

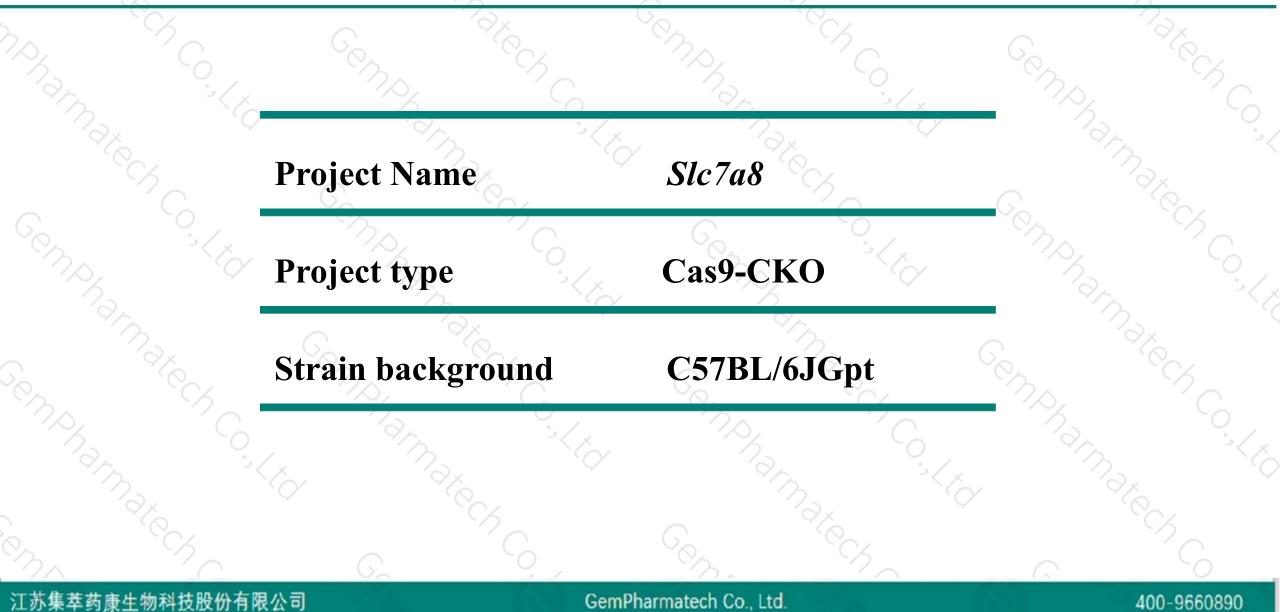
Slc7a8 Cas9-CKO Strategy

Designer: Reviewer: Design Date: Ruirui Zhang Huimin Su

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Project Overview

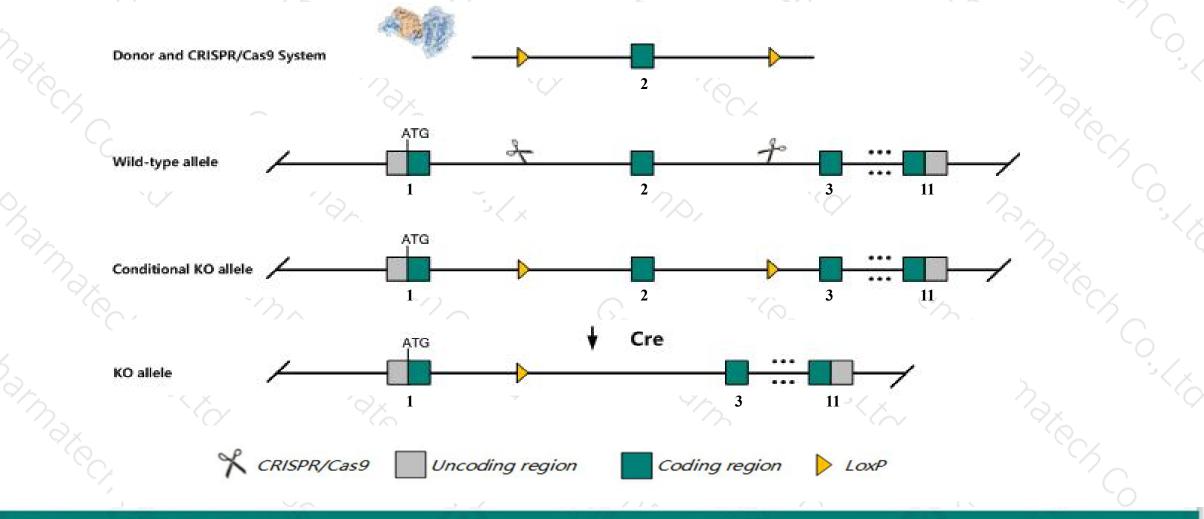




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Slc7a8* gene. The schematic diagram is as follows:



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The Slc7a8 gene has 2 transcripts. According to the structure of Slc7a8 gene, exon2 of Slc7a8-201 (ENSMUST00000022787.7) transcript is recommended as the knockout region. The region contains 205bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Slc7a8* 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 targeted mutation display hypoactivity, decreased motor performance, and resistance to pharmacologically induced seizures.
 - > The Slc7a8 gene is located on the Chr14. 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)



SIc7a8 solute carrier family 7 (cationic amino acid transporter, y+ system), member 8 [Mus musculus (house mouse)]

Gene ID: 50934, updated on 12-Aug-2019

Summary

Official Symbol SIc7a8 provided by MGI Official Full Name solute carrier family 7 (cationic amino acid transporter, y+ system), member 8 provided by MGI Primary source MGI:MGI:1355323 See related Ensembl:ENSMUSG00000022180 protein coding Gene type RefSeq status PROVISIONAL Organism Mus musculus Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Lineage Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus Also known as LAT2; AA408822 Biased expression in kidney adult (RPKM 137.5), duodenum adult (RPKM 56.6) and 13 other tissues See more Expression Orthologs human all

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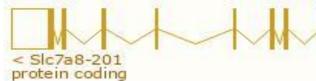


The gene has 2 transcripts, all transcripts are shown below:

Name 🖕	Transcript ID 🖕	bp 🛔	Protein 🖕	Biotype 💧	CCDS 🖕	UniProt 🖕	Flags 🖕		
SIc7a8-201	ENSMUST0000022787.7	4084	<u>531aa</u>	Protein coding	<u>CCDS27101</u> 교	<u>Q9QXW9</u> &	TSL:1	GENCODE basic	APPRIS P1
SIc7a8-202	ENSMUST00000226646.1	2155	No protein	Retained intron	-	÷	-		

59.72

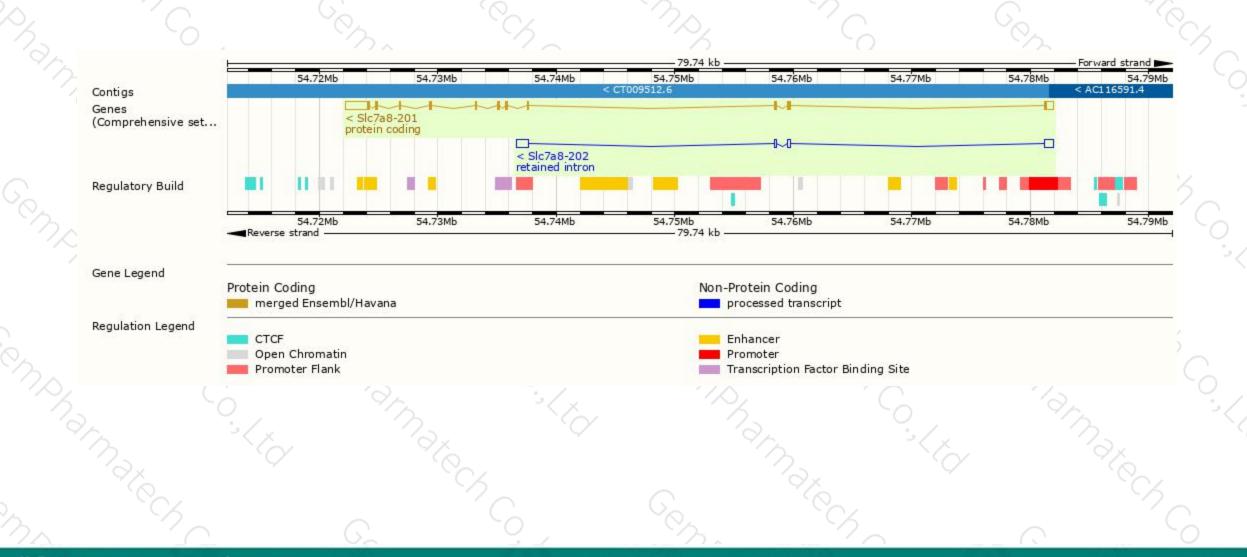
The strategy is based on the design of Slc7a8-201 transcript, The transcription is shown below



Reverse strand

Genomic location distribution





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Protein domain



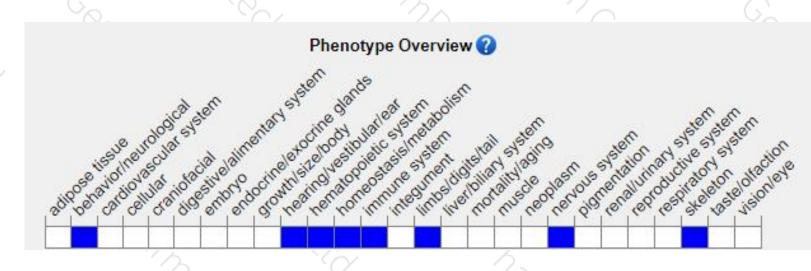
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1	ENSMUSP00000022 Transmembrane heli MobiDB lite Low complexity (Seg) TIGRFAM	L-type amino acid tra	nsporter						
	Pfam	Amino acid/p	olyamine transpo	orter I				200 10 10 10	
	PIRSF	Amino acid/polya	mine transporter	I					
	PANTHER.	PTHR11785 PTHR11785:SF113							
	Gene3D	1.20.1740.10							
	All sequence SNPs/i	Sequence variants	(dbSNP and all (other source:	5)	$\overline{\mathbf{u}}$	uù j	1 11 11	~
	Variant Legend	missense varia							°-< ×
	Scale bar	o 60	120	180	240	300	360	420	531
	Y Contraction		C'A C.	/					°C C

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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 targeted mutation display hypoactivity, decreased motor performance, and resistance to pharmacologically induced seizures.

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



