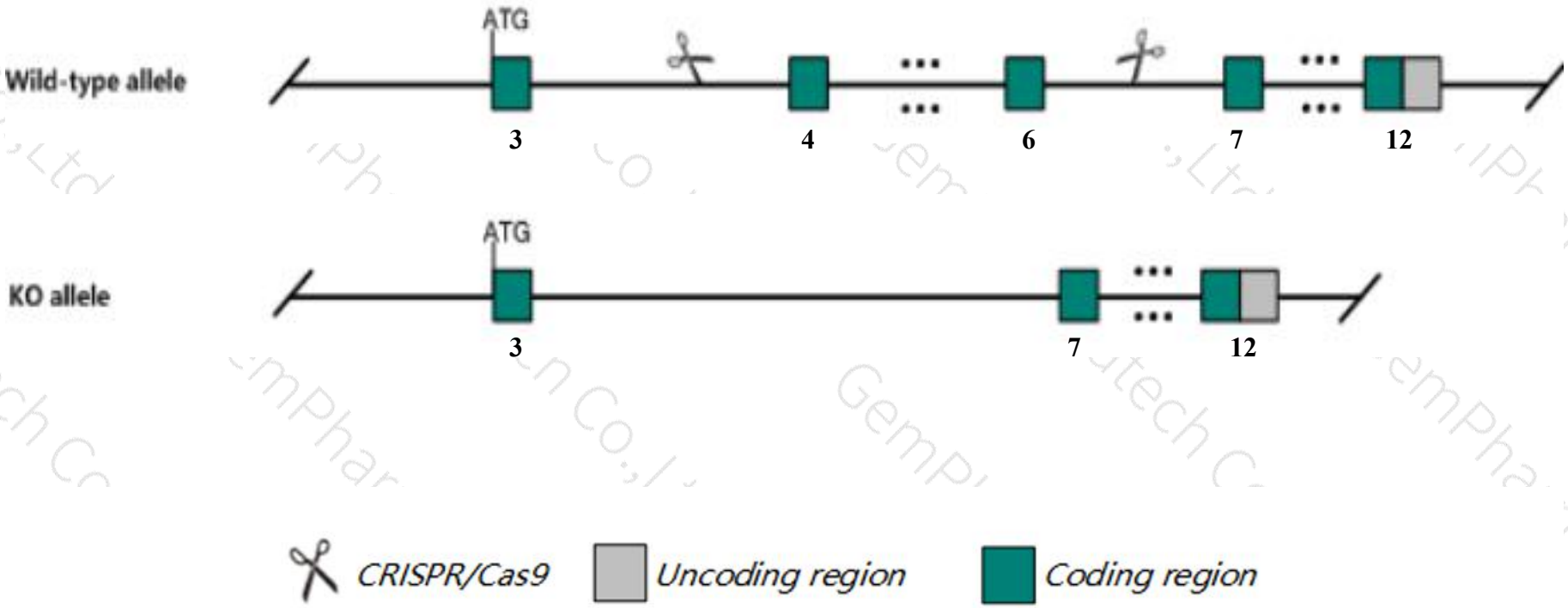
Design Date: 2020-9-16

Project Overview

Project Name	***Slc22a14***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Slc22a14* gene. The schematic diagram is as follows:



Technical routes

➢ The *Slc22a14* gene has 4 transcripts. According to the structure of *Slc22a14* gene, exon4-exon6 of *Slc22a14-201*(ENSMUST00000093775.11) transcript is recommended as the knockout region.The region contains 428bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Slc22a14* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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

➢ According to the existing MGI data,mice homozygous for a knock-out allele exhibit severe male infertility associated with asthenozoospermia, impaired sperm capacitation, decreased fertilization frequency, abnormal sperm flagellar bending, and abnormal sperm annulus morphology.

➢ Transcript *Slc22a14*-202, *Slc22a14*-203 may not be affected.

➢ The *Slc22a14* gene is located on the Chr9. 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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Slc22a14 solute carrier family 22 (organic cation transporter), member 14 [Mus musculus (house mouse)]

Gene ID: 382113, updated on 13-Mar-2020

Summary

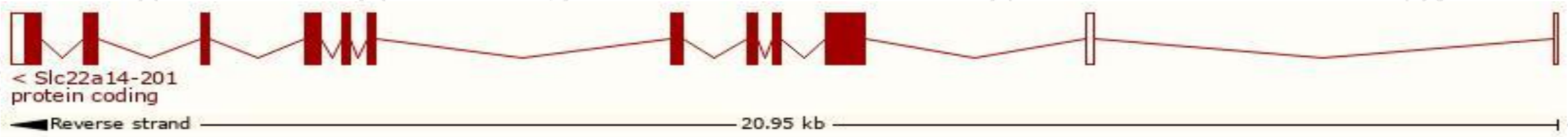
Official Symbol	Slc22a14 provided by MGI
Official Full Name	solute carrier family 22 (organic cation transporter), member 14 provided byMGI
Primary source	MGI:MGI:2685974
See related	Ensembl:ENSMUSG00000070280
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Gm1128
Expression	Restricted expression toward testis adult (RPKM 114.3)See more
Orthologs	human all

Transcript information（Ensembl）

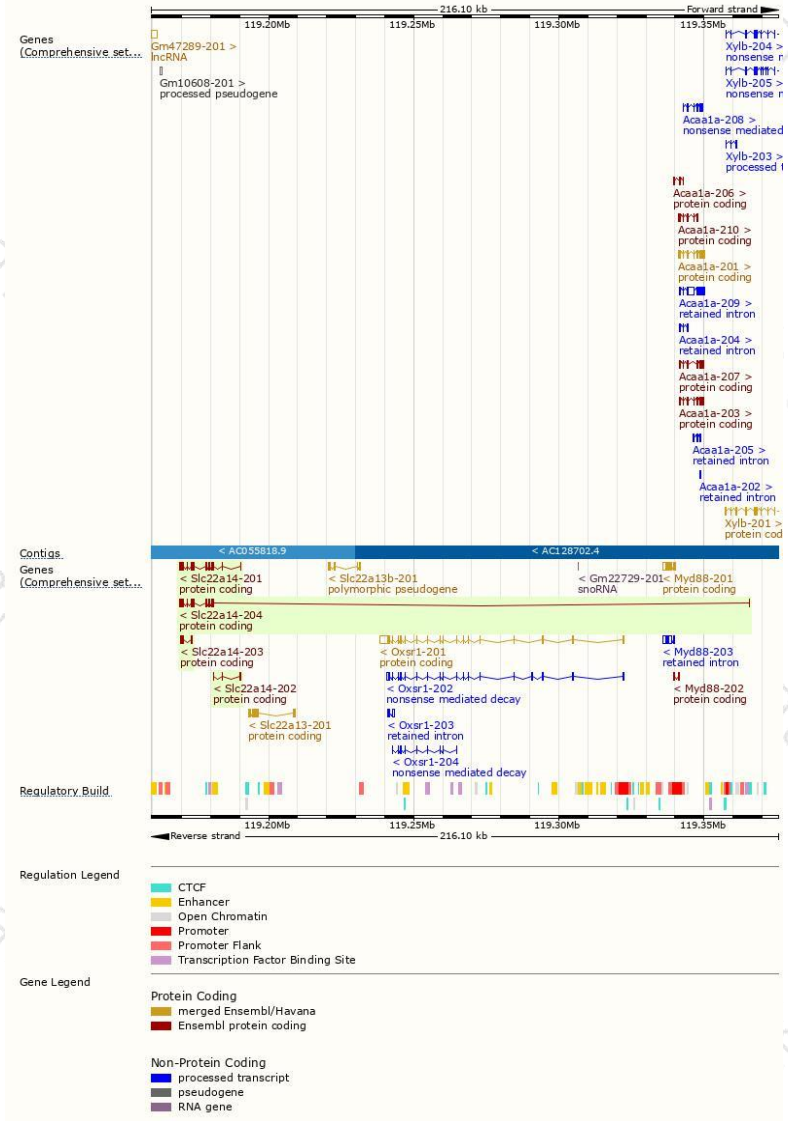
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Slc22a14-201	ENSMUST00000093775.11	2213	629aa	Protein coding	CCDS23609	Q497L9	TSL:1 GENCODE basic APPRIS P1
Slc22a14-204	ENSMUST00000170400.8	2110	629aa	Protein coding	CCDS23609	Q497L9	TSL:5 GENCODE basic APPRIS P1
Slc22a14-203	ENSMUST00000152061.1	589	196aa	Protein coding	-	F7AMC9	CDS 5' and 3' incomplete TSL:3
Slc22a14-202	ENSMUST00000127794.1	340	20aa	Protein coding	-	D3YUH1	CDS 3' incomplete TSL:5

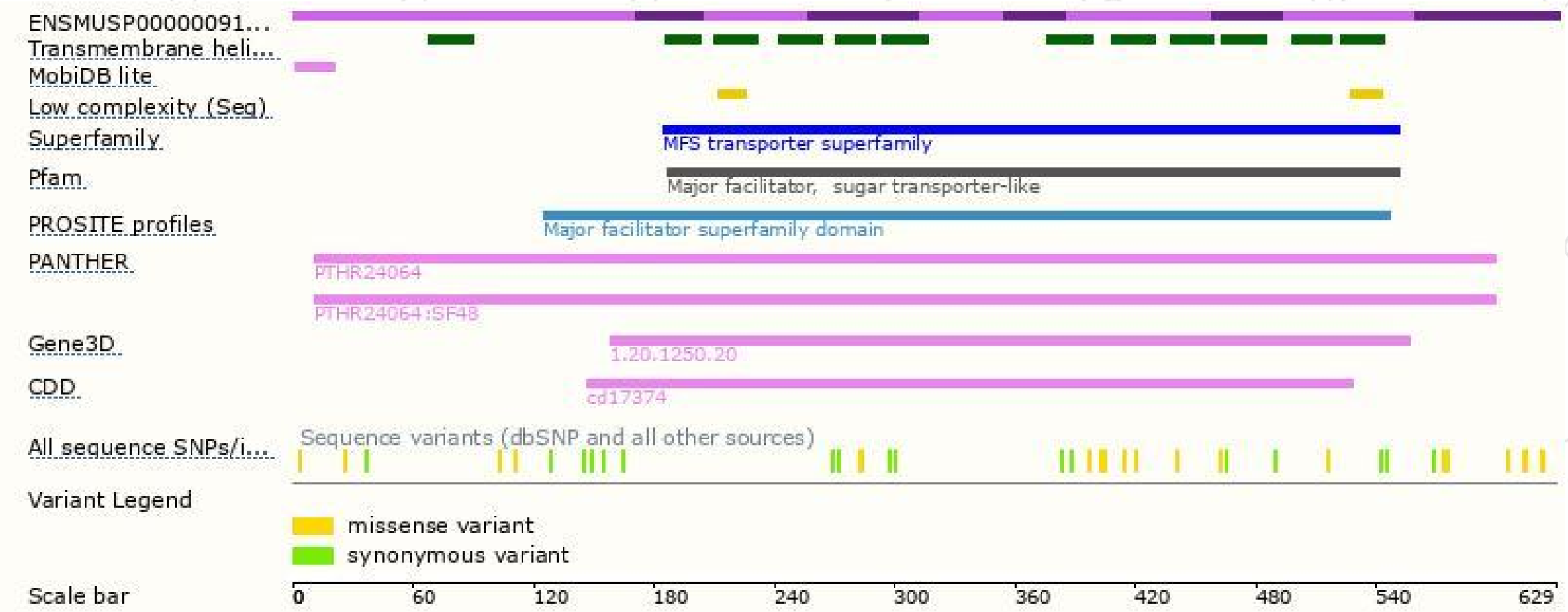
The strategy is based on the design of *Slc22a14-201* transcript,the transcription is shown below:



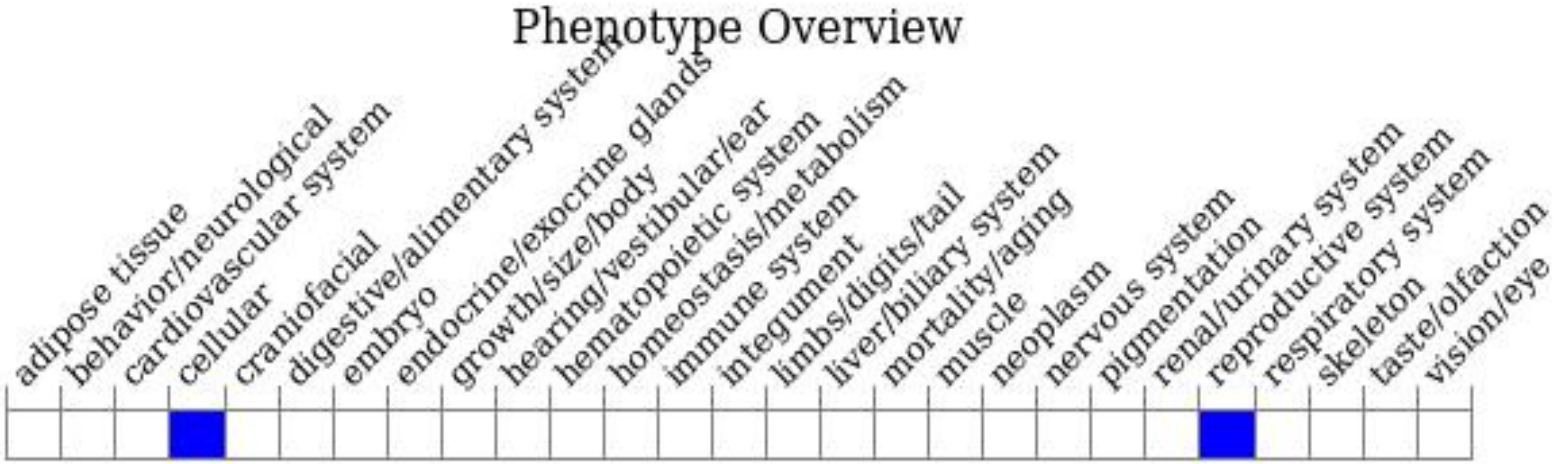
Genomic location distribution



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/).

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

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