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Ppp1r12b Cas9-CKO Strategy

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Reviewer: Yao Yu

Design Date: 2023-9-26

Overview

Target Gene Name

- *Ppp1r12b*

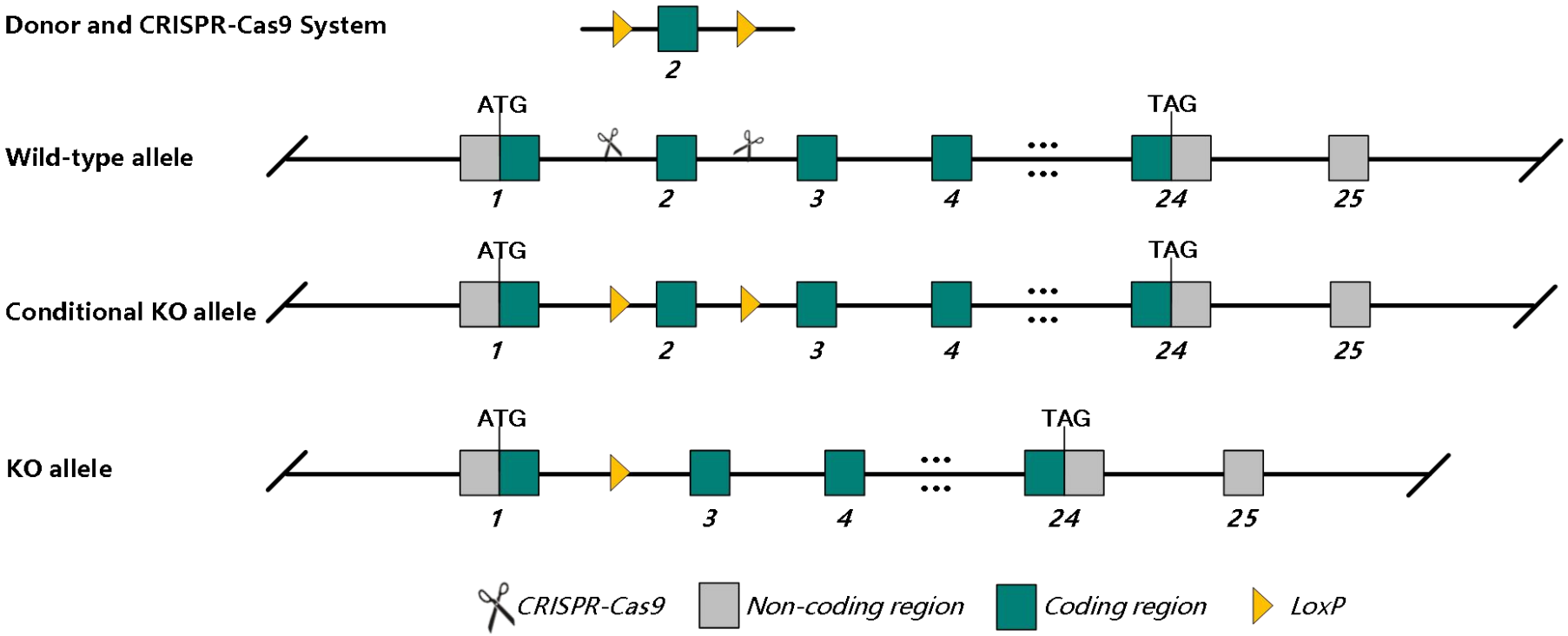
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Ppp1r12b* gene.

Technical Information

- The *Ppp1r12b* gene has 11 transcripts. According to the structure of *Ppp1r12b* gene, exon 2 of *Ppp1r12b*-209 (ENSMUST00000168381.8) is recommended as the knockout region. The region contains 131 bp of coding sequence. Knocking out the region will result in disruption of gene function.
- In this project we use CRISPR-Cas9 technology to modify *Ppp1r12b* 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 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.
- 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.

Gene Information

Ppp1r12b protein phosphatase 1, regulatory subunit 12B [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 329251, updated on 7-Sep-2023

Summary

Official Symbol	Ppp1r12b provided by MGI
Official Full Name	protein phosphatase 1, regulatory subunit 12B provided by MGI
Primary source	MGI:MGI:1916417
See related	Ensembl:ENSMUSG00000073557 AllianceGenome:MGI:1916417
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	Mypt2; 1810037O03Rik; 9530009M10Rik
Summary	Predicted to enable enzyme inhibitor activity and phosphatase regulator activity. Predicted to be involved in signal transduction. Located in A band and Z disc. Orthologous to human PPP1R12B (protein phosphatase 1 regulatory subunit 12B). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Broad expression in bladder adult (RPKM 30.2), heart adult (RPKM 14.8) and 21 other tissues See more
Orthologs	human all

NEW **Try the new Gene table**
Try the new Transcript table

Genomic context

Location: 1 E4; 1 58.24 cM — See Ppp1r12b in Genome Data Viewer

Exon count: 29

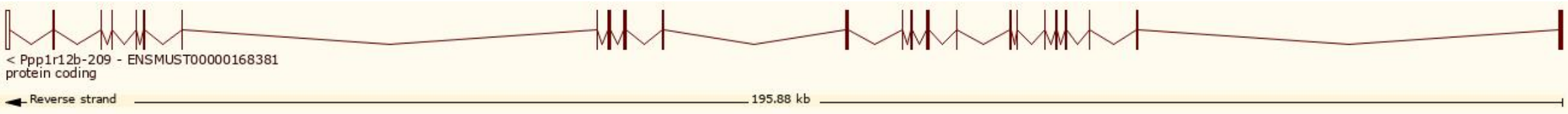
https://www.ncbi.nlm.nih.gov/gene/329251

Transcript Information

The gene has 11 transcripts, all transcripts are shown below:

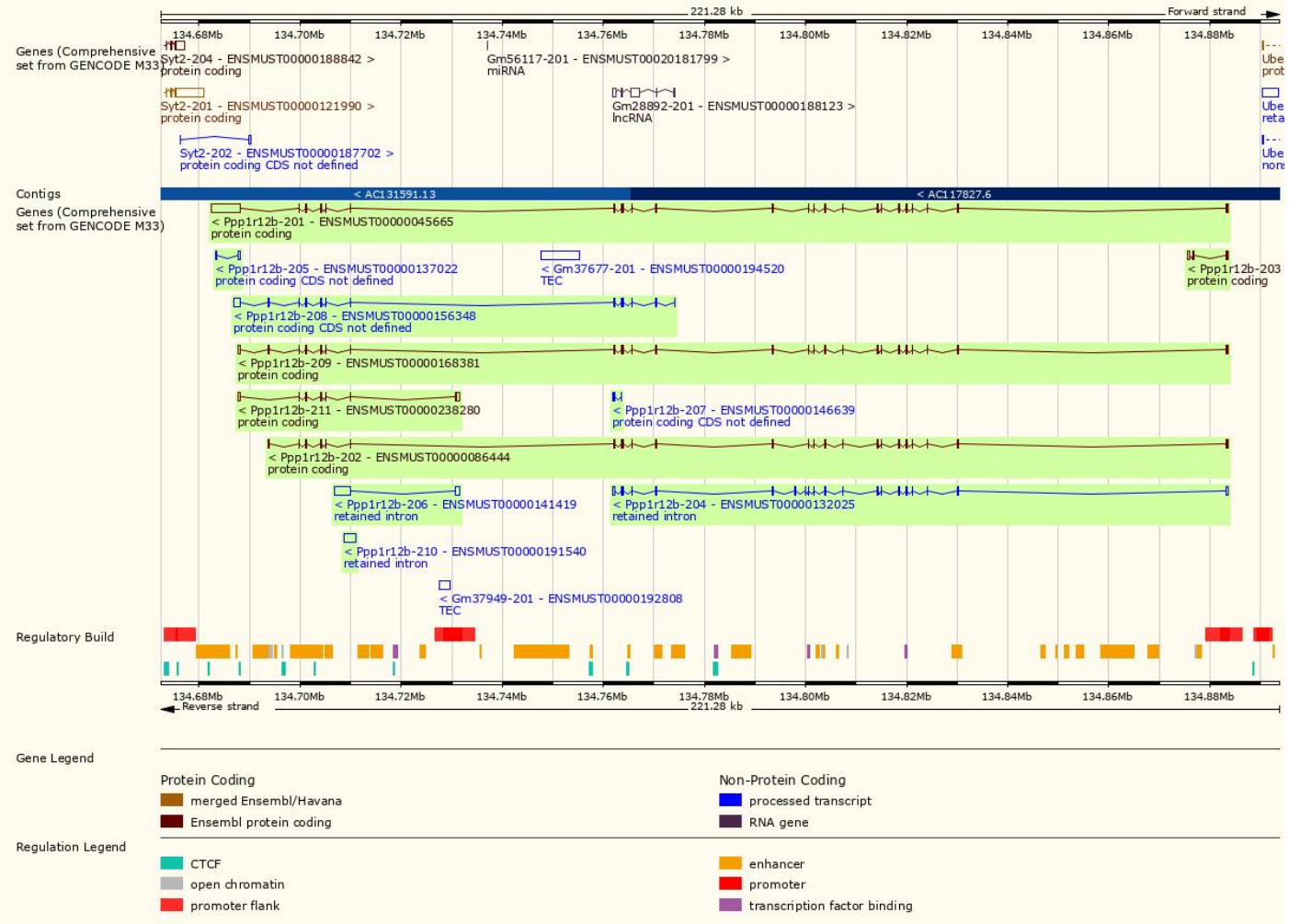
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000045665.13	Ppp1r12b-201	8840	976aa	Protein coding		Q8BG95-1	GENCODE basic APPRIS P1 TSL:5
ENSMUST00000086444.6	Ppp1r12b-202	3137	992aa	Protein coding	CCDS35717	A6H644	GENCODE basic TSL:5
ENSMUST00000112163.2	Ppp1r12b-203	828	105aa	Protein coding		Q8BG95-2	GENCODE basic TSL:1
ENSMUST00000132025.2	Ppp1r12b-204	3213	No protein	Retained intron		-	TSL:1
ENSMUST00000137022.2	Ppp1r12b-205	660	No protein	Protein coding CDS not defined		-	TSL:2
ENSMUST00000141419.2	Ppp1r12b-206	3808	No protein	Retained intron		-	TSL:1
ENSMUST00000146639.2	Ppp1r12b-207	277	No protein	Protein coding CDS not defined		-	TSL:5
ENSMUST00000156348.8	Ppp1r12b-208	2671	No protein	Protein coding CDS not defined		-	TSL:1
ENSMUST00000168381.8	Ppp1r12b-209	3621	992aa	Protein coding	CCDS35717	A6H644	Ensembl Canonical GENCODE basic TSL:1
ENSMUST00000191540.2	Ppp1r12b-210	2351	No protein	Retained intron		-	TSL:NA
ENSMUST00000238280.2	Ppp1r12b-211	1521	186aa	Protein coding		A0A571BGD8	GENCODE basic

The strategy is based on the design of *Ppp1r12b*-209 transcript, the transcription is shown below:



Source: http://asia.ensembl.org/

Genomic Information



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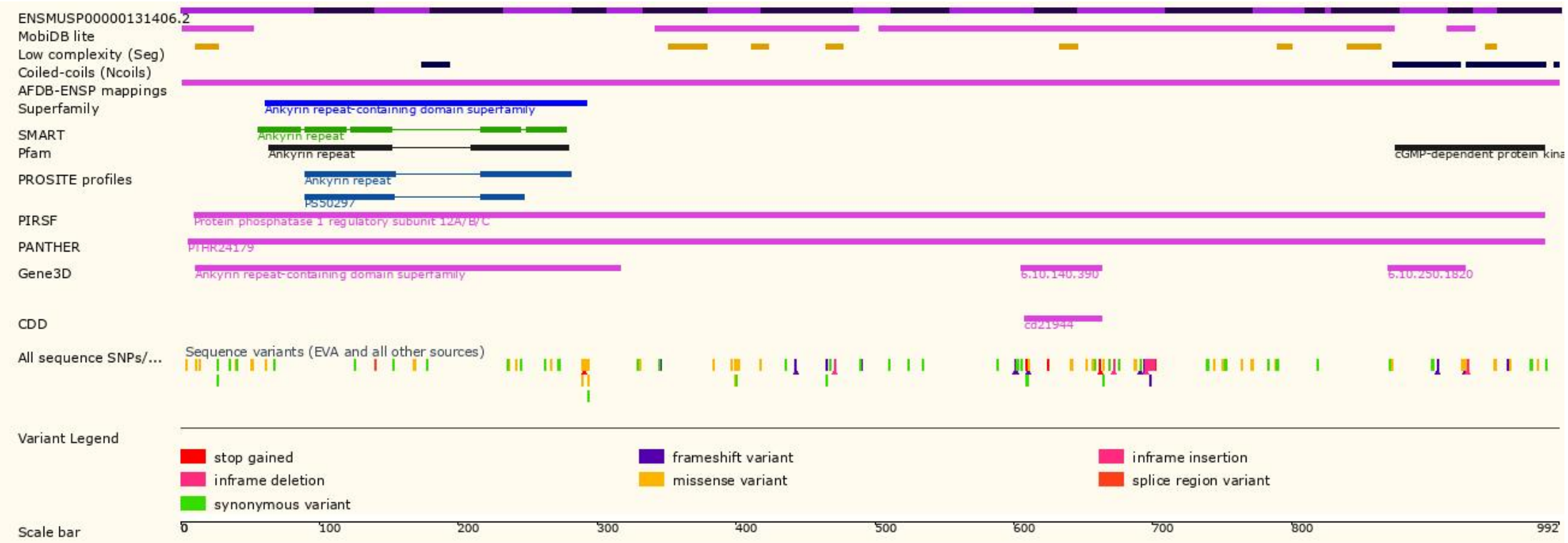
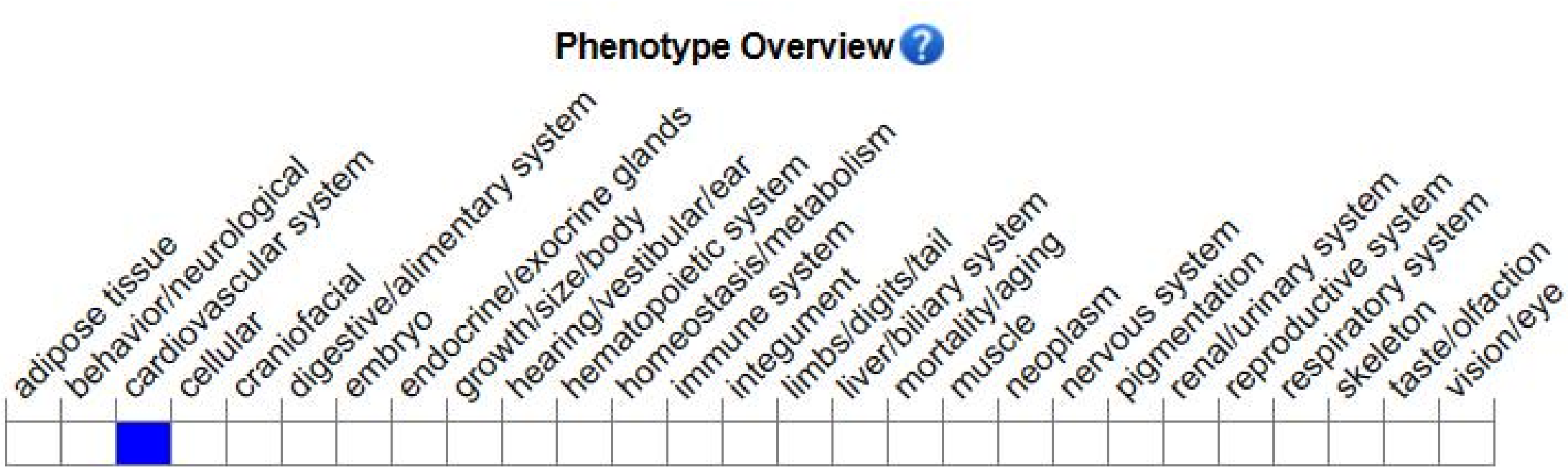
Source: http://asia.ensembl.org/

Protein Information



Source: https://www.ensembl.org

Mouse Phenotype Information (MGI)

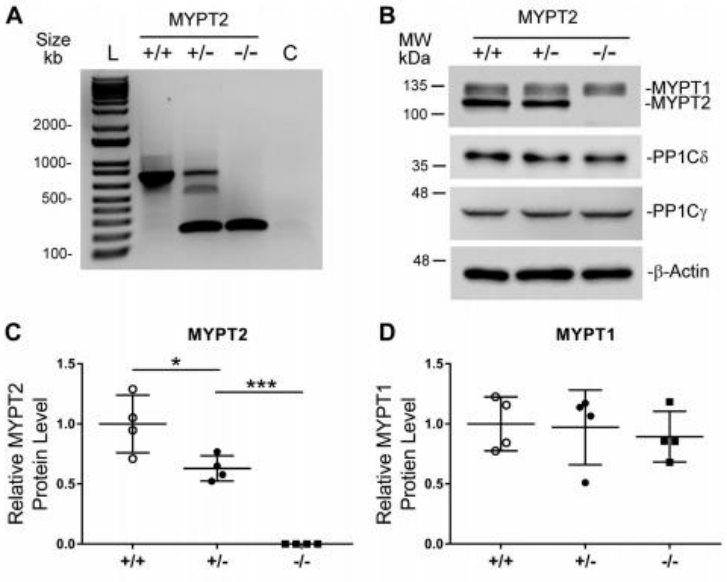


Source: https://www.informatics.jax.org

Important Information

- This stratergy may not affect *Ppp1r12b*-203, *Ppp1r12b*-205, *Ppp1r12b*-206, *Ppp1r12b*-207, *Ppp1r12b*-208, *Ppp1r12b*-210 and *Ppp1r12b*-211 transcript.
- *Ppp1r12b* is located on Chr 1. 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 risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Reference



In this paper, we characterize MYPT2 KO mice, which were developed by the Mutant Mouse Resource and Research Center. The mice contain a 580-nucleotide deletion in the gene encoding MYPT2 (*Ppp1r12b*), which removes exon 2, creating a frameshift in the mRNA-coding sequence and producing a stop codon 12 amino acids past the splice site. These mice are in a C57BL/6N background, which has been recently shown to lack MLCK3 protein expression, due to a point mutation that produces an out of frame start codon five nucleotides upstream from the normal start codon (20). The C57BL/6N substrains are known to be susceptible to heart failure (21), and the lack of MLCK3 is likely a major contributor to this phenotype. In this study, we found that the

[1] Hu T, Kalyanaraman H, Pilz RB, Casteel DE. Phosphatase regulatory subunit MYPT2 knockout partially compensates for the cardiac dysfunction in mice caused by lack of myosin light chain kinase 3. J Biol Chem. 2023 Apr;299(4):104584. doi: 10.1016/j.jbc.2023.104584. Epub 2023 Mar 7. PMID: 36889588; PMCID: PMC10124902.