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P2ry14 Cas9-CKO Strategy

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Design Date: 2019-12-24

Overview

Target Gene Name

- P2ry14

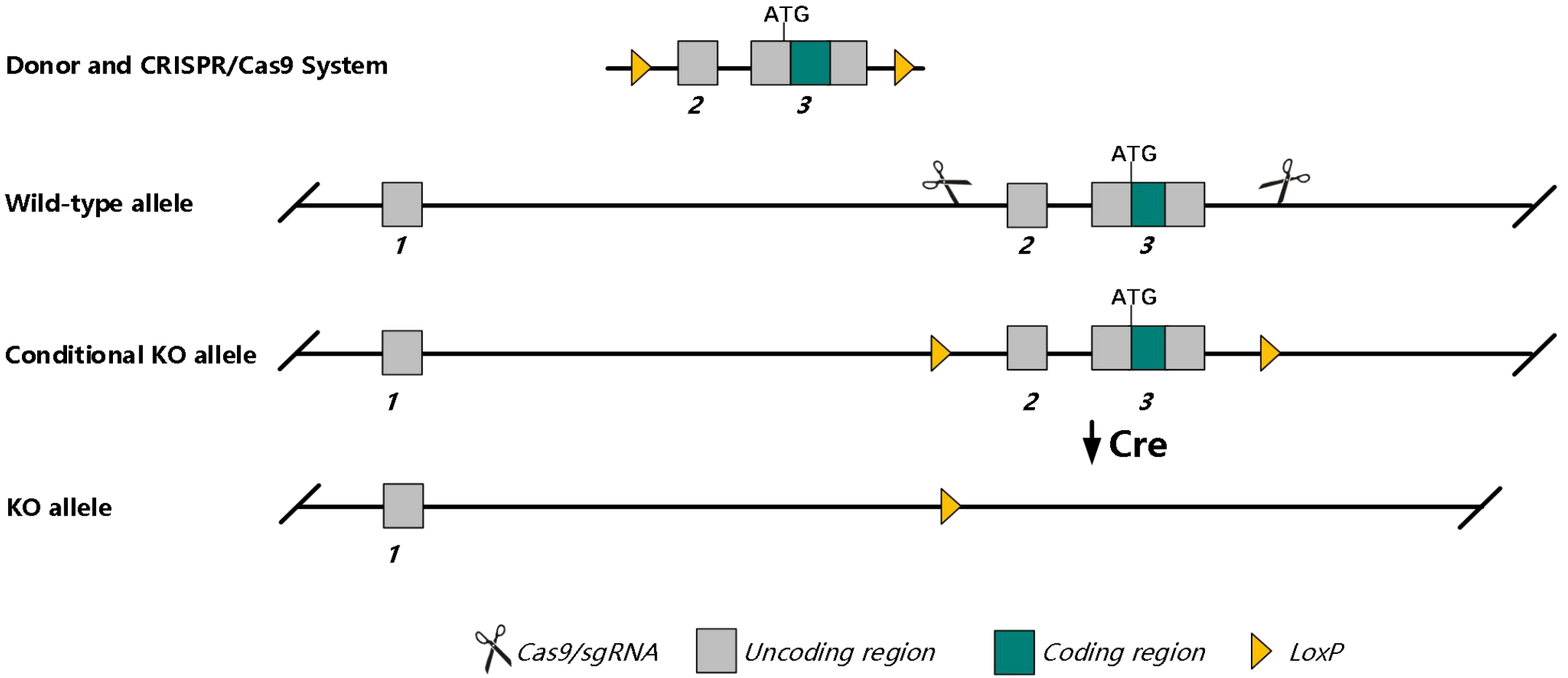
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *P2ry14* gene.

Technical Information

- The *P2ry14* gene has 9 transcripts. According to the structure of *P2ry14* gene, exon2-exon3 of *P2ry14*-201 (ENSMUST00000065220.13) 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 *P2ry14* 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

P2ry14 purinergic receptor P2Y, G-protein coupled, 14 [Mus musculus (house mouse)]

Gene ID: 140795, updated on 12-Apr-2023

Summary

Official Symbol	P2ry14 provided by MGI
Official Full Name	purinergic receptor P2Y, G-protein coupled, 14 provided by MGI
Primary source	MGI:MGI:2155705
See related	Ensembl:ENSMUSG00000036381
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	A330108O13Rik, Gpr105, P2Y14
Summary	Predicted to enable G protein-coupled purinergic nucleotide receptor activity. Acts upstream of or within hematopoietic stem cell homeostasis. Predicted to be located in plasma membrane. Predicted to be integral component of membrane. Orthologous to human P2RY14 (purinergic receptor P2Y14). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Broad expression in subcutaneous fat pad adult (RPKM 13.2), large intestine adult (RPKM 3.9) and 20 other tissues See more
Orthologs	human all

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

Transcript Information

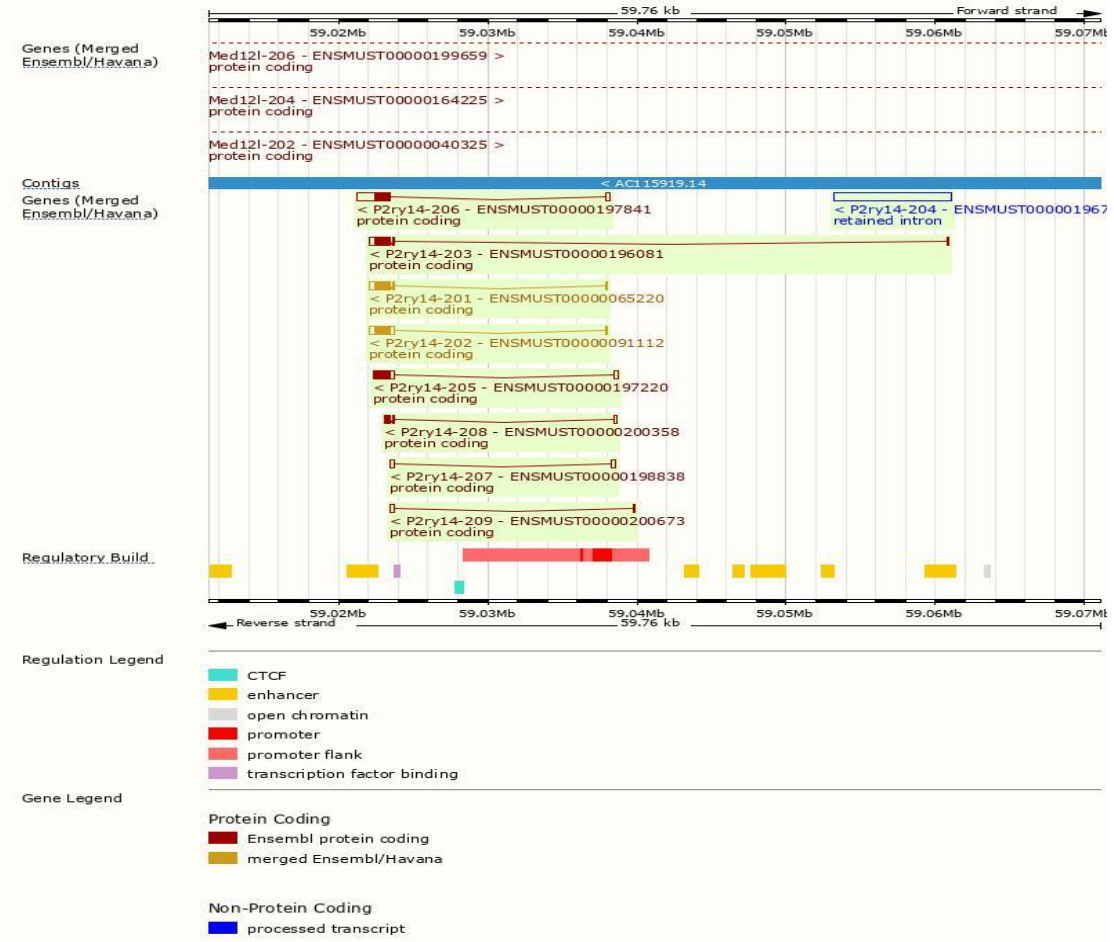
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
P2ry14-206	ENSMUST00000197841.2	2476	347aa	Protein coding	CCDS79913		A single transcript chosen for a gene which is the most conserved, most highly expressed, has the longest coding sequence and is represented in other key resources, such as NCBI and UniProt. This is defined in detail on http://www.ensembl.org/info/genome/genebuild/canonical.html Ensembl Canonical , The GENCODE set is the gene set for human and mouse. GENCODE basic , TSL:5 ,
P2ry14-202	ENSMUST00000091112.6	1795	338aa	Protein coding	CCDS17371		The GENCODE set is the gene set for human and mouse. GENCODE basic , APPRIS P1 , TSL:1 ,
P2ry14-205	ENSMUST00000197220.2	1684	338aa	Protein coding	CCDS17371		The GENCODE set is the gene set for human and mouse. GENCODE basic , APPRIS P1 , TSL:1 ,
P2ry14-201	ENSMUST00000065220.13	1672	338aa	Protein coding	CCDS17371		The GENCODE set is the gene set for human and mouse. GENCODE basic , APPRIS P1 , TSL:1 ,
P2ry14-203	ENSMUST00000196081.5	1615	338aa	Protein coding	CCDS17371		The GENCODE set is the gene set for human and mouse. GENCODE basic , APPRIS P1 , TSL:1 ,
P2ry14-208	ENSMUST00000200358.2	669	110aa	Protein coding			TSL:2 , CDS 3' incomplete ,
P2ry14-207	ENSMUST00000198838.2	557	9aa	Protein coding			TSL:3 , CDS 3' incomplete ,
P2ry14-209	ENSMUST00000200673.2	339	4aa	Protein coding			TSL:2 , CDS 3' incomplete ,
P2ry14-204	ENSMUST00000196716.2	7795	No protein	Retained intron			TSL:NA ,

The strategy is based on the design of *P2ry14*-201 transcript, the transcription is shown below:

Source: https://www.ensembl.org

Genomic Information



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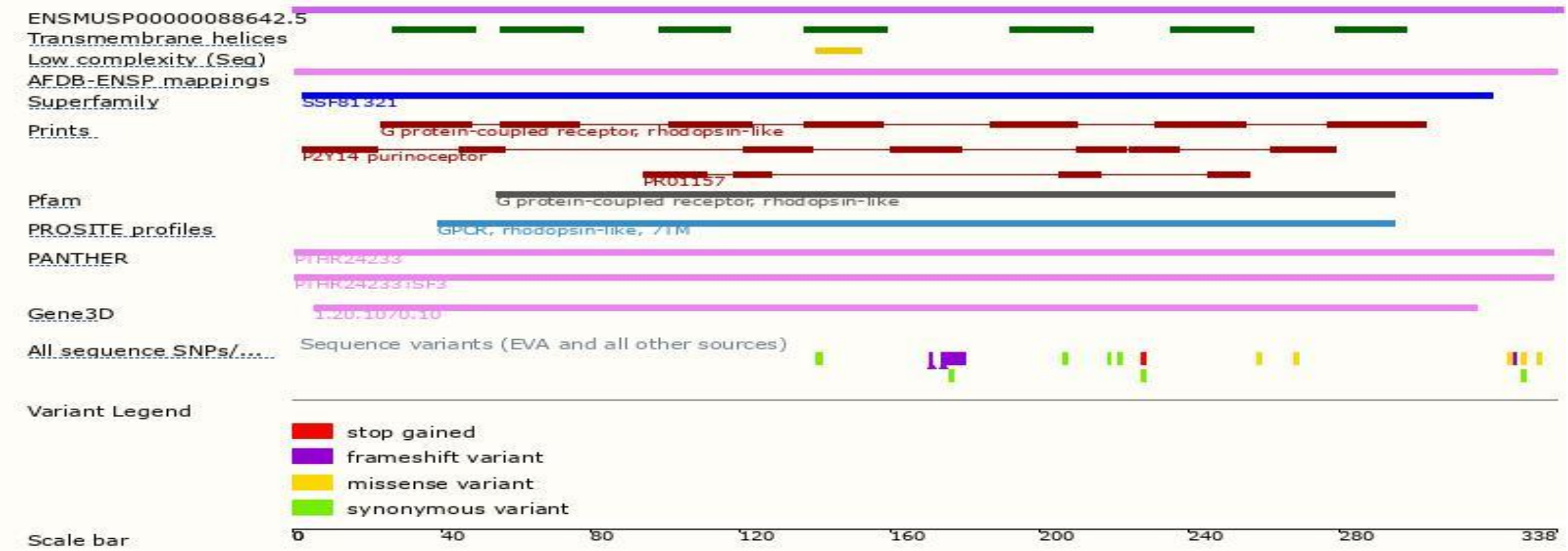
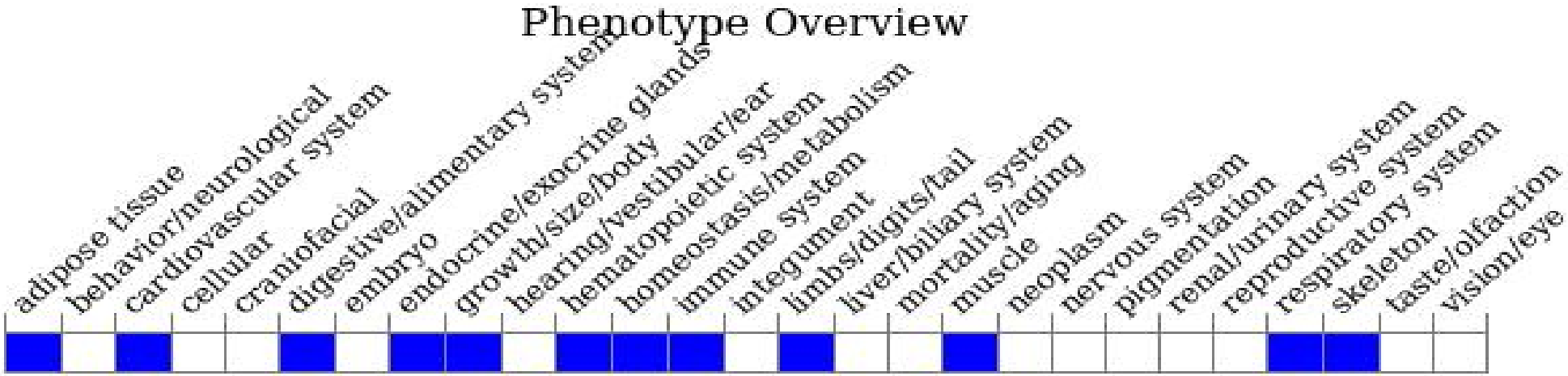
Source: : https://www.ensembl.org

Protein Information



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

Mouse Phenotype Information (MGI)



- Mice homozygous for a null allele fail to exhibit increased glucose mediated forestomach muscle tension. Mice homozygous for a different null allele show decreased gastrointestinal emptying, impaired glucose tolerance, decreased glucose-stimulated insulin release, and reduced airway responsiveness.

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

Important Information

- *P2ry14* is located on Chr3. 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.
- The flox region overlaps with the gene *Med12l,*Knockout the region may affect its function of *Med12l* gene.