

***Cd274* Cas9-CKO Strategy**

Designer:Lixin Lv

Project Overview

Project Name

Cd274

Project type

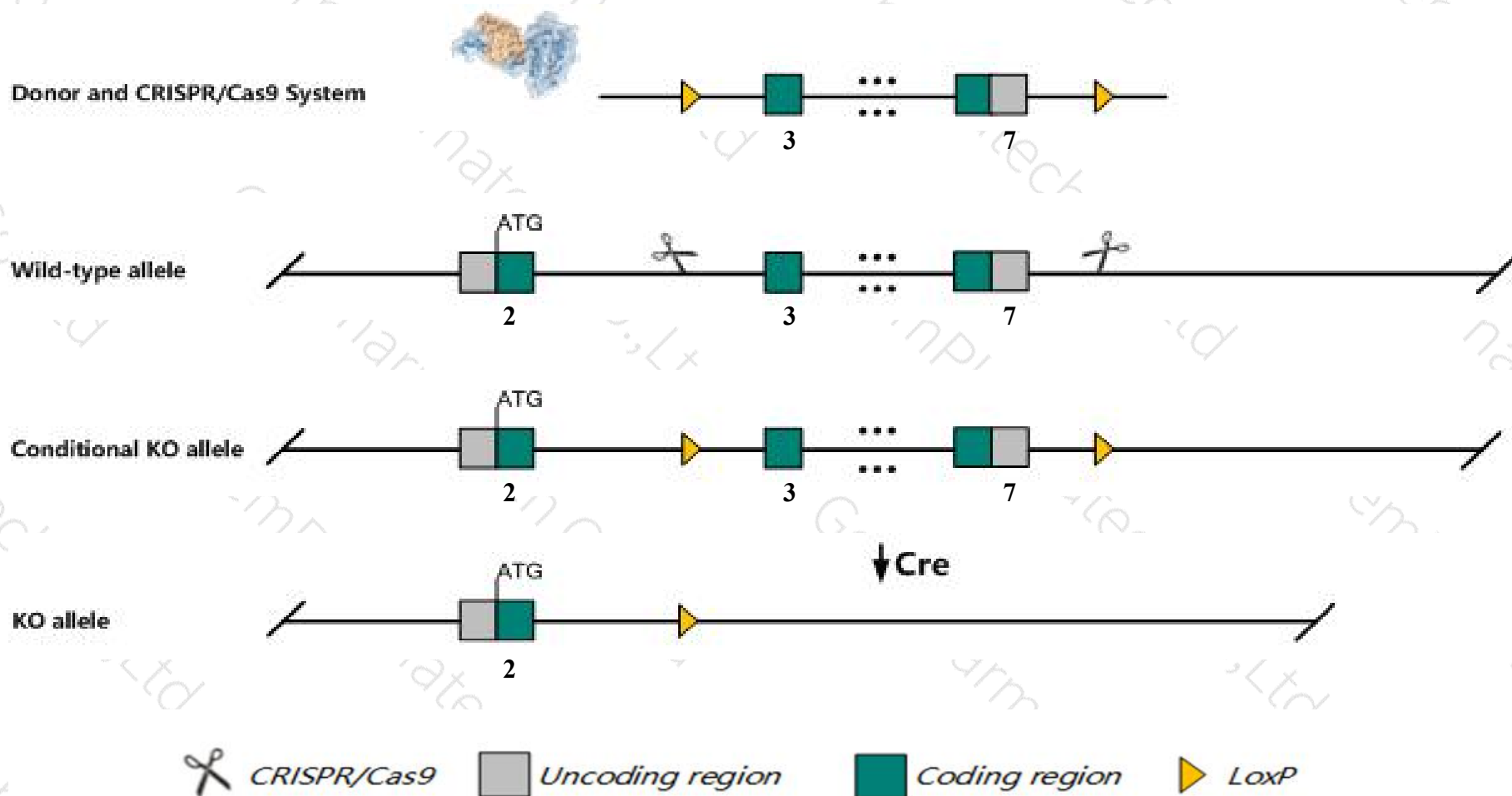
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cd274* gene. The schematic diagram is as follows:



- The *Cd274* gene has 1 transcript. According to the structure of *Cd274* gene, exon3-exon7 of *Cd274-201* (ENSMUST00000016640.7) transcript is recommended as the knockout region. The region contains 821bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cd274* 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.

- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit altered susceptibility to experimental autoimmune encephalomyelitis, induced arthritis, nerve injury, autoimmune diabetes, bacterial infection, viral infection, and parasitic infection due to abnormal T cell morphology and physiology.
- The *AC119228.1* and *Cd274* are overlap, so the *AC119228.1* gene will be knockout together.
- The *Cd274* 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)

Cd274 CD274 antigen [Mus musculus (house mouse)]

Gene ID: 60533, updated on 9-Apr-2019

Summary

Official Symbol Cd274 provided by [MGI](#)

Official Full Name CD274 antigen provided by [MGI](#)

Primary source [MGI:MGI:1926446](#)

See related [Ensembl:ENSMUSG00000016496](#)

Gene type protein coding

RefSeq status REVIEWED

Organism [Mus musculus](#)

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus

Also known as A530045L16Rik, B7h1, Pdcd1l1, Pdcd1lg1, Pdl1

Summary The protein encoded by this gene is an immune inhibitory receptor ligand that is expressed by hematopoietic and non-hematopoietic cells, such as T cells and B cells and various types of tumor cells. The encoded protein is a type I transmembrane protein that has immunoglobulin V-like and C-like domains. Interaction of this ligand with its receptor inhibits T-cell activation and cytokine production. During infection or inflammation of normal tissue, this interaction is important for preventing autoimmunity by maintaining homeostasis of the immune response. In tumor microenvironments, this interaction provides an immune escape for tumor cells through cytotoxic T-cell inactivation. Mice deficient for this gene display a variety of phenotypes including decreased allogeneic fetal survival rates and severe experimental autoimmune encephalomyelitis. [provided by RefSeq, Sep 2015]

Expression Broad expression in thymus adult (RPKM 7.4), mammary gland adult (RPKM 5.3) and 23 other tissues [See more](#)

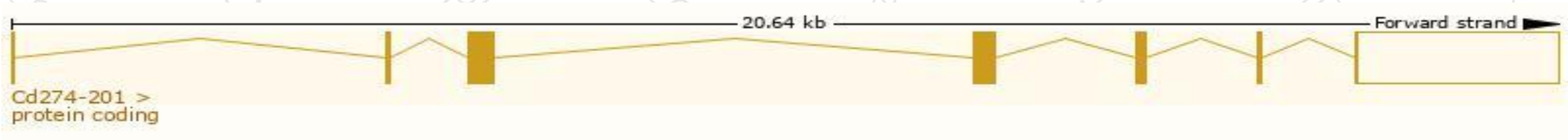
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

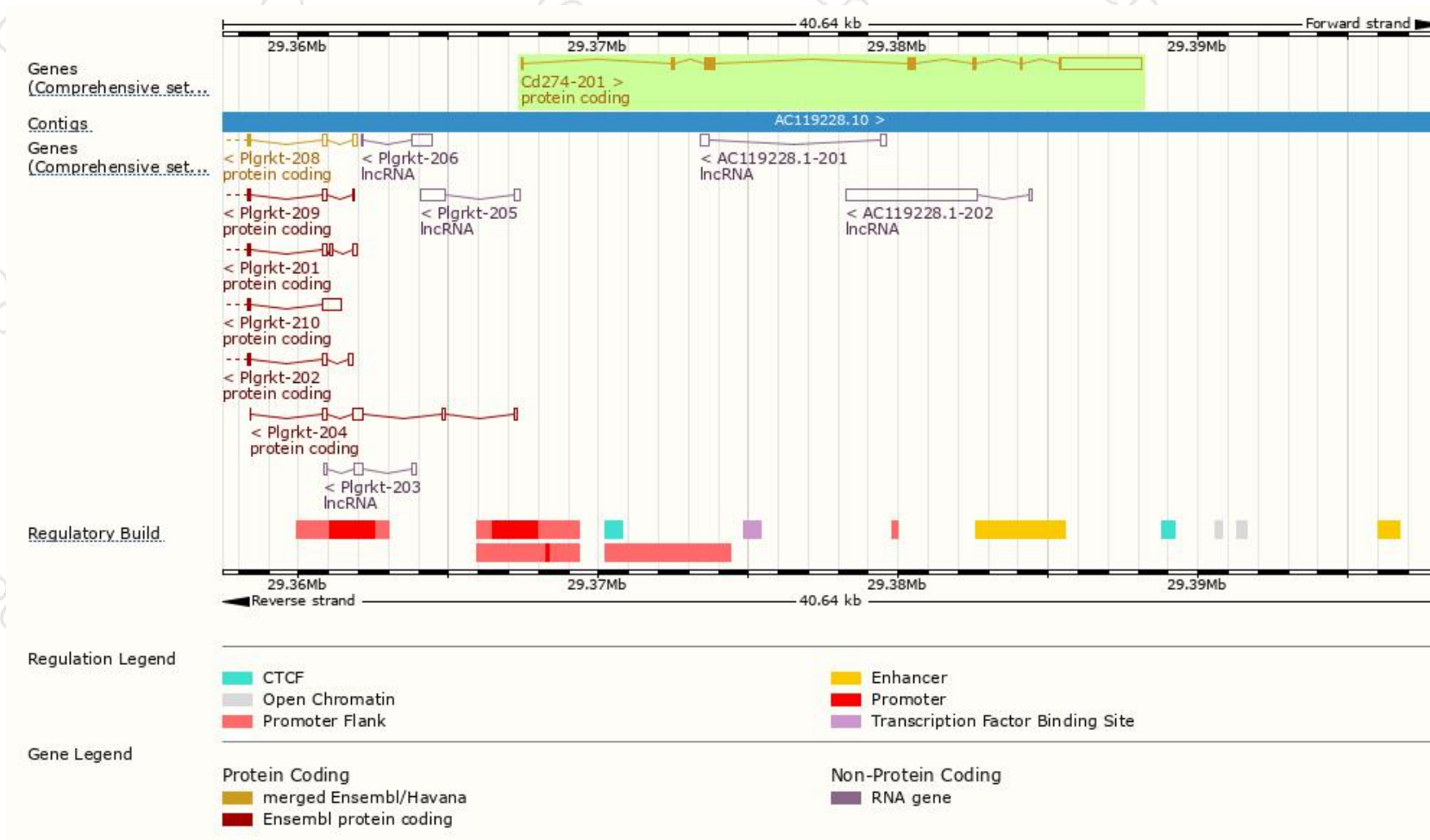
The gene has 1 transcript, and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cd274-201	ENSMUST00000016640.7	3622	290aa	Protein coding	CCDS29735	Q3U472 Q9EP73	TSL:1 GENCODE basic APPRIS P1

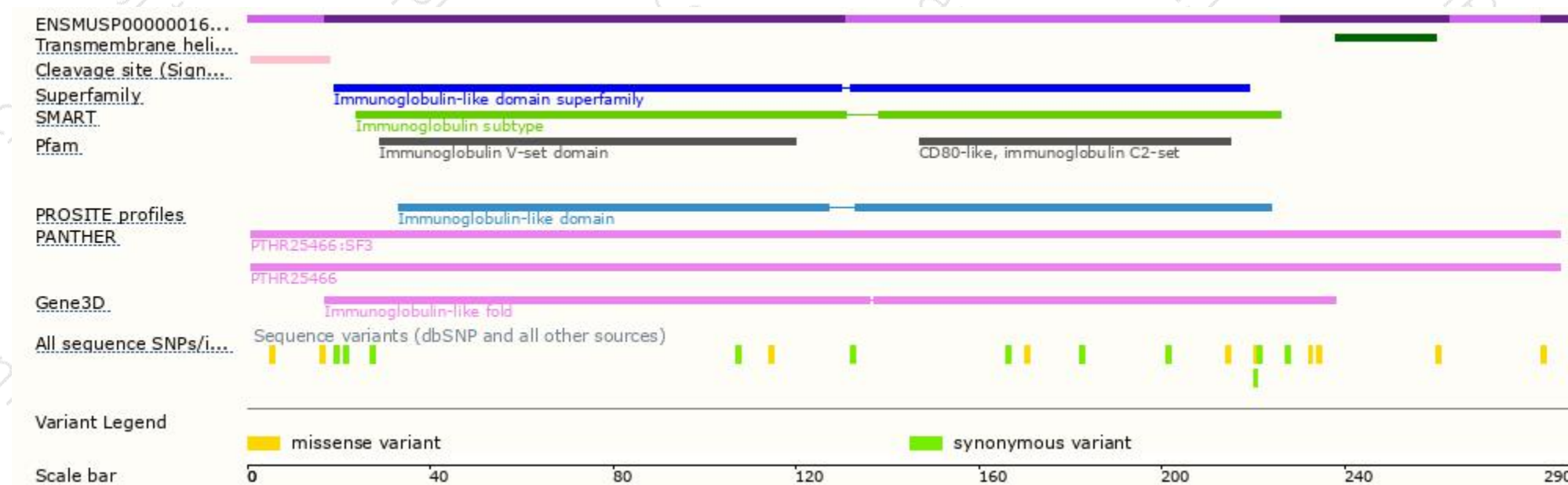
The strategy is based on the design of *Cd274-201* transcript, The transcription is shown below



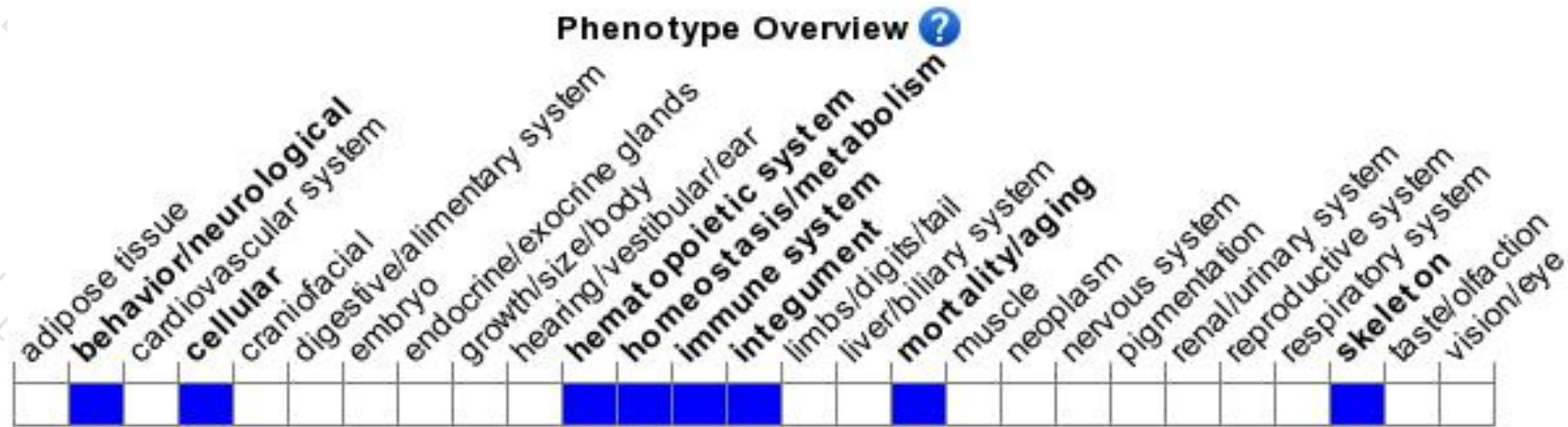
Genomic location distribution



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 knock-out allele exhibit altered susceptibility to experimental autoimmune encephalomyelitis, induced arthritis, nerve injury, autoimmune diabetes, bacterial infection, viral infection, and parasitic infection due to abnormal T cell morphology and physiology.

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

Tel: 400-9660890

