

Pdcd1lg2 Cas9-CKO Strategy

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Reviewer:

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Project Overview

Project Name

Pdcd1lg2

Project type

Cas9-CKO

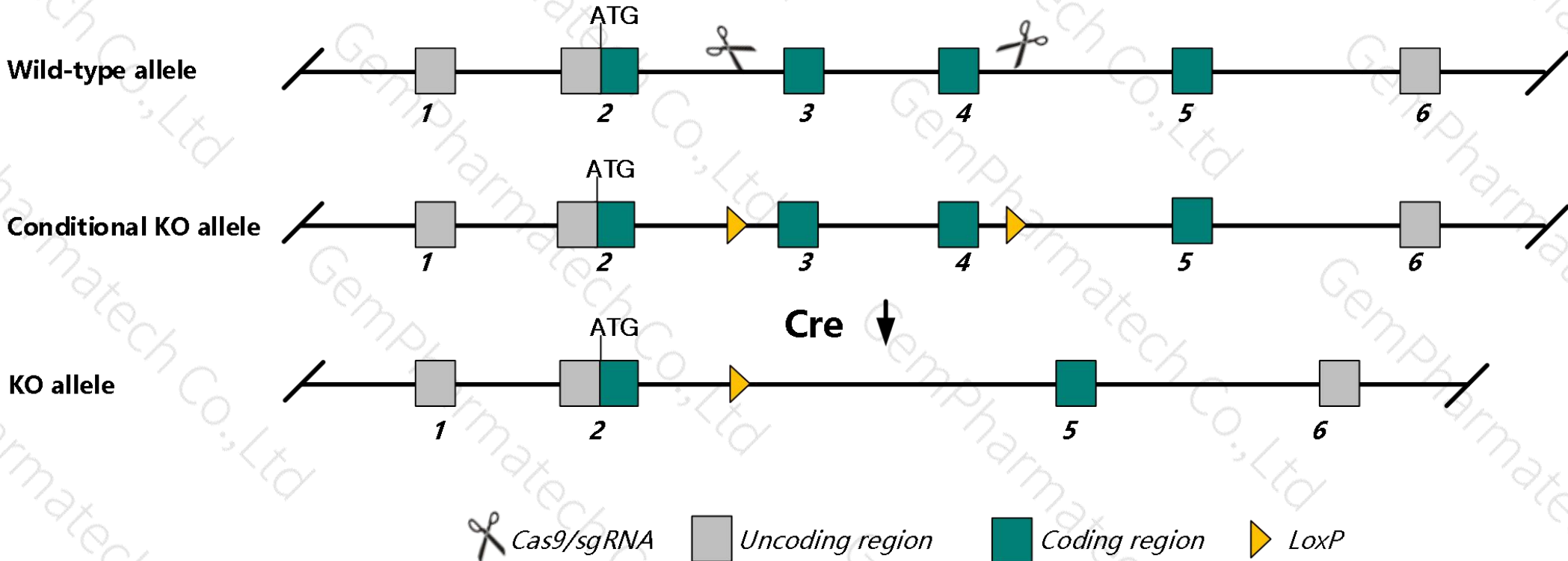
Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pdcd1lg2* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System



- The *Pdcd1lg2* gene has 2 transcripts. According to the structure of *Pdcd1lg2* gene, exon3-exon4 of *Pdcd1lg2*-201 (ENSMUST00000112576.2) transcript is recommended as the knockout region. The region contains 576bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Pdcd1lg2* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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.

- According to the existing MGI data, Mice homozygous for disruptions in this gene have dendritic cells that display a diminished ability to activate CD4⁺ T cells.
- The KO region deletes most of the coding sequence, but does not result in frameshift.
- The *Pdcd1lg2* gene is located on the Chr19. 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)

Pdcd1lg2 programmed cell death 1 ligand 2 [*Mus musculus* (house mouse)]

Gene ID: 58205, updated on 12-Aug-2019

Summary



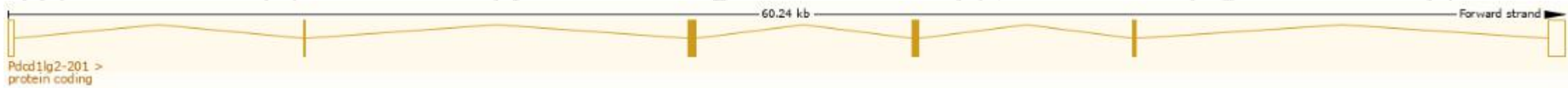
Official Symbol	Pdcd1lg2 provided by MGI
Official Full Name	programmed cell death 1 ligand 2 provided by MGI
Primary source	MGI:MGI:1930125
See related	Ensembl:ENSMUSG00000016498
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Btdc; B7-DC; PD-L2; F730015O22Rik
Expression	Low expression observed in reference dataset See more
Orthologs	human all

Transcript information (Ensembl)

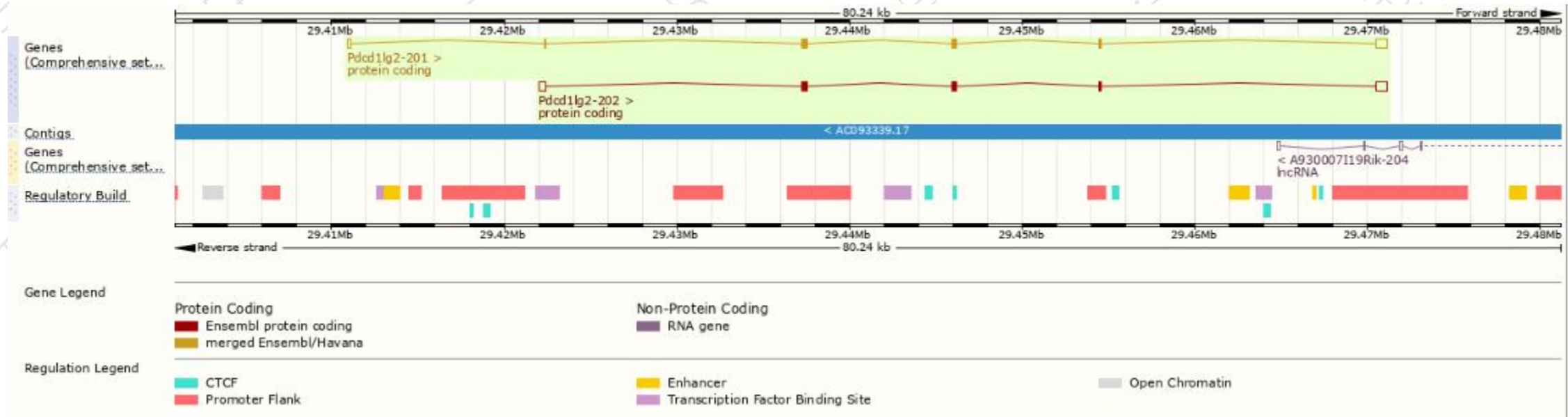
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pdcd1lg2-202	ENSMUST00000235164.1	1729	247aa	Protein coding	CCDS29736	Q3U304	GENCODE basic APPRIS P1
Pdcd1lg2-201	ENSMUST00000112576.3	1649	247aa	Protein coding	CCDS29736	Q3U304 Q9WUL5	TSL:1 GENCODE basic APPRIS P1

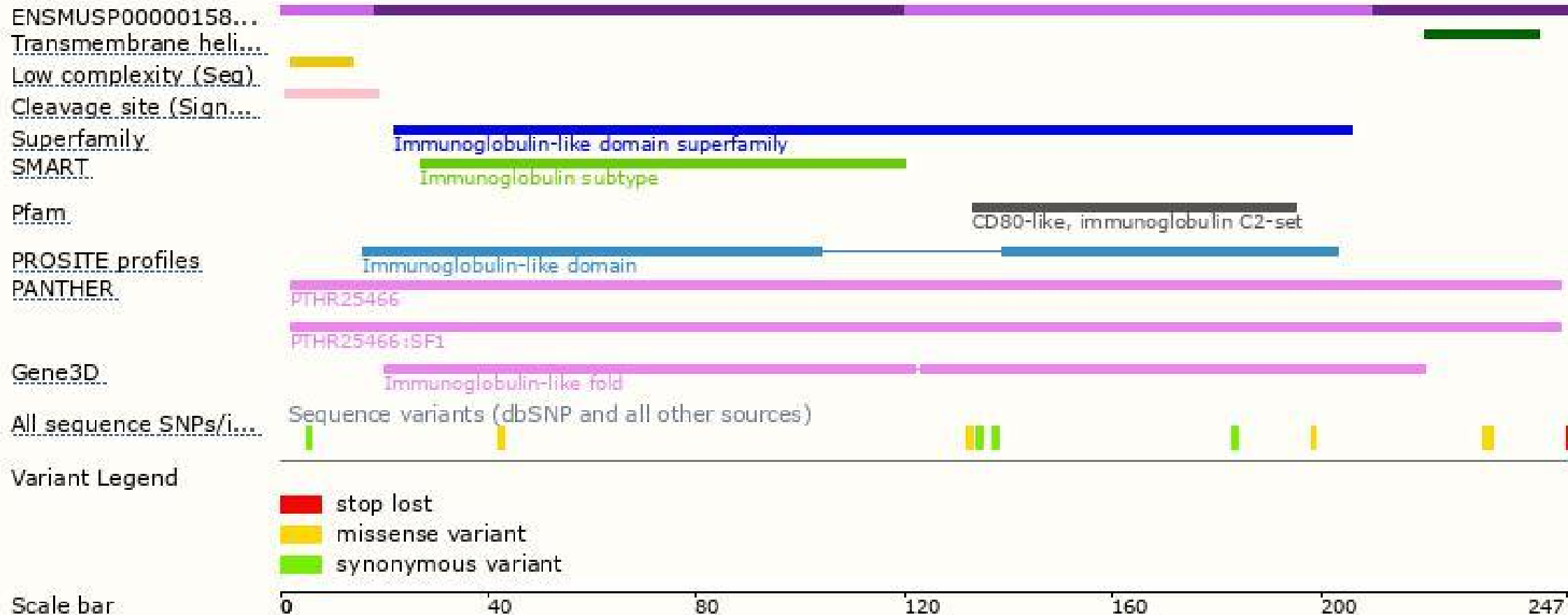
The strategy is based on the design of *Pdcd1lg2-201* transcript,The transcription is shown below



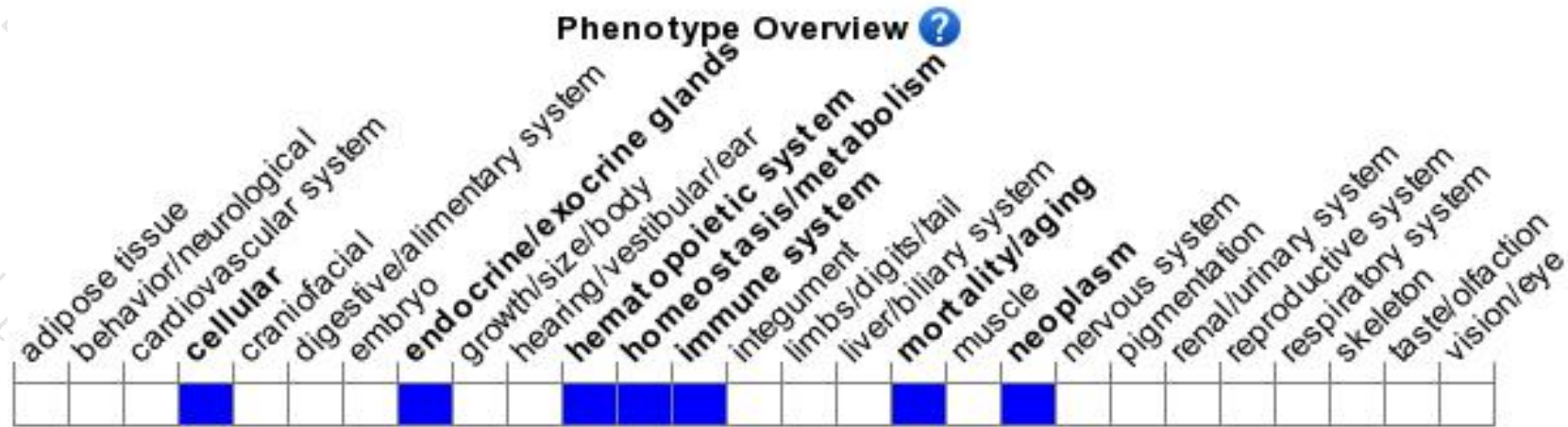
Genomic location distribution



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 disruptions in this gene have dendritic cells that display a diminished ability to activate CD4⁺ T cells.

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

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