

Ppp1r3g Cas9-KO Strategy

Designer: XiaoJing Li

Reviewer: Lu Chen

Design Date: 2023-9-26

Overview

Target Gene Name

• *Ppp1r3g*

Project Type

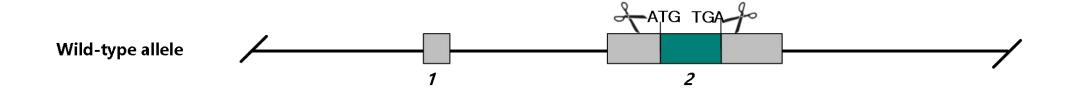
• Cas9-KO

Genetic Background

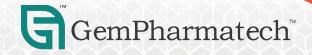
• C57BL/6JGpt



Strain Strategy

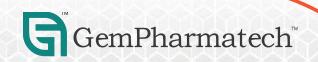


Schematic representation of CRISPR-Cas9 engineering used to edit the *Ppp1r3g* gene.



Technical Information

- The *Ppp1r3g* gene has 3 transcripts. According to the structure of *Ppp1r3g* gene, exon 2 of *Ppp1r3g*-203 (ENSMUST00000225537.2) 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 *Ppp1r3g* 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 on-target 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

Ppp1r3g protein phosphatase 1, regulatory subunit 3G [Mus musculus (house mouse)]

Gene ID: 76487, updated on 10-Oct-2020



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



Transcript Information

The gene has 3 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ppp1r3g-203	ENSMUST00000225537.2	2389	<u>347aa</u>	Protein coding	CCDS49237		GENCODE basic , APPRIS P1 ,
Ppp1r3g-201	ENSMUST00000132661.2	2361	<u>347aa</u>	Protein coding	CCDS49237		TSL:1 , GENCODE basic , APPRIS P1 ,
Ppp1r3g-202	ENSMUST00000172766.3	571	No protein	Processed transcript	I I		TSL:3,

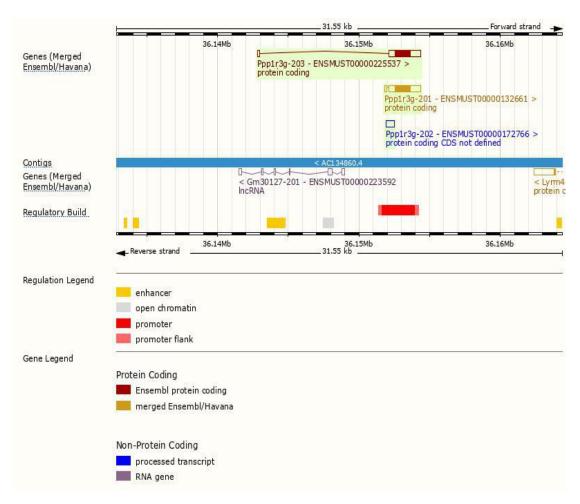
The strategy is based on the design of *Ppp1r3g*-203 transcript, the transcription is shown below:



Source: https://www.ensembl.org



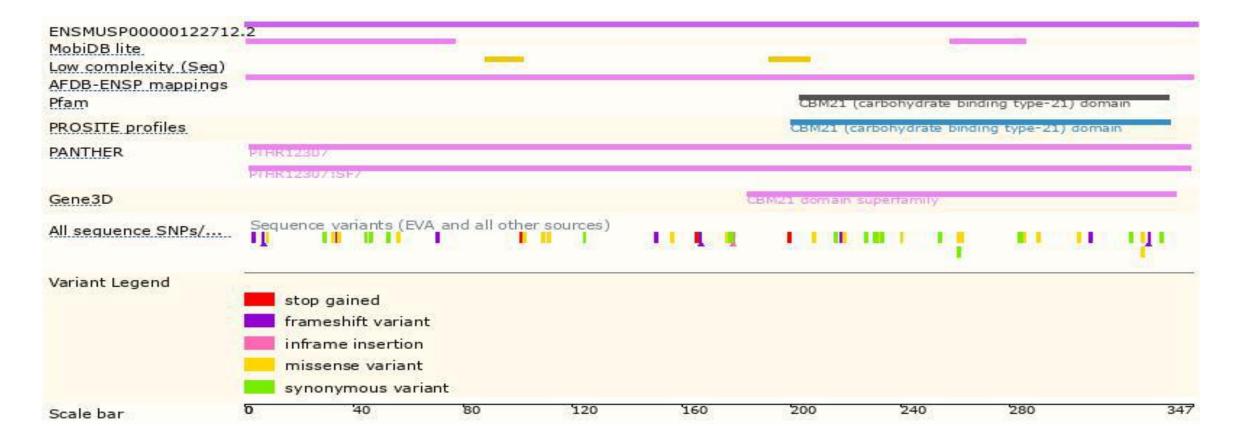
Genomic Information





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

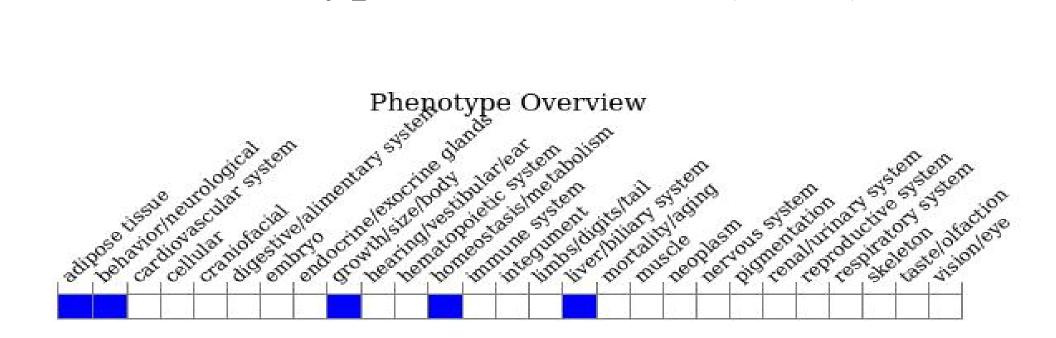
Protein Information





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

Mouse Phenotype Information (MGI)



• Under a high-fat diet, mice homozygous for a knock-out allele show decreased susceptibility to diet-induced obesity and hepatic steatosis along with a higher metabolic rate, increased food intake, and decreased glycogen levels in liver and adipose tissue.

GemPharmatech™

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

Important Information

- *Ppp1r3g* 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.
- The knockout region is about 3.5 kb away from the 5'terminal of *Gm30127*-201 lncRNA gene, which may affect its 5'terminal regulatory function.
- 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.

