

Slc2a9 Cas9-CKO Strategy

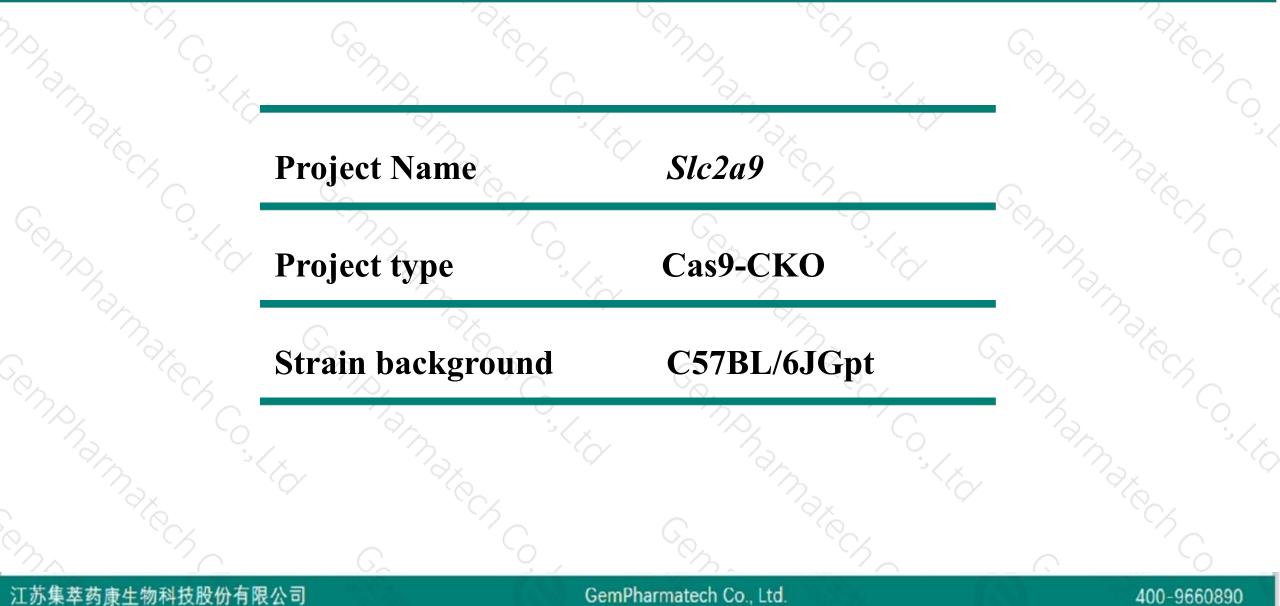
Designer: Huimin Su

Reviewer: Ruiuri Zhang

Design Date: 2020-7-22

Project Overview



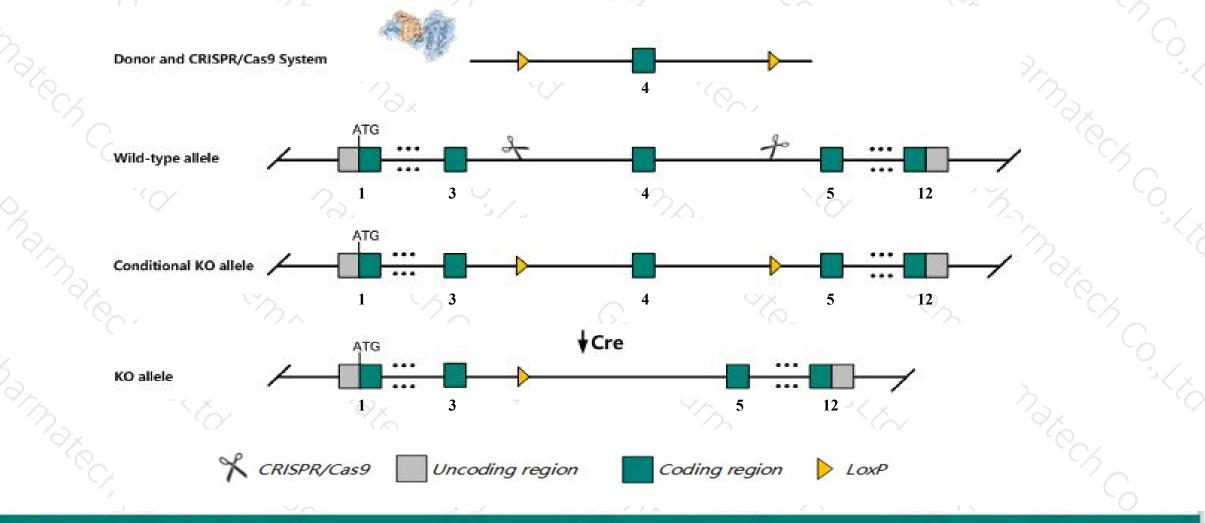


Conditional Knockout strategy



400-9660890

This model will use CRISPR/Cas9 technology to edit the *Slc2a9* gene. The schematic diagram is as follows:



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The Slc2a9 gene has 12 transcripts. According to the structure of Slc2a9 gene, exon4 of Slc2a9-203(ENSMUST00000067886.11) transcript is recommended as the knockout region. The region contains 125bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Slc2a9* 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.



> According to the existing MGI data, mice homozygous for a knock-out allele show partial prenatal lethality, polydipsia, hyperuricemia, hyperuricosuria and polyuria, and develop urate nephropathy, characterized by obstructive lithiasis, tubulointerstitial inflammation, cortical fibrosis, renal insufficiency and reduced male weight.

➤ Transcript *Slc2a9-212* may not be affected.

> The *Slc2a9* 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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SIc2a9 solute carrier family 2 (facilitated glucose transporter), member 9 [Mus musculus (house mouse)]

Gene ID: 117591, updated on 26-Jun-2020

Summary

 Official Symbol
 Slc2a9 provided by MGI

 Official Full Name
 solute carrier family 2 (facilitated glucose transporter), member 9 provided by MGI

 Primary source
 MGI:MGI:2152844

 See related
 Ensembl:ENSMUSG0000005107

 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
 Glut9; GLUT-9; SLC2A9B; SLC2a9A

 Expression
 Broad expression in liver adult (RPKM 6.8), large intestine adult (RPKM 5.0) and 16 other tissues <u>See more</u>

 Orthologs
 human all

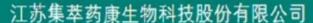
Transcript information (Ensembl)



The gene has 12 transcripts, all transcripts are shown below:

Name 🖕	Transcript ID	bp 🖕	Protein A	Biotype 🖕	CCDS 🍦	UniProt 🖕	Flags		
SIc2a9-209	ENSMUST00000147664.7	652	<u>144aa</u>	Protein coding	-	A0A5H1ZRL9@	CDS 3' incomplete TSL:2		
SIc2a9-212	ENSMUST00000156272.7	526	<u>158aa</u>	Protein coding	-	A0A5H1ZRN5@	CDS 3' incomplete TSL:5		
SIc2a9-204	ENSMUST00000122970.7	2900	<u>281aa</u>	Nonsense mediated decay	<u> </u>	A0A5H1ZRL6@	TSL:1		
SIc2a9-201	ENSMUST0000005238.12	3243	<u>416aa</u>	Protein coding	<u>CCDS19256</u> 률	<u>Q3T9X0</u> &	TSL:1 GENCODE basic		
SIc2a9-207	ENSMUST00000143758.7	1296	<u>431aa</u>	Protein coding	<u>CCDS51482</u> &	<u>Q3T9X0</u> ┏	TSL:1 GENCODE basic		
SIc2a9-205	ENSMUST00000129099.7	3462	<u>523aa</u>	Protein coding	<u>CCDS51484</u> &	<u>Q3T9X0</u> @	TSL:5 GENCODE basic APPRIS ALT2		
SIc2a9-202	ENSMUST0000067872.11	3403	<u>523aa</u>	Protein coding	CCDS51484@	<u>Q3T9X0</u> &	TSL:1 GENCODE basic APPRIS ALT2		
SIc2a9-210	ENSMUST00000155634.7	3164	<u>523aa</u>	Protein coding	<u>CCDS51484</u> 교	<u>Q3T9X0</u> ഭ	TSL:5 GENCODE basic APPRIS ALT2		
SIc2a9-203	ENSMUST0000067886.11	3602	<u>538aa</u>	Protein coding	CCDS51483@	<u>Q3T9X0</u> @	TSL:1 GENCODE basic APPRIS P4		
SIc2a9-206	ENSMUST00000140462.1	776	No protein	Processed transcript	-		TSL:3		
SIc2a9-208	ENSMUST00000144290.1	770	No protein	Processed transcript	<u> </u>	121	TSL:3		
SIc2a9-211	ENSMUST00000156076.1	427	No protein	Retained intron	2	3 <u>8</u> 3	TSL:3		

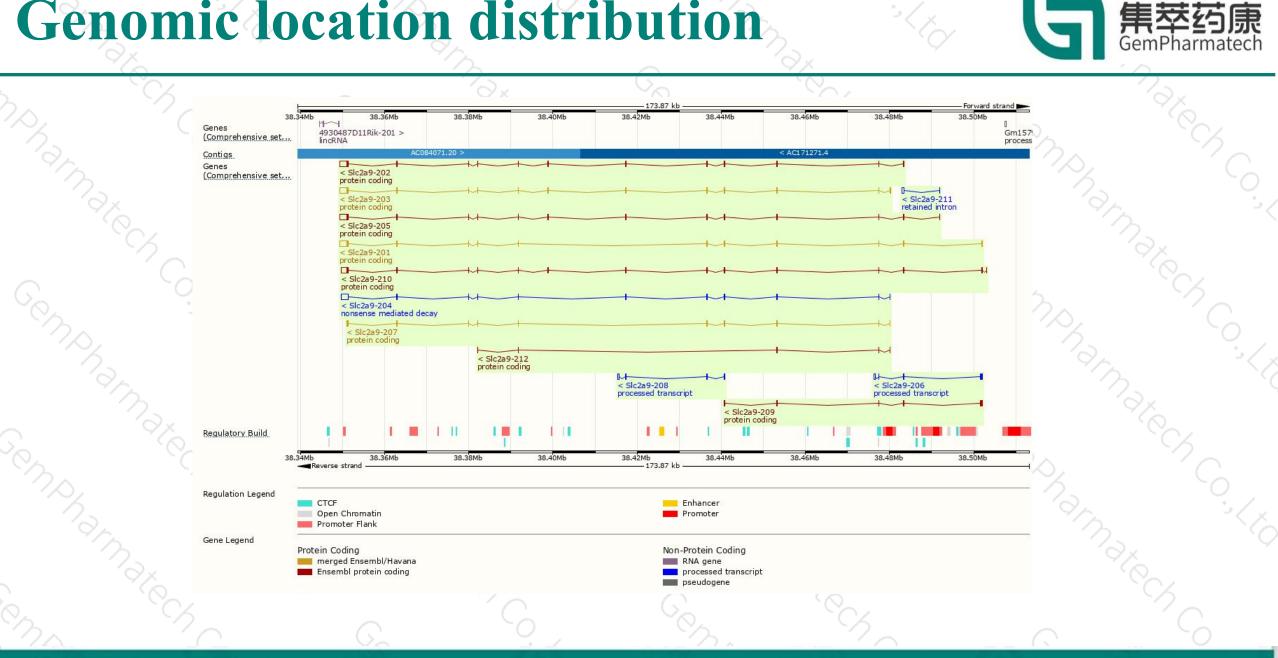
The strategy is based on the design of *Slc2a9-203* transcript, the transcription is shown below:



< Slc2a9-203

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Genomic location distribution



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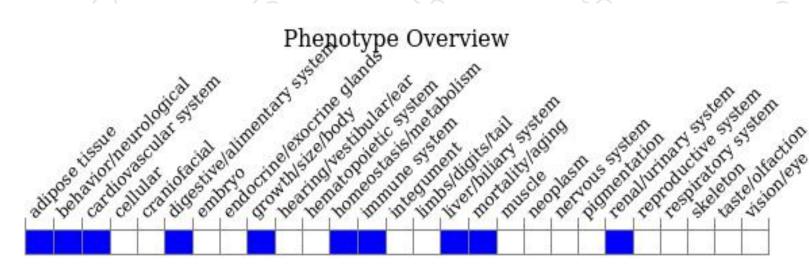
Protein domain



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Prints Pfam	Sugar/inositol transporter					
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Gene3D	PTHR23503					
CDD	1,20,1250,20 cd17432					
All sequence SNPs/i	Sequence variants (dbSNP and all other	ources)	i i ii	ant i tra	n an	
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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 a knock-out allele show partial prenatal lethality, polydipsia, hyperuricemia, hyperuricosuria and polyuria, and develop urate nephropathy, characterized by obstructive lithiasis, tubulointerstitial inflammation, cortical fibrosis, renal insufficiency and reduced male weight.

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If you have any questions, you are welcome to inquire. Tel: 400-9660890



