

Slc5a9 Cas9-CKO Strategy

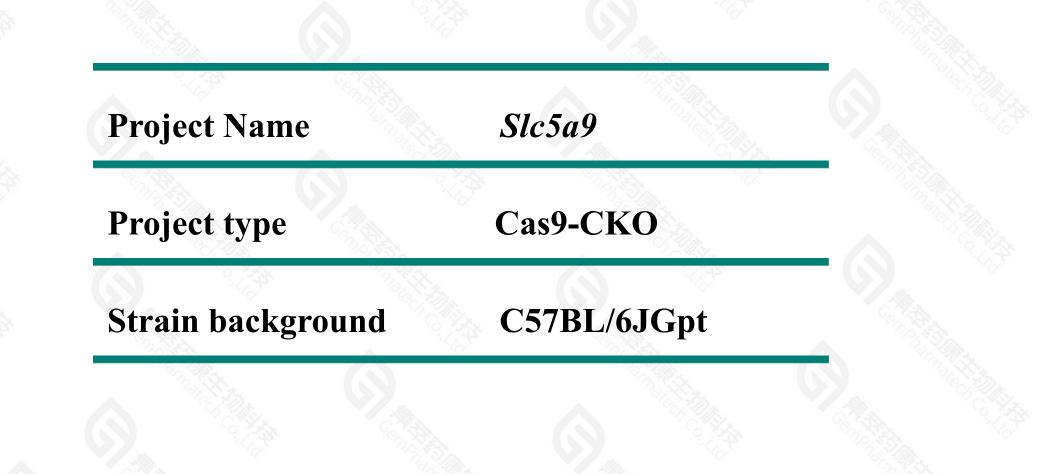
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Reviewer: Miaomiao Cui

Design Date: 2021-5-26

Project Overview

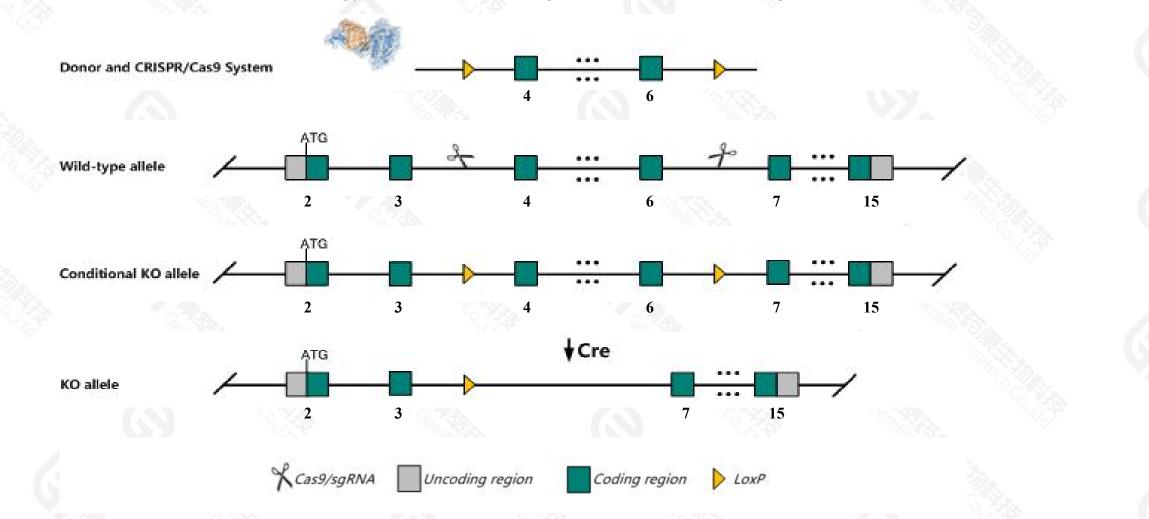




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Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Slc5a9* gene. The schematic diagram is as follows:



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Technical routes



The Slc5a9 gene has 8 transcripts. According to the structure of Slc5a9 gene, exon4-exon6 of Slc5a9-203(ENSMUST00000102721.8) transcript is recommended as the knockout region. The region contains 376bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Slc5a9* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor was constructed.Cas9, sgRNA 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.

 \succ The flox mice was 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.



- > The *Slc5a9* gene is located on the Chr4. 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)

Slc5a9 solute carrier family 5 (sodium/glucose cotransporter), member 9 [Mus musculus (house mouse)]

Gene ID: 230612, updated on 17-Dec-2020

Summary

Official Symbol	SIc5a9 provided by MGI
Official Full Name	solute carrier family 5 (sodium/glucose cotransporter), member 9 provided by MGI
Primary source	MGI:MGI:2140201
See related	Ensembl:ENSMUSG0000028544
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	AI159731, SGL, SGLT4
Expression	Biased expression in kidney adult (RPKM 7.9), thymus adult (RPKM 7.5) and 7 other tissuesSee more
Orthologs	human all

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Transcript information (Ensembl)

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

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Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Slc5a9-203	ENSMUST00000102721.8	4484	<u>685aa</u>	Protein coding	CCD518474		TSL:1 , GENCODE basic , APPRIS P1 ,
Slc5a9-202	ENSMUST00000102720.8	3393	<u>685aa</u>	Protein coding	CCDS18474		TSL:1 , GENCODE basic , APPRIS P1 ,
Slc5a9-201	ENSMUST00000102719.8	2460	<u>685aa</u>	Protein coding	CCDS18474		TSL:1 , GENCODE basic , APPRIS P1 ,
Slc5a9-205	ENSMUST00000128340.3	766	<u>255aa</u>	Protein coding			CDS 5' and 3' incomplete , TSL:3 ,
Slc5a9-204	ENSMUST00000128224.2	2814	No protein	Processed transcript	<u>a</u>		TSL:1,
Slc5a9-208	ENSMUST00000154838.2	672	No protein	Processed transcript			TSL:2,
Slc5a9-207	ENSMUST00000149922.2	497	No protein	Processed transcript	-		TSL:5,
Slc5a9-206	ENSMUST00000131772.2	494	No protein	Processed transcript	3 3		TSL:3,

The strategy is based on the design of *Slc5a9-203* transcript, the transcription is shown below:

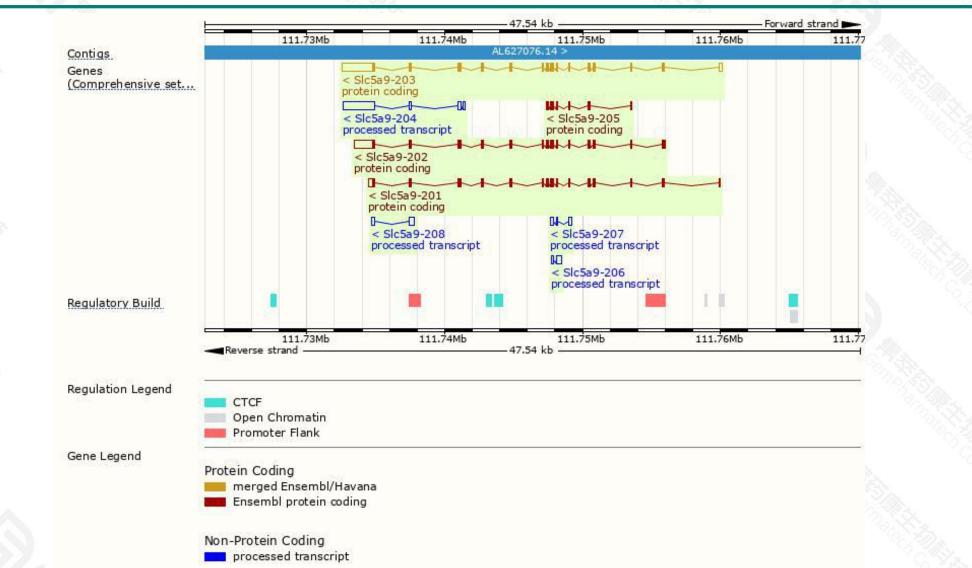


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Genomic location distribution





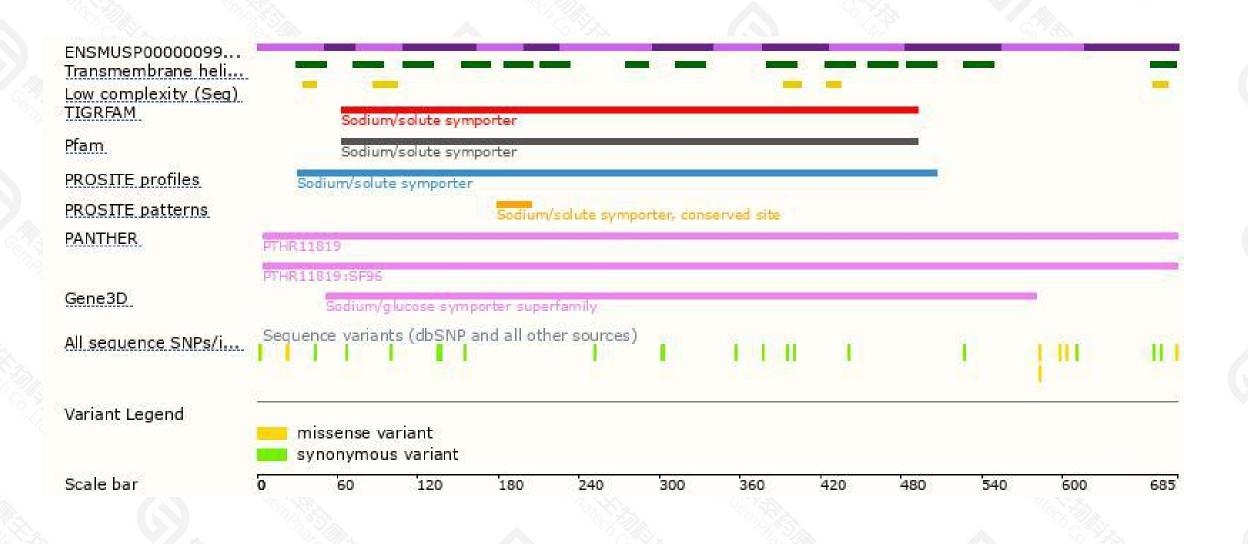
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Protein domain





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If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



