

Glrx Cas9-CKO Strategy

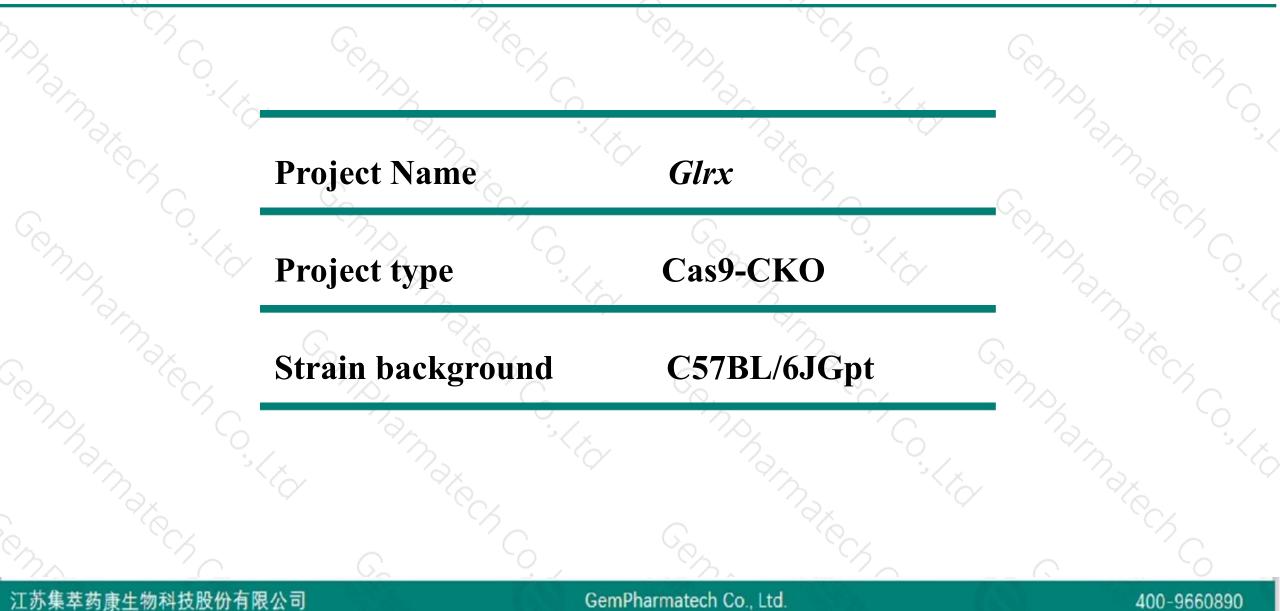
Designer: Huan Wang

Reviewer: Huan Fan

Design Date: 2019-1-8

Project Overview



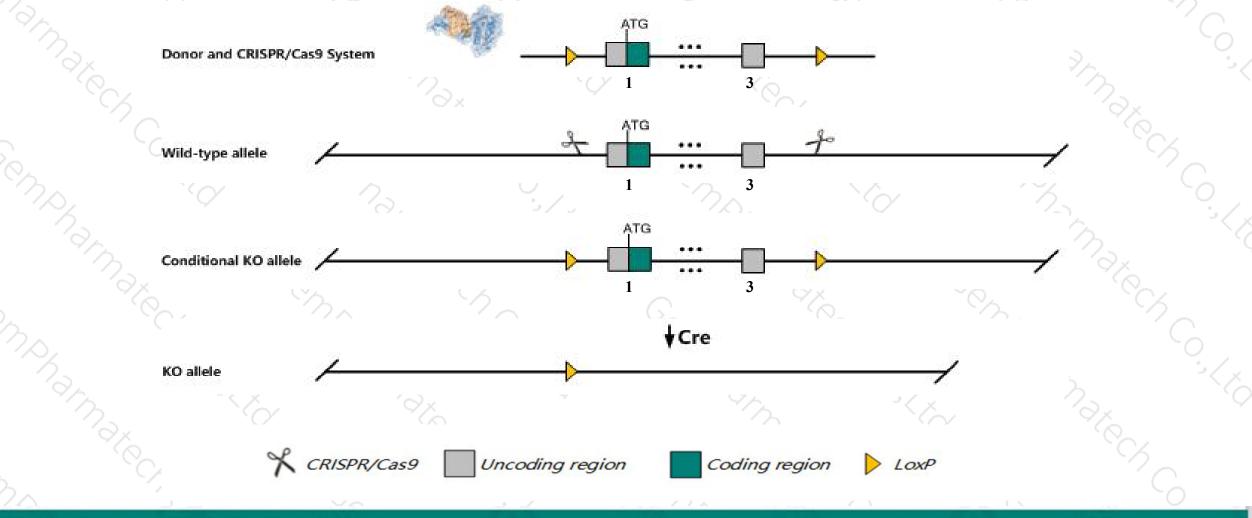


Conditional Knockout strategy



400-9660890

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



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> The *Glrx* gene has 4 transcripts. According to the structure of *Glrx* gene, exon1-exon3 of *Glrx-201*(ENSMUST0000022082.7) transcript is recommended as the

knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.

> In this project we use CRISPR/Cas9 technology to modify *Glrx* 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 null allele do not exhibit any increased injury in response to oxidative insults to the heart or lungs but mouse embryonic fibroblast derived from these embryos are more sensative to diquat and paraqut and more resistant to apoptosis induced by TNF-alpha plus actinomyosin D.
The *Glrx* gene is located on the Chr13. 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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GIrx glutaredoxin [Mus musculus (house mouse)]

Gene ID: 93692, updated on 13-Mar-2020

Summary

Official Symbol	GIrx provided by MGI
Official Full Name	glutaredoxin provided by MGI
Primary source	MGI:MGI:2135625
See related	Ensembl:ENSMUSG0000021591
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	C86710, D13Wsu156e, Glrx1, Grx1, TTase
Expression	Broad expression in large intestine adult (RPKM 132.0), placenta adult (RPKM 90.4) and 25 other tissues See more
Orthologs	human all

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Transcript information (Ensembl)



The gene has 4 transcripts, all transcripts are shown below:

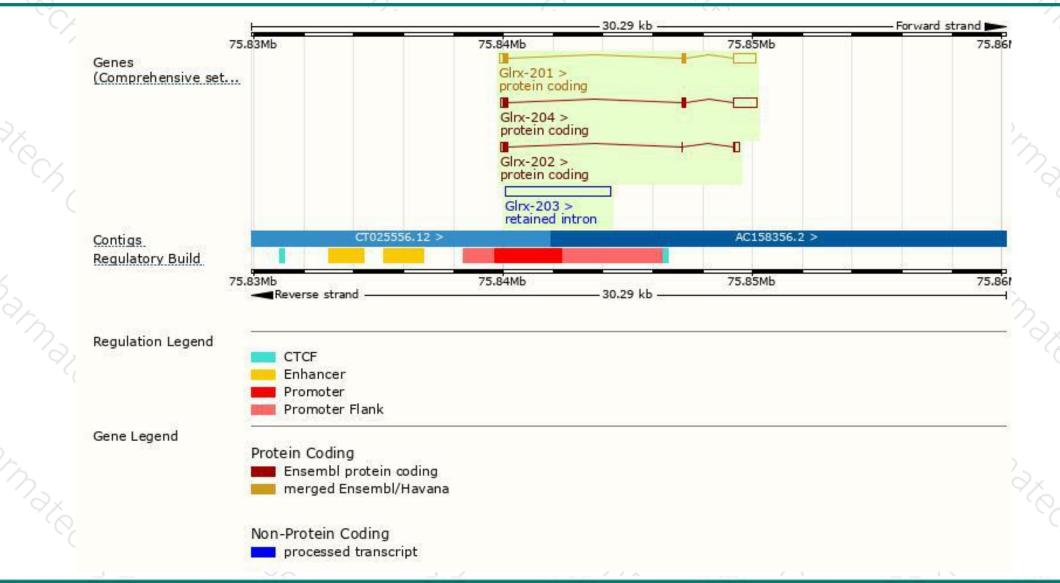
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Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Glrx-201	ENSMUST0000022082.7	1345	<u>107aa</u>	Protein coding	CCDS26651	Q3U6L3 Q9QUH0	TSL:1 GENCODE basic APPRIS P1
Glrx-204	ENSMUST00000223120.1	1333	<u>107aa</u>	Protein coding	CCDS26651	Q3U6L3 Q9QUH0	TSL:5 GENCODE basic APPRIS P1
Glrx-202	ENSMUST00000220523.1	493	<u>84aa</u>	Protein coding	1 <u>1</u> 1	A0A1Y7VM65	TSL:3 GENCODE basic
Glrx-203	ENSMUST00000222530.1	4228	No protein	Retained intron			TSL:NA
C. Jun	1 200	100			N / N		

The strategy is based on the design of *Glrx-201* transcript, the transcription is shown below:

			10.	Forward strand			
Glrx-201 > protein codi	ng						
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Genomic location distribution





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Protein domain



				°S		20j				200	°°C	
ENSMUSP00000152 TIGRFAM		Glu	taredoxin, i	eukaryotic/vi	ria						-	Ś
Superfamily	Thiore	daxin-like s	uperfamily									2<
Prints Pfam		indexed all the	taredoxin s taredoxin	ubgroup	_				2	i i		
PROSITE profiles	Glutare	doxin		-								
PROSITE patterns		e	ilutaredoxii	active site							, O	
PANTHER	PTHR4618	5									<u>.</u>	
Gene3D	3,40,30,1	0										
CDD.		cd03	419									
All sequence SNPs/i	Sequence	e variants	(dbSNP ar	id all other :	sources)	M					6	
Variant Legend	miss	ense varia	ant									
Scale bar	ю	10	20	30	40	50	60	70	80	90	107	~
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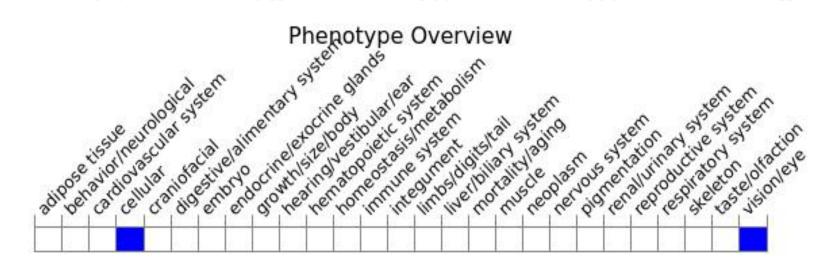
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Mouse phenotype description(MGI)





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 null allele do not exhibit any increased injury in response to oxidative insults to the heart or lungs but mouse embryonic fibroblast derived from these embryos are more sensative to diquat and paraqut and more resistant to apoptosis induced by TNF-alpha plus actinomyosin D



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



