

Slc22a3 Cas9-CKO Strategy

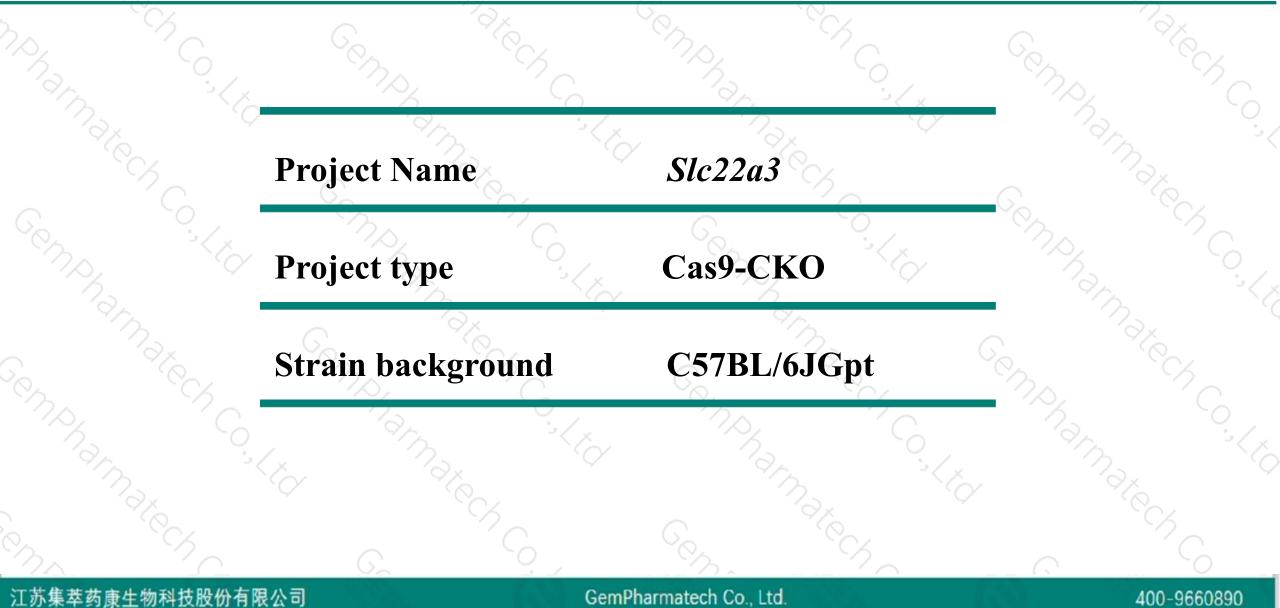
Designer: Reviewer:

Design Date:

Daohua Xu Huimin Su 2020-1-19

Project Overview



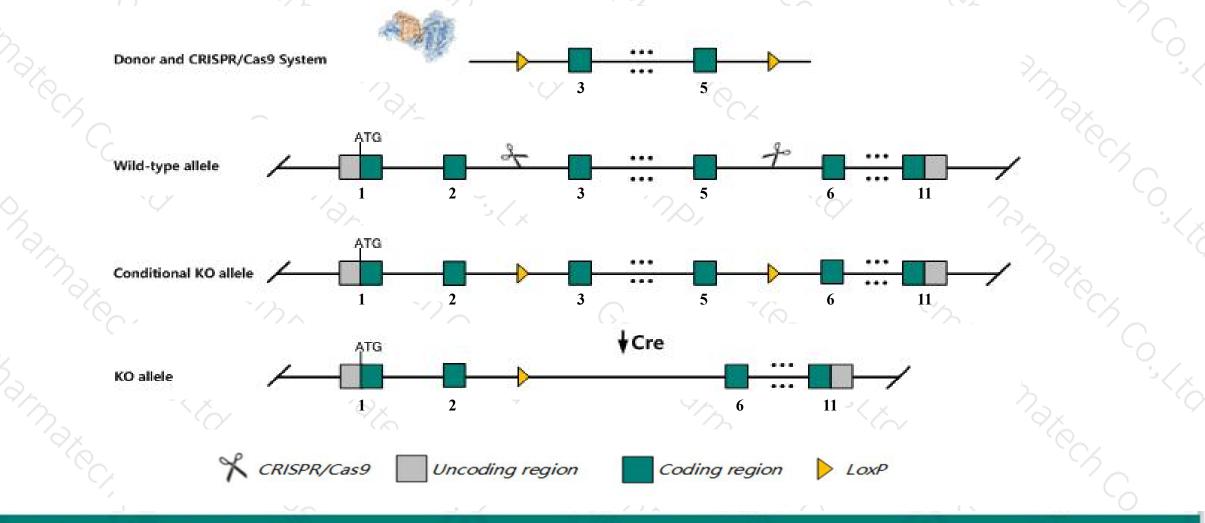


Conditional Knockout strategy



400-9660890

This model will use CRISPR/Cas9 technology to edit the *Slc22a3* gene. The schematic diagram is as follows:



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The Slc22a3 gene has 2 transcripts. According to the structure of Slc22a3 gene, exon3-exon5 of Slc22a3-201 (ENSMUST00000024595.3) transcript is recommended as the knockout region. The region contains 442bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Slc22a3* 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 exhibit increased susceptibility to paraquat-induced dopamine neuron neurotoxicity.
 - > The Slc22a3 gene is located on the Chr17. 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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SIc22a3 solute carrier family 22 (organic cation transporter), member 3 [Mus musculus (house mouse)]

Gene ID: 20519, updated on 19-Feb-2019

Summary

Official Symbol	SIc22a3 provided by MGI
Official Full Name	solute carrier family 22 (organic cation transporter), member 3 provided by MGI
Primary source	MGI:MGI:1333817
See related	Ensembl:ENSMUSG0000023828
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
	Muroidea; Muridae; Murinae; Mus; Mus
Also known as	EMT, Oct3, Orct3, SIca22a3
Expression	Biased expression in subcutaneous fat pad adult (RPKM 16.2), genital fat pad adult (RPKM 10.1) and 10 other tissues See more
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
SIc22a3-201	ENSMUST0000024595.3	3504	<u>551aa</u>	Protein coding	CCDS28391	Q547K2 Q9WTW5	TSL:1 GENCODE basic APPRIS P1
SIc22a3-202	ENSMUST00000233535.1	4127	<u>363aa</u>	Nonsense mediated decay	(.	A0A3B2W7B3	

The strategy is based on the design of Slc22a3-201 transcript, The transcription is shown below

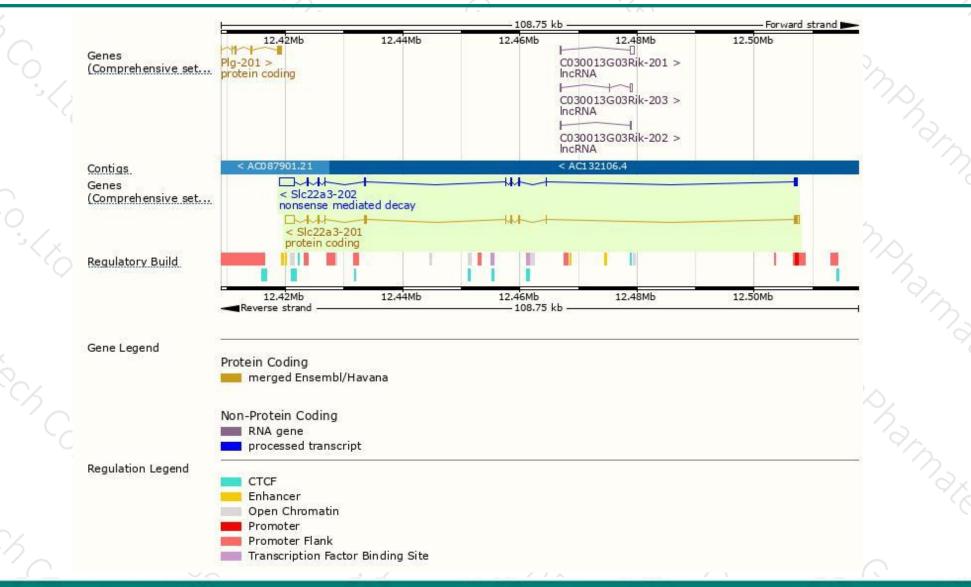
< Slc22a3-201 protein coding

- 87.74 kb -

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Genomic location distribution





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Protein domain



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	ENSMUSP00000024 Transmembrane heli		•					-	-		-	3
	Low complexity (Seg) TIGRFAM	Organi	c cation trans	port protein/S\	VOP							Ó
	Superfamily			MR	S transporter	superfamily						
	Pfam			М	ajor facilitator	, sugar transp	orter-like					
	PROSITE profiles			Major facilitator	- superfamily	domain						
	PROSITE patterns							s	ugar transpor	ter, conserve	ed site	C
2	PANTHER	PTHR2406	4:SF52									-0 .,/
		PTHR2406	14									
	Gene3D			1.20.1250	,20							
	CDD			Maj	or facilitator :	superfamily do	main					
	All sequence SNPs/i	Sequenc	e variants (dbSNP and al	l other sourc	es)	1.11-1	RE	11	1704	11	6
	Variant Legend	📕 spli	sense varia ce region va onymous va	ariant	6				80	103		
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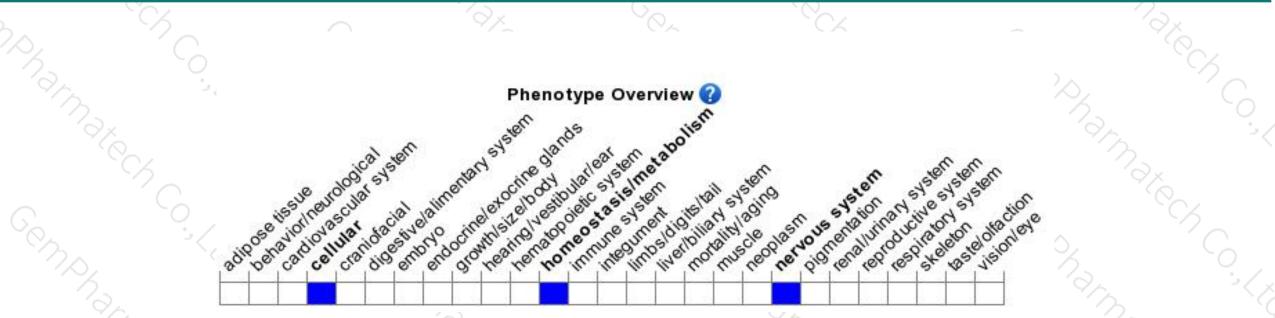
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Mouse phenotype description(MGI)





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 exhibit increased susceptibility to paraquat-induced dopamine neuron neurotoxicity.



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



