

Ppp1r15a Cas9-CKO Strategy

Designer: Huan Wang

Reviewer: Lingyan Wu

Design Date: 2021-6-29

Project Overview

Project Name

Ppp1r15a

Project type

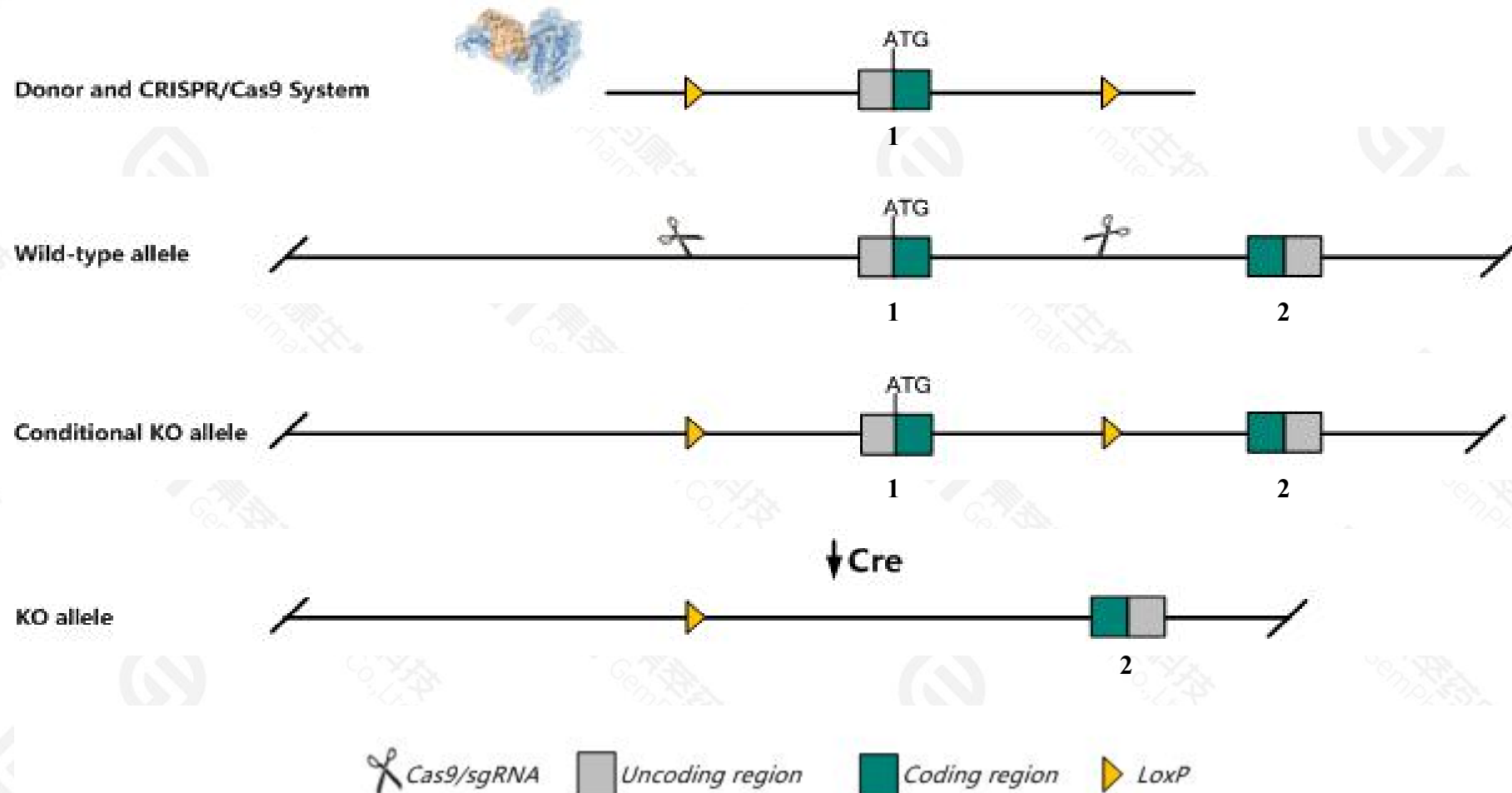
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ppp1r15a* gene. The schematic diagram is as follows:



- The *Ppp1r15a* gene has 3 transcripts. According to the structure of *Ppp1r15a* gene, exon1 of *Ppp1r15a*-202(ENSMUST00000167273.2) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ppp1r15a* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor was constructed. Cas9, sgRNA 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 was 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, homozygous mutant mice show abnormal cellular responses to either ER- or oxidative-stress.
- The floxed region contains functional region of the *Plekha4* gene. Knockout the region may affect the function of *Plekha4* gene.
- The Intron1 is only 416bp, loxp insertion may affect mRNA splicing.
- The floxed region is about 50bp from the 3'UTR of *Tulp2* gene. Knockout the region may affect the function of *Tulp2* gene.
- The *Ppp1r15a* gene is located on the Chr7. 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)

Ppp1r15a protein phosphatase 1, regulatory subunit 15A [Mus musculus (house mouse)]

Gene ID: 17872, updated on 2-Feb-2021

Summary



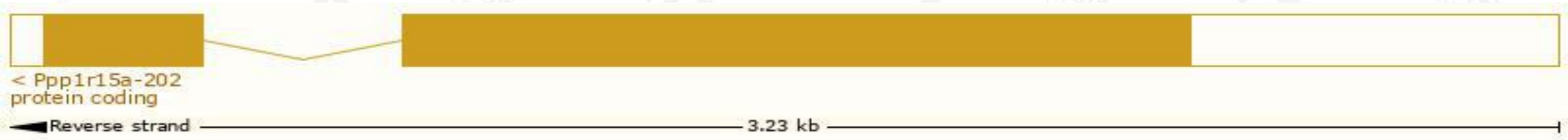
Official Symbol	Ppp1r15a provided by MGI
Official Full Name	protein phosphatase 1, regulatory subunit 15A provided by MGI
Primary source	MGI:MGI:1927072
See related	Ensembl:ENSMUSG00000040435
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	9630030H21, Gadd3, Gadd34, Myd116
Expression	Broad expression in liver E14 (RPKM 79.4), liver E14.5 (RPKM 76.2) and 26 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

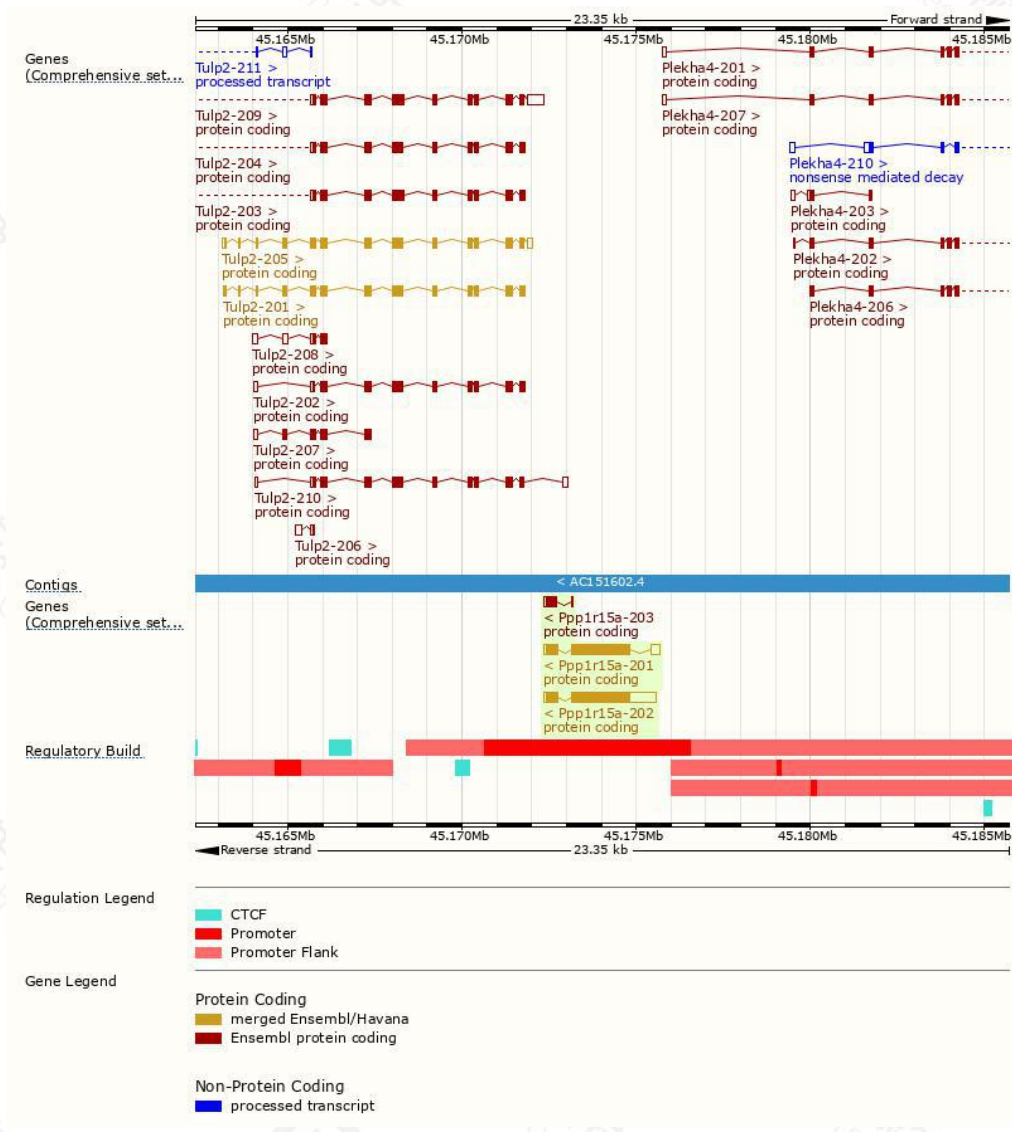
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ppp1r15a-202	ENSMUST00000167273.2	2809	657aa	Protein coding	CCDS21249		TSL:2 , GENCODE basic , APPRIS P1 ,
Ppp1r15a-201	ENSMUST00000042105.11	2333	657aa	Protein coding	CCDS21249		TSL:1 , GENCODE basic , APPRIS P1 ,
Ppp1r15a-203	ENSMUST00000211212.2	389	103aa	Protein coding	-		CDS 5' incomplete , TSL:3 ,

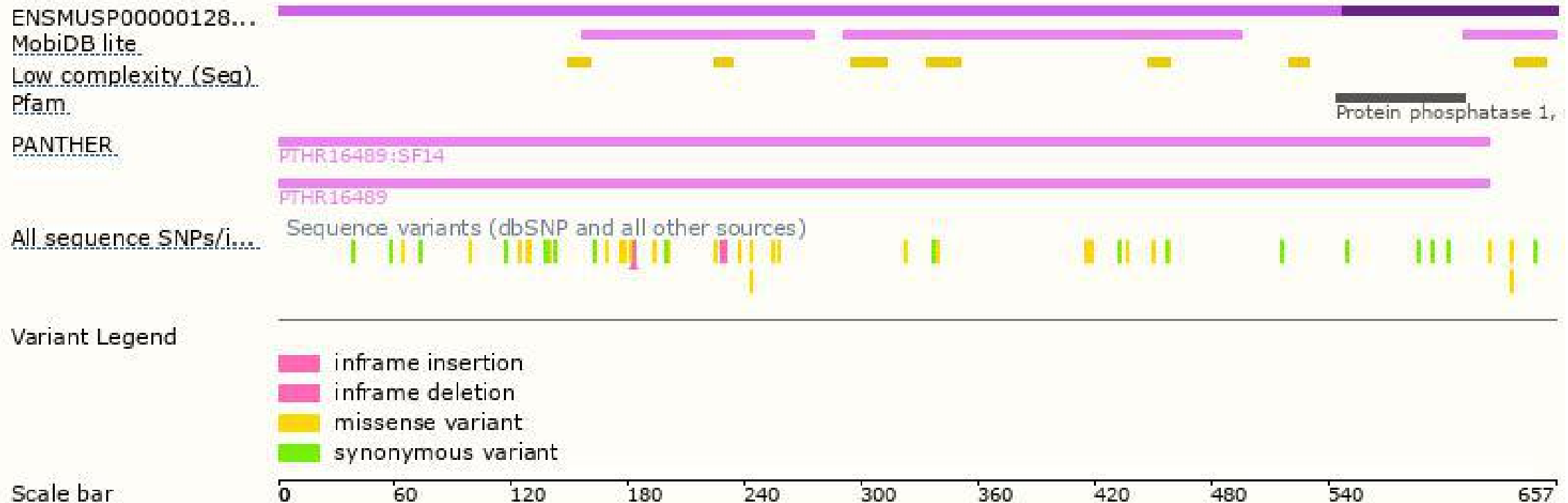
The strategy is based on the design of *Ppp1r15a-202* transcript,the transcription is shown below:



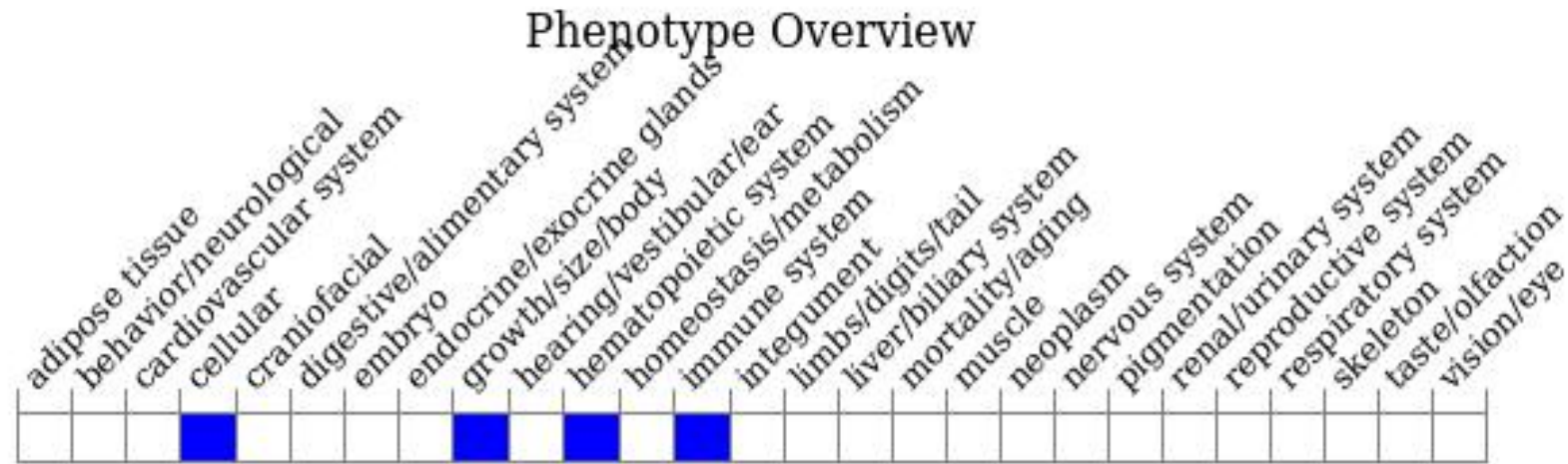
Genomic location distribution



Protein domain



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, homozygous mutant mice show abnormal cellular responses to either ER- or oxidative- stress.

If you have any questions, you are welcome to inquire.

Tel: 025-5864 1534

