Ppp2r5c Cas9-CKO Strategy

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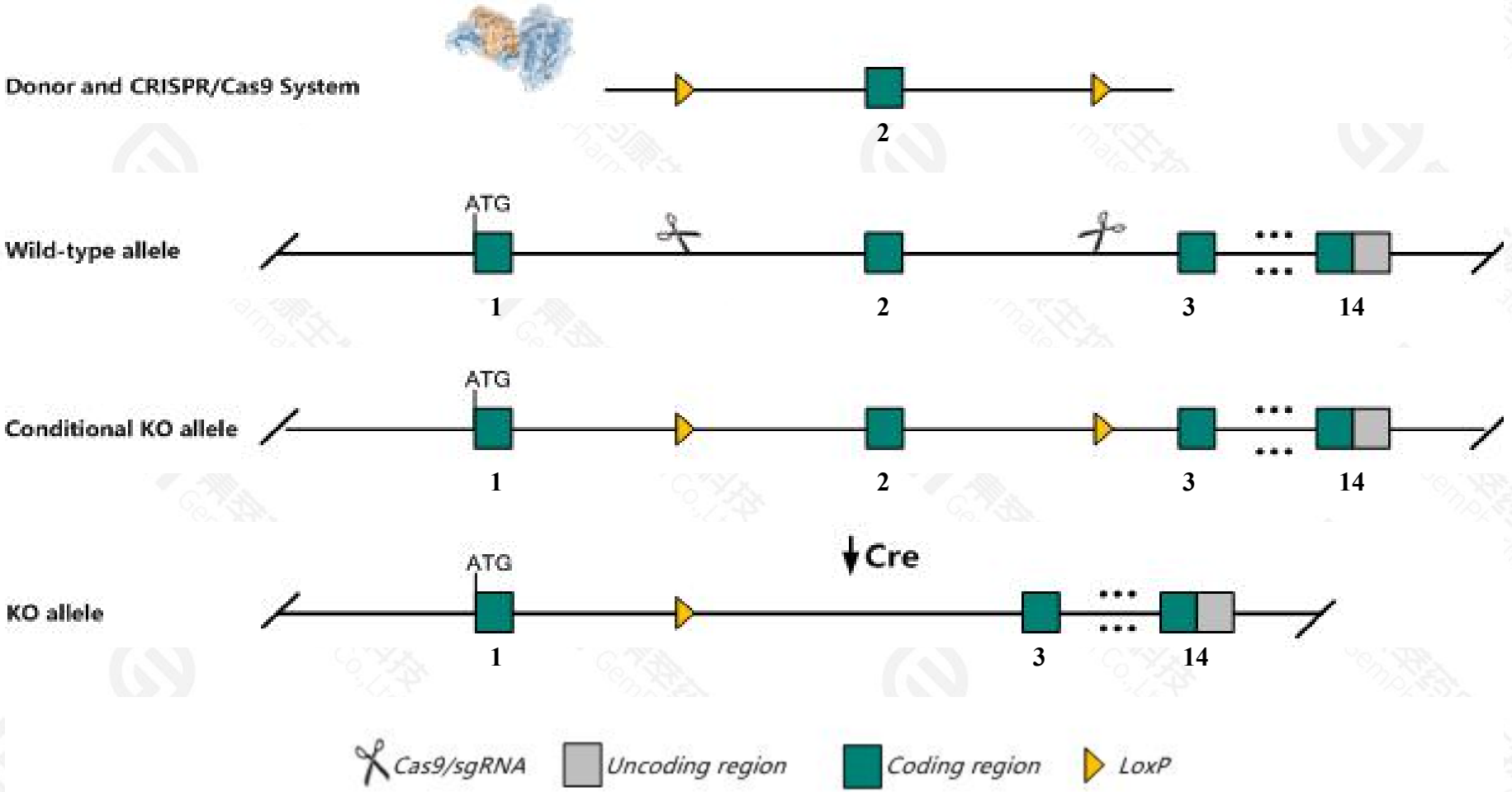
Project Overview

Project Name	***Ppp2r5c***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ppp2r5c* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ppp2r5c* gene has 7 transcripts. According to the structure of *Ppp2r5c* gene, exon2 of *Ppp2r5c-201*(ENSMUST00000084985.10) transcript is recommended as the knockout region.The region contains 200bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Ppp2r5c* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector 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.

Notice

➢ According to the existing MGI data,mice homozygous for a gene-trapped allele show partial neonatal lethality, hypoactivity, and abnormal ventricular septum formation associated with increased fetal cardiomyocyte apoptosis. Surviving homozygotes develop obesity and show an abnormal gait, decreased grip strength, and impaired balance.

➢ Transcript *Ppp2r5c-206* may not be affected.

➢ The *Ppp2r5c* gene is located on the Chr12. 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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Ppp2r5c protein phosphatase 2, regulatory subunit B', gamma [Mus musculus (house mouse)]

Gene ID: 26931, updated on 13-Mar-2020

Summary

Official Symbol	Ppp2r5c provided by MGI
Official Full Name	protein phosphatase 2, regulatory subunit B', gamma provided byMGI
Primary source	MGI:MGI:1349475
See related	Ensembl:ENSMUSG00000017843
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	2610043M05Rik, 2700063L20Rik, AI060890, AW545884, C85228, D12Bwg0916e
Expression	Ubiquitous expression in testis adult (RPKM 41.8), cortex adult (RPKM 18.2) and 27 other tissuesSee more
Orthologs	human all

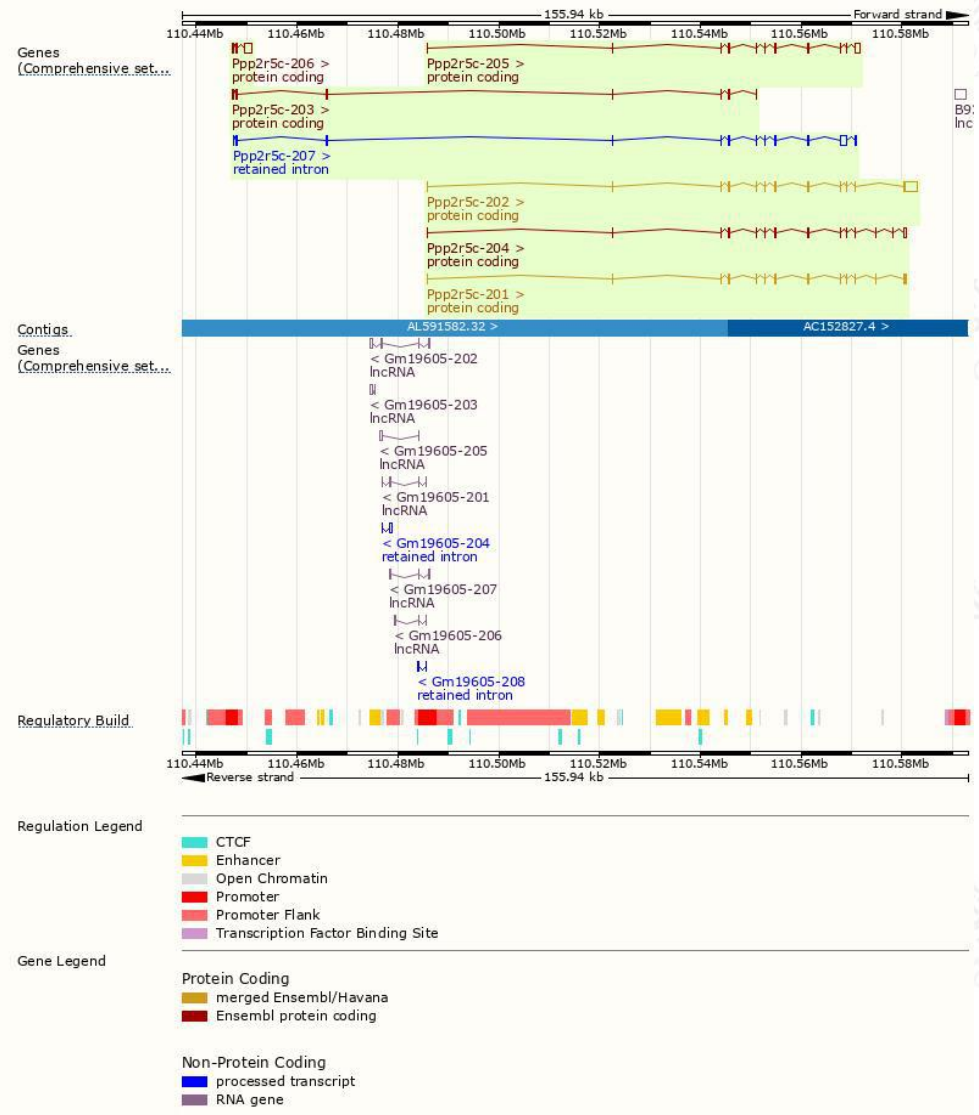
Transcript information（Ensembl）

The gene has 7 transcripts,all transcripts are shown below:

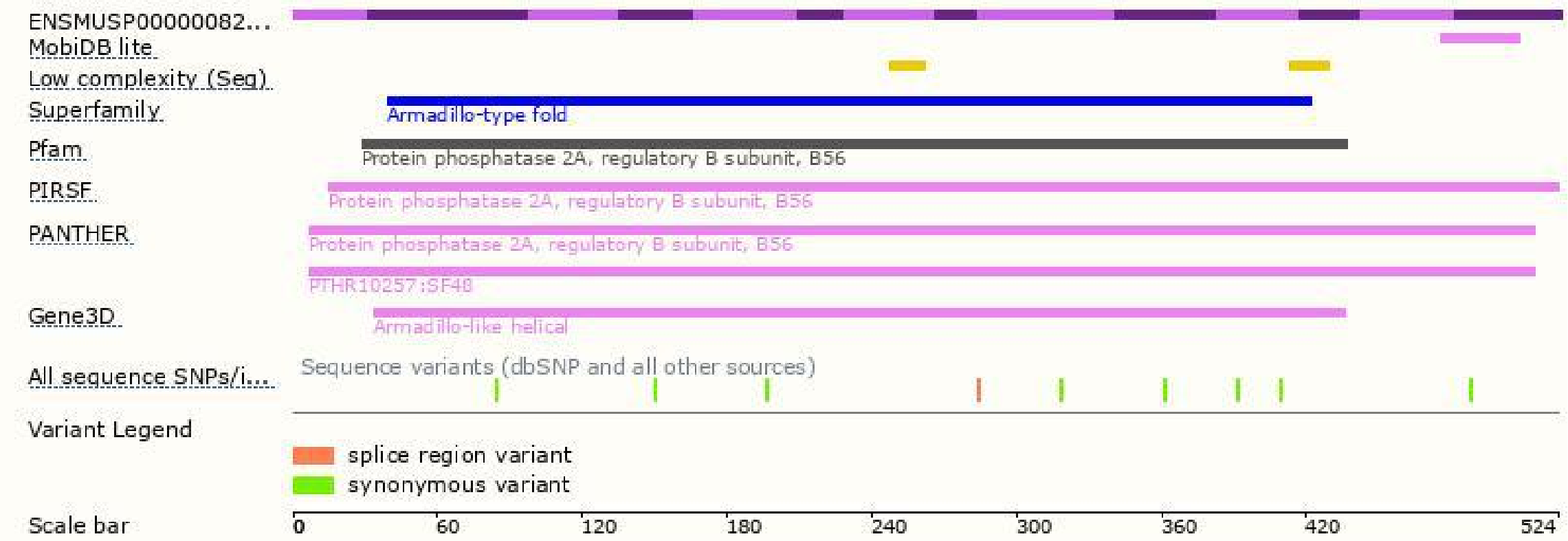
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ppp2r5c-202	ENSMUST00000109832.2	4108	485aa	Protein coding	CCDS36558	Q60996	TSL:2 GENCODE basic APPRIS ALT1
Ppp2r5c-201	ENSMUST00000084985.10	1847	524aa	Protein coding	CCDS36557	Q60996	TSL:2 GENCODE basic APPRIS P4
Ppp2r5c-205	ENSMUST00000221715.1	2367	452aa	Protein coding	-	Q60996	TSL:1 GENCODE basic
Ppp2r5c-204	ENSMUST00000221074.1	1971	496aa	Protein coding	-	Q60996	TSL:5 GENCODE basic
Ppp2r5c-206	ENSMUST00000222276.1	1802	49aa	Protein coding	-	A0A1Y7VJC8	TSL:2 GENCODE basic
Ppp2r5c-203	ENSMUST00000220509.1	956	283aa	Protein coding	-	A0A1Y7VIR0	CDS 3' incomplete TSL:5
Ppp2r5c-207	ENSMUST00000223168.1	2790	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Ppp2r5c-201* transcript,the transcription is shown below:

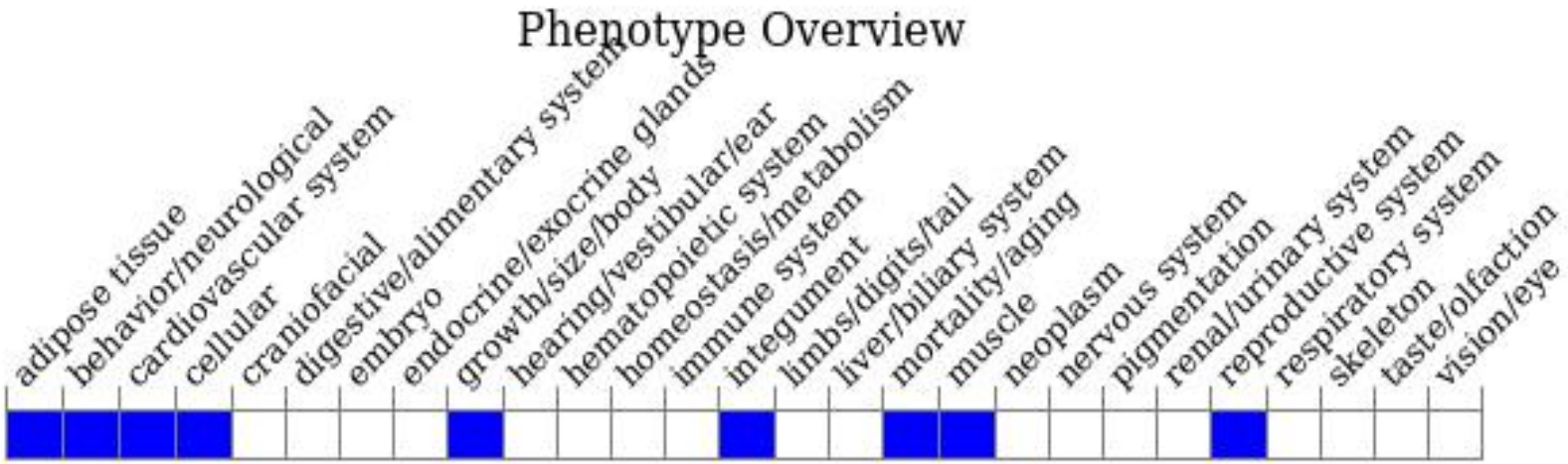
Genomic location distribution



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,mice homozygous for a gene-trapped allele show partial neonatal lethality, hypoactivity, and abnormal ventricular septum formation associated with increased fetal cardiomyocyte apoptosis. Surviving homozygotes develop obesity and show an abnormal gait, decreased grip strength, and impaired balance.

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

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