

Glrx Cas9-KO Strategy

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Reviewer: Yumeng Wang

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Overview

Target Gene Name

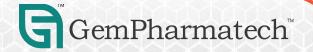
• Glrx

Project Type

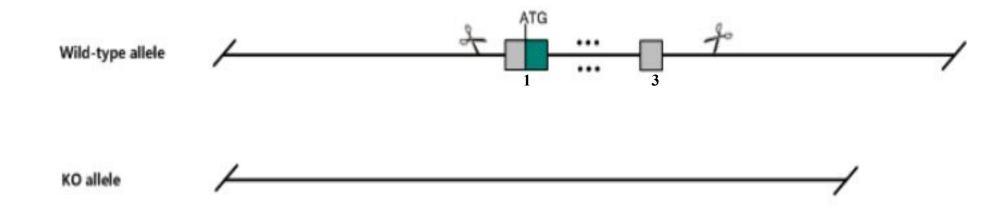
• Cas9-KO

Genetic Background

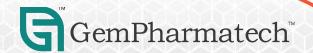
• C57BL/6JGpt



Strain Strategy







Technical Information

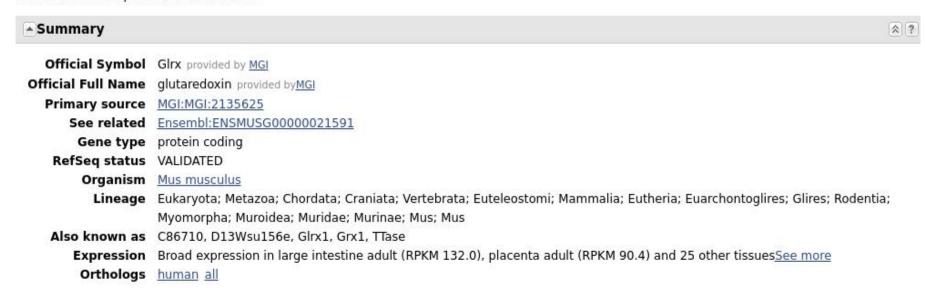
- The *Glrx* gene has 4 transcripts. According to the structure of *Glrx* gene, exon1-exon3 of *Glrx*-201 (ENSMUST00000022082.8) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Glrx* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs 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 ontarget amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.



Gene Information

Glrx glutaredoxin [Mus musculus (house mouse)]

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



Source: https://www.ncbi.nlm.nih.gov/



Transcript Information

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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Glrx-201	ENSMUST00000022082.7	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	10	A0A1Y7VM65	TSL:3 GENCODE basic
Glrx-203	ENSMUST00000222530.1	4228	No protein	Retained intron		70	TSL:NA

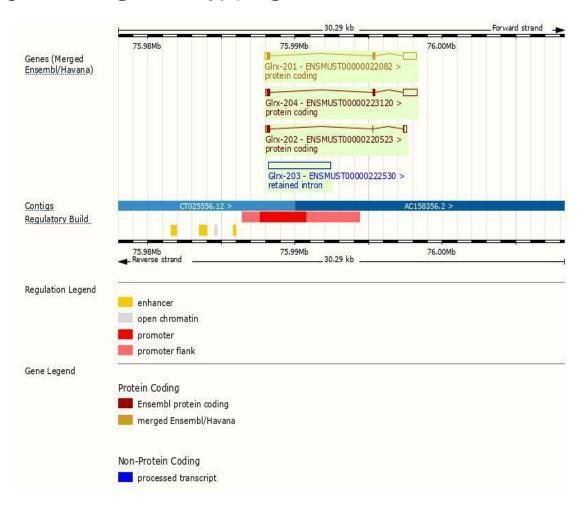
The strategy is based on the design of *Glrx*-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org



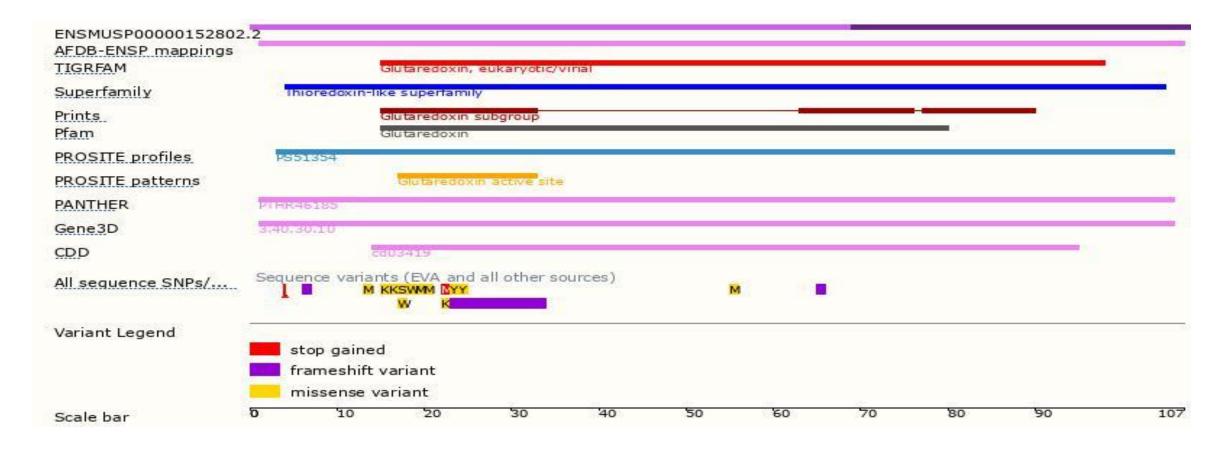
Genomic Information





Source: : https://www.ensembl.org

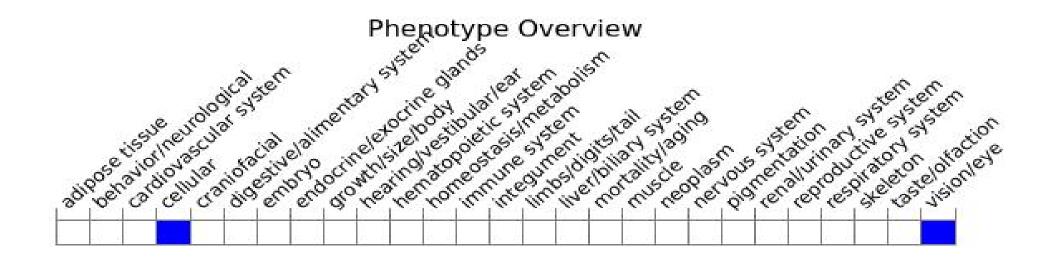
Protein Information





Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



• 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.

GemPharmatech

Source: https://www.informatics.jax.org

Important Information

- *Glrx* is located on Chr13. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

