Slc4a4 Cas9-CKO Strategy

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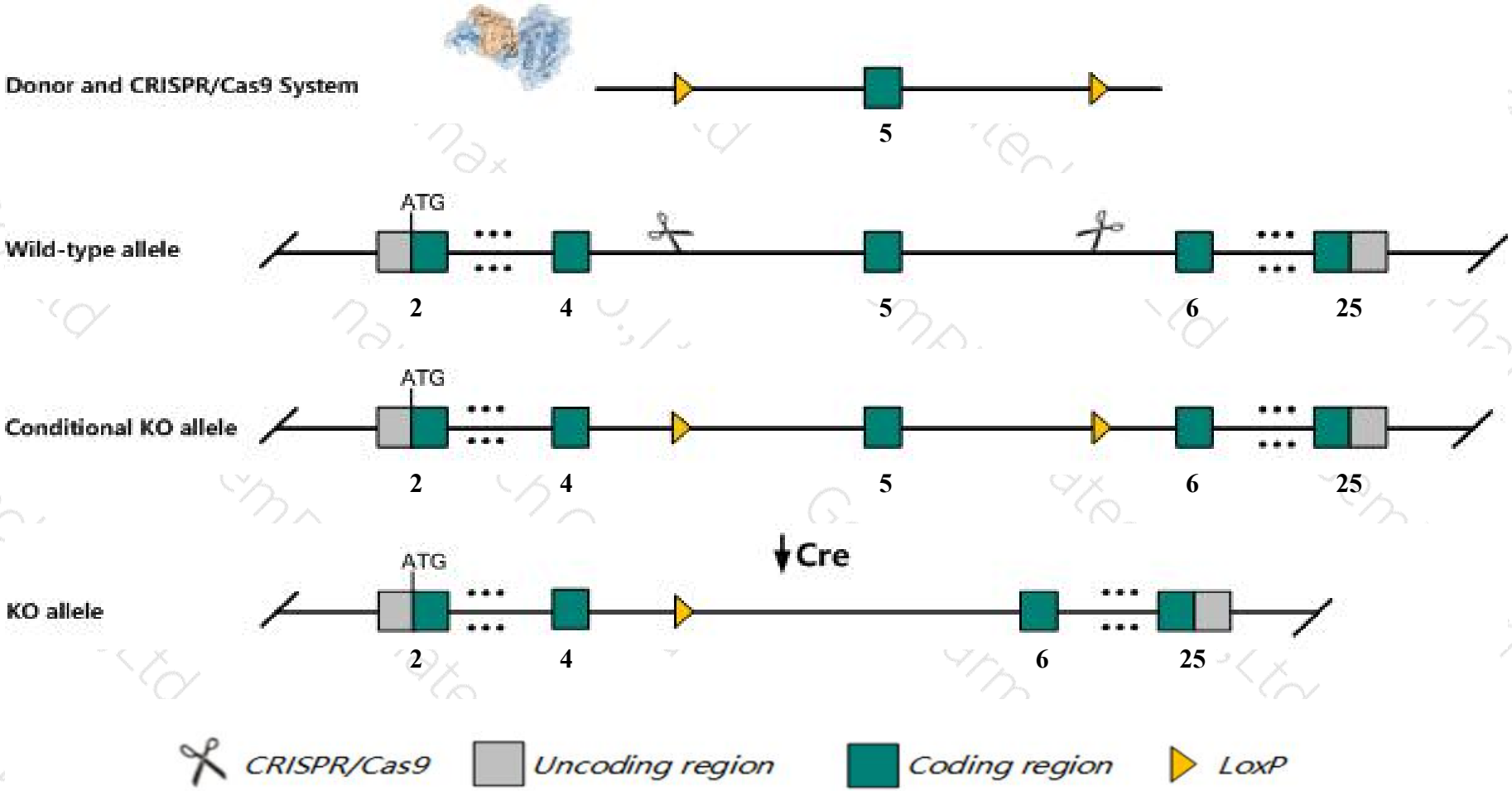
Project Overview

Project Name	***Slc4a4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Slc4a4* gene. The schematic diagram is as follows:



Technical routes

- The *Slc4a4* gene has 10 transcripts. According to the structure of *Slc4a4* gene, exon5 of *Slc4a4-208* (ENSMUST00000156238.7) transcript is recommended as the knockout region.The region contains 161bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Slc4a4* 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,nullizygous mice show postnatal growth retardation and lethality, bowel obstructions, metabolic acidosis and abnormal urine homeostasis. additional phenotypes include altered blood, ion and ammonia homeostasis, renal tubular acidosis/atrophy, corneal opacities, and bone, muscle and spleen defects.
- Transcripts *Slc4a4-206* and *Slc4a4-209* are incomplete, so the effect on them are unknown.
- The *Slc4a4* gene is located on the Chr5. 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）

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Slc4a4 solute carrier family 4 (anion exchanger), member 4 [*Mus musculus* (house mouse)]

Gene ID: 54403, updated on 20-Apr-2020

Summary

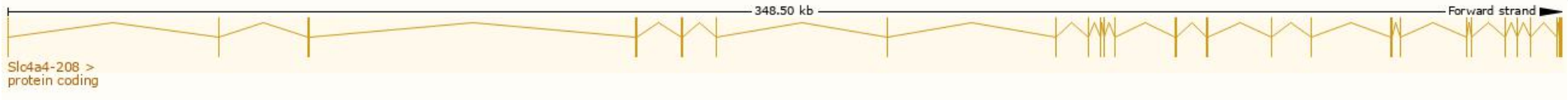
Official Symbol	Slc4a4 provided by MGI
Official Full Name	solute carrier family 4 (anion exchanger), member 4 provided by MGI
Primary source	MGI:MGI:1927555
See related	Ensembl:ENSMUSG00000060961
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	NBC; NBC1; AI835705
Expression	Biased expression in kidney adult (RPKM 60.8), cerebellum adult (RPKM 27.2) and 9 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

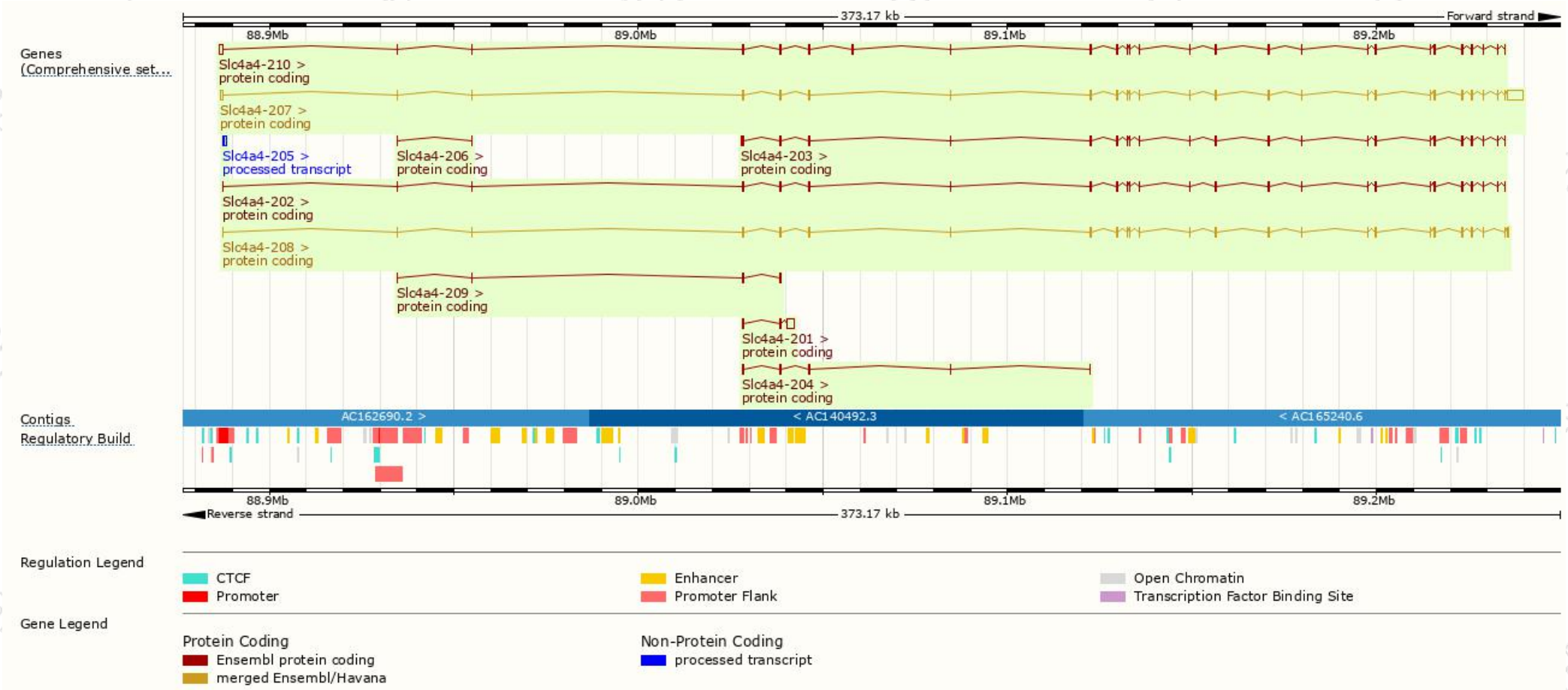
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Slc4a4-207	ENSMUST00000148750.7	7931	1079aa	Protein coding	CCDS19406	O88343	TSL:5 GENCODE basic APPRIS P1
Slc4a4-208	ENSMUST00000156238.7	3494	1094aa	Protein coding	CCDS51541	E9Q8N8	TSL:5 GENCODE basic
Slc4a4-202	ENSMUST00000113218.9	3269	1070aa	Protein coding	CCDS57352	A7E1Z5	TSL:1 GENCODE basic
Slc4a4-210	ENSMUST00000239214.1	4212	1111aa	Protein coding	-	-	GENCODE basic
Slc4a4-203	ENSMUST00000130041.7	3335	1035aa	Protein coding	-	E1AWU4 O88343	TSL:1 GENCODE basic
Slc4a4-201	ENSMUST00000113216.8	2518	157aa	Protein coding	-	O88343	TSL:1 GENCODE basic
Slc4a4-204	ENSMUST00000134303.1	780	233aa	Protein coding	-	D3Z7G8	CDS 3' incomplete TSL:5
Slc4a4-209	ENSMUST00000238265.1	587	171aa	Protein coding	-	-	CDS 3' incomplete
Slc4a4-206	ENSMUST00000144713.1	371	86aa	Protein coding	-	D3Z7I7	CDS 3' incomplete TSL:3
Slc4a4-205	ENSMUST00000135283.1	489	No protein	Processed transcript	-	-	TSL:3

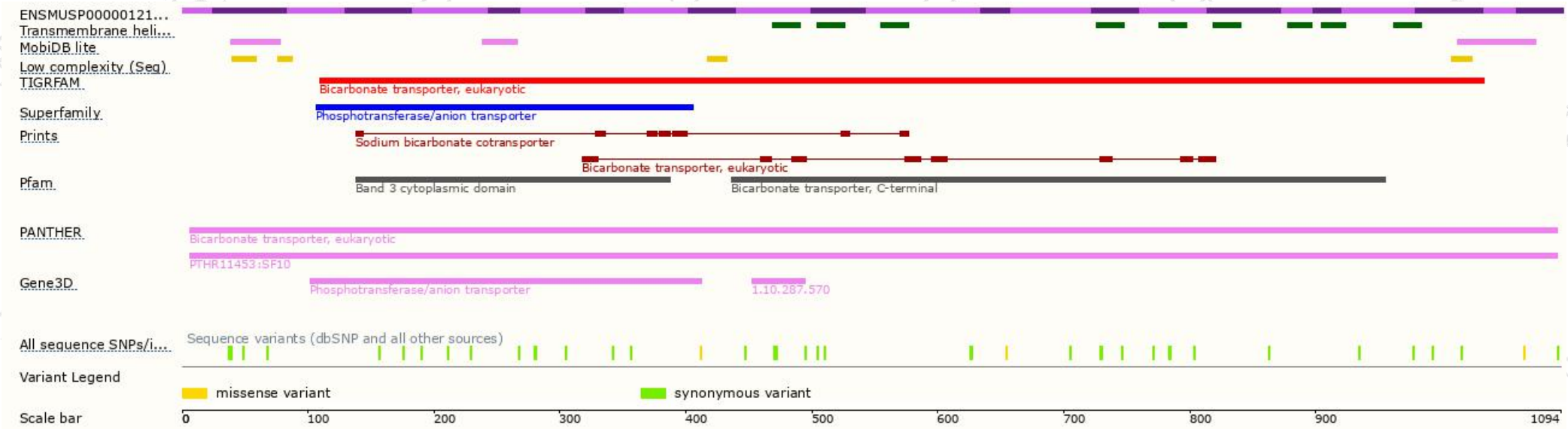
The strategy is based on the design of *Slc4a4-208* transcript, the transcription is shown below



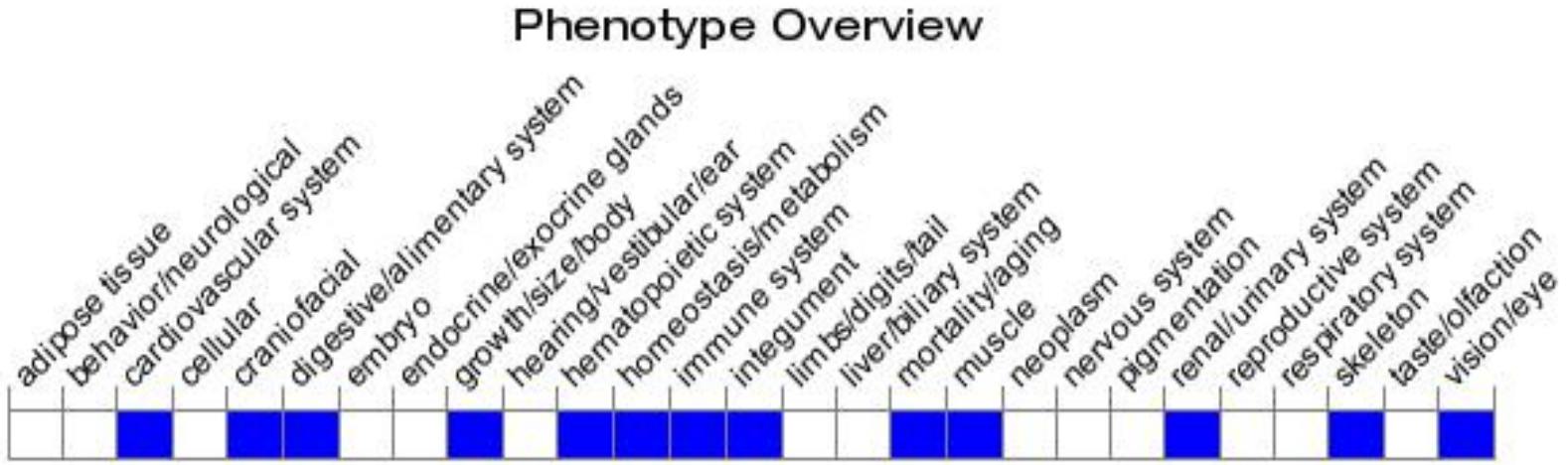
Genomic location distribution



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, nullizygous mice show postnatal growth retardation and lethality, bowel obstructions, metabolic acidosis and abnormal urine homeostasis. Additional phenotypes include altered blood, ion and ammonia homeostasis, renal tubular acidosis/atrophy, corneal opacities, and bone, muscle and spleen defects.

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

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