

P2ry1 Cas9-CKO Strategy

Designer: Longyun Hu

Reviewer: Yun Li

Design Date: 2019-12-18

Project Overview



Project Name

P2ry1

Project type

Cas9-CKO

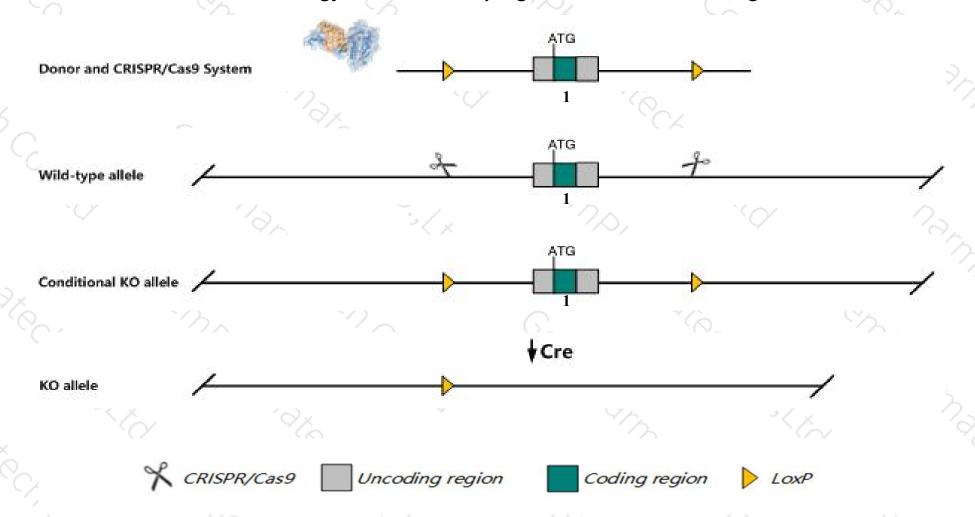
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *P2ry1* gene. The schematic diagram is as follows:



Technical routes



- The *P2ry1* gene has 3 transcripts. According to the structure of *P2ry1* gene, exon1 of *P2ry1-201* (ENSMUST00000029331.6) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *P2ry1* 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.
- 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.

Notice



- ➤ According to the existing MGI data, Mice homozygous for either one of two independently generated knock-out alleles exhibit decreased platelet aggregation, increased bleeding time, and resistance to induced thromboembolism.
- The *P2ry1* gene is located on the Chr3. 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.
- ➤ The CKO region contains functional region of the Gm37035 gene. Knockout the region affect the function of Gm37035 gene.
- > 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)



P2ry1 purinergic receptor P2Y, G-protein coupled 1 [Mus musculus (house mouse)]

Gene ID: 18441, updated on 19-Mar-2019

Summary

☆ ?

Official Symbol P2ry1 provided by MGI

Official Full Name purinergic receptor P2Y, G-protein coupled 1 provided by MGI

Primary source MGI:MGI:105049

See related Ensembl: ENSMUSG00000027765

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 P2Y1

Expression Ubiquitous expression in colon adult (RPKM 5.3), large intestine adult (RPKM 4.9) and 27 other tissuesSee more

Orthologs <u>human</u> all

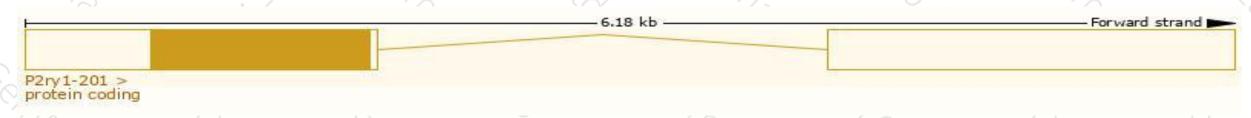
Transcript information (Ensembl)



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

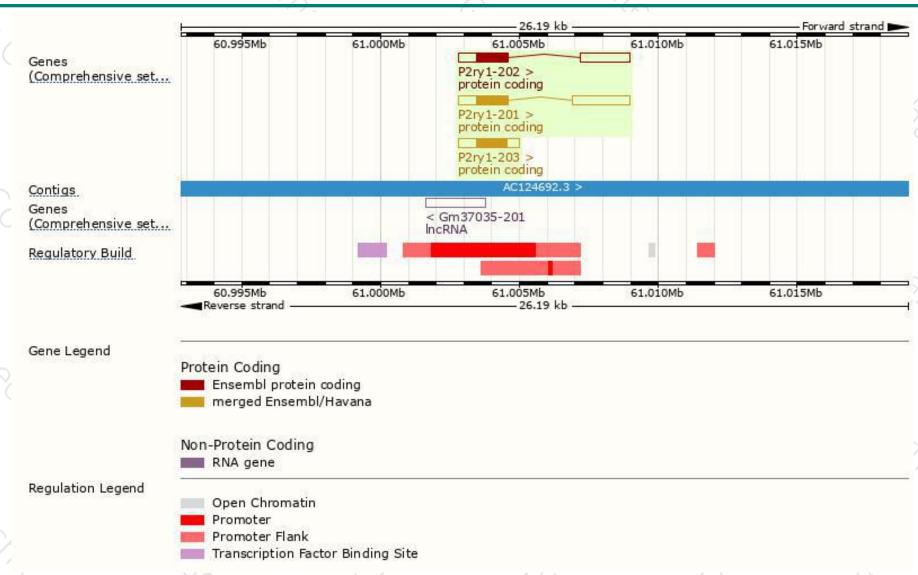
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
P2ry1-201	ENSMUST00000029331.6	3886	<u>373aa</u>	Protein coding	CCDS17377	P49650 Q544J5	TSL:1 GENCODE basic APPRIS P1
P2ry1-202	ENSMUST00000193201.1	3614	<u>373aa</u>	Protein coding	CCDS17377	P49650 Q544J5	TSL:1 GENCODE basic APPRIS P1
P2ry1-203	ENSMUST00000193943.1	2203	<u>373aa</u>	Protein coding	CCDS17377	P49650 Q544J5	TSL:NA GENCODE basic APPRIS P1

The strategy is based on the design of *P2ry1-201* transcript, The transcription is shown below



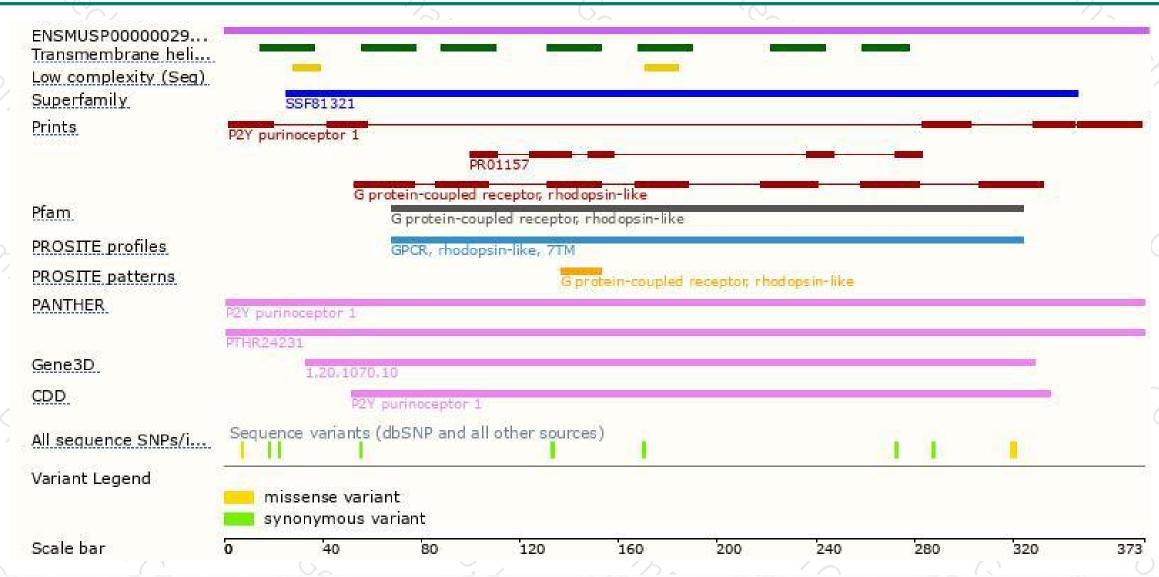
Genomic location distribution





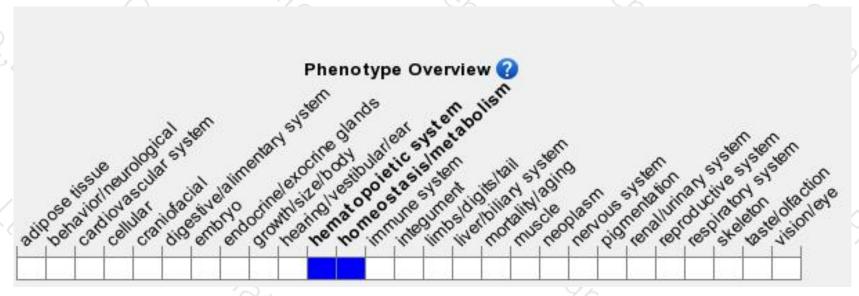
Protein domain





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 either one of two independently generated knock-out alleles exhibit decreased platelet aggregation, increased bleeding time, and resistance to induced thromboembolism.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





