# *Cd274* Cas9-KO Strategy

Designer:Lixin Lv

# Project Overview

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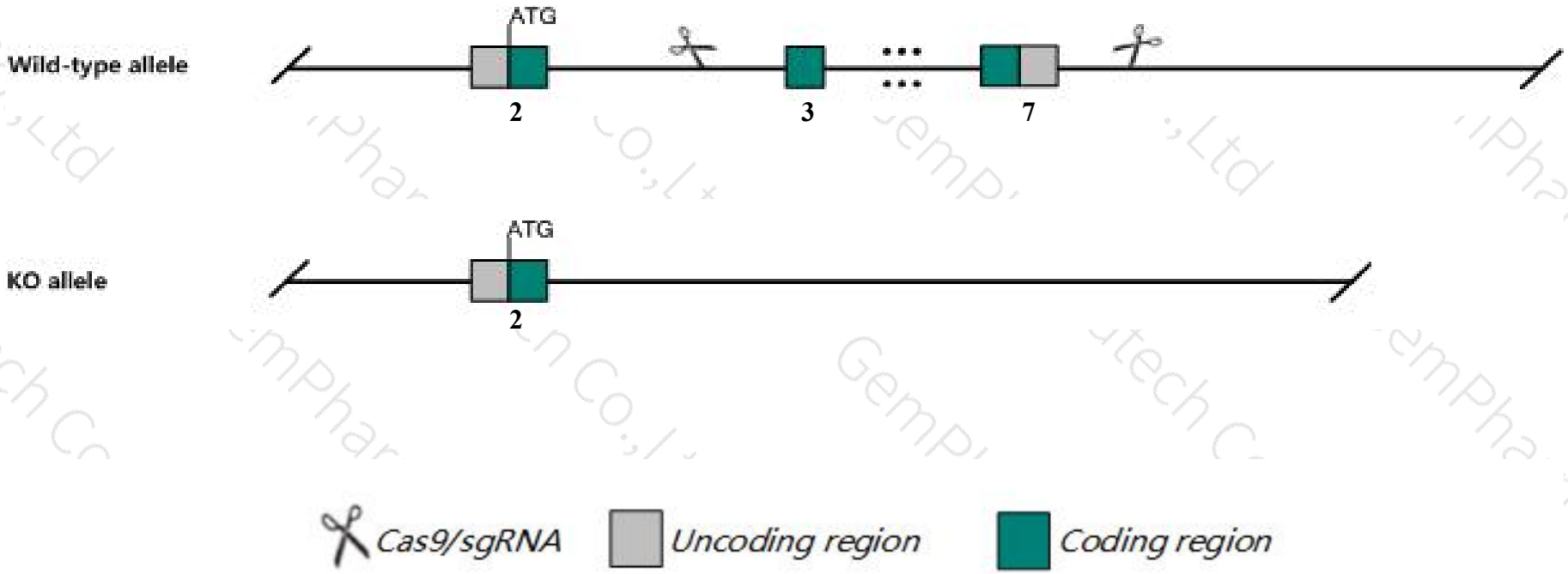
| **Project Name** | ***Cd274*** |
| **Project type** | **Cas9-KO** |
| **Strain background** | **C57BL/6J** |

# Knockout strategy

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



# Technical routes

➢ 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: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.

# Notice

- 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 cellmorphology 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

# Gene information（NCBI）

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## Cd274 CD274 antigen [Mus musculus (house mouse)]

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

### Summary

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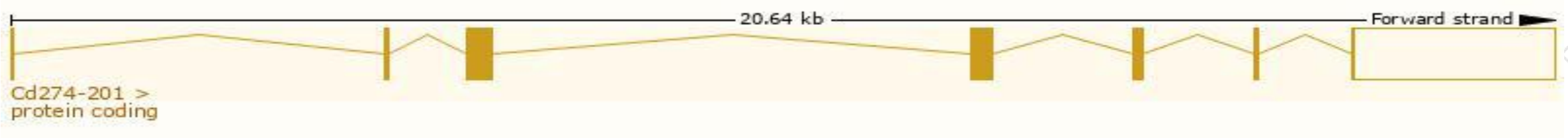
|---|---|
| **Official Symbol** | Cd274 provided by MGI |
| **Official Full Name** | CD274 antigen provided by MGI |
| **Primary source** | MGI:MGI:1926446 |
| **See related** | Ensembl:ENSMUSG00000016496 |
| **Gene type** | protein coding |
| **RefSeq status** | REVIEWED |
| **Organism** | Mus musculus |
| **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）

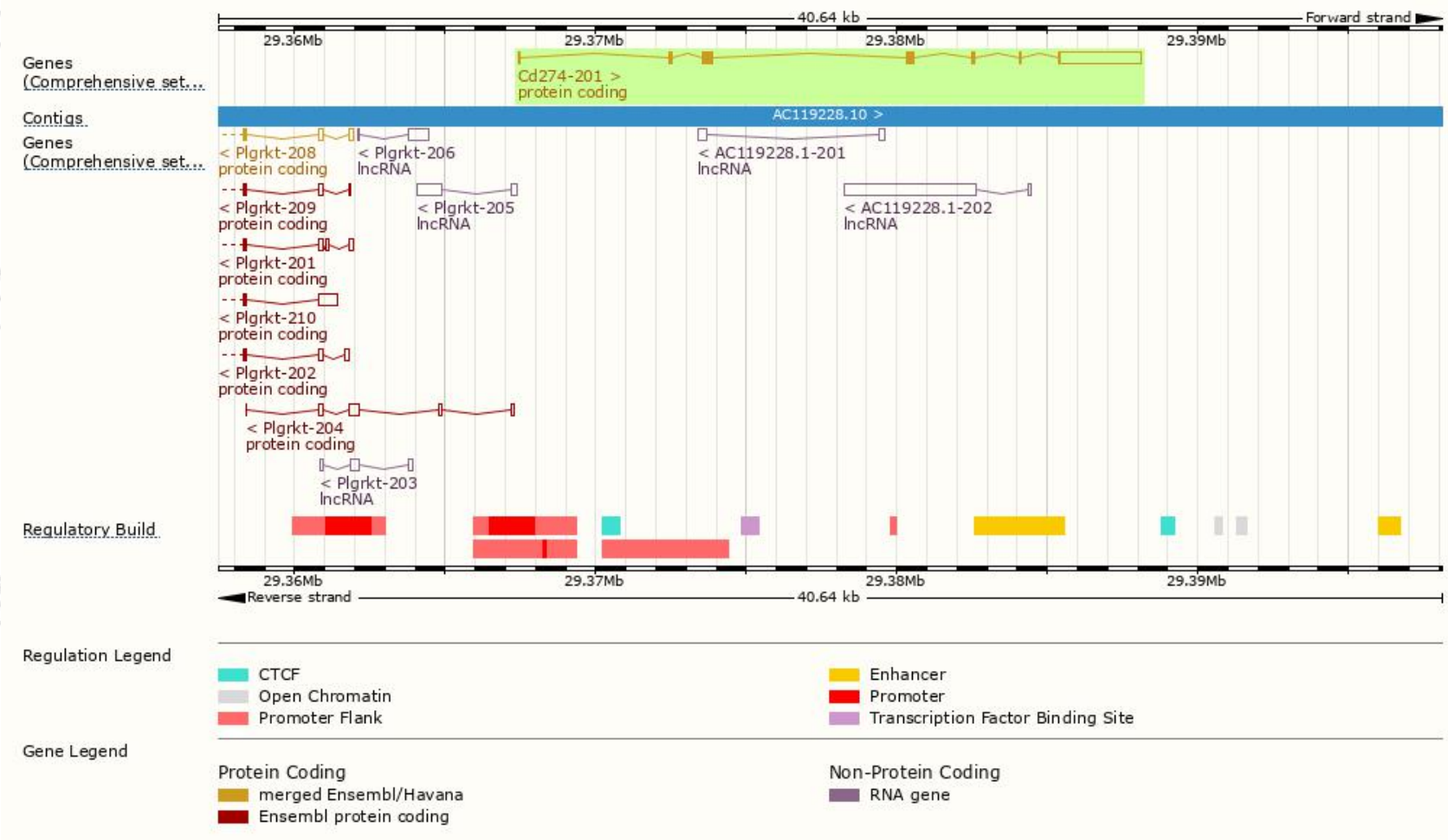
The gene has 1 transcript,and the transcript is shown below:

| 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 |

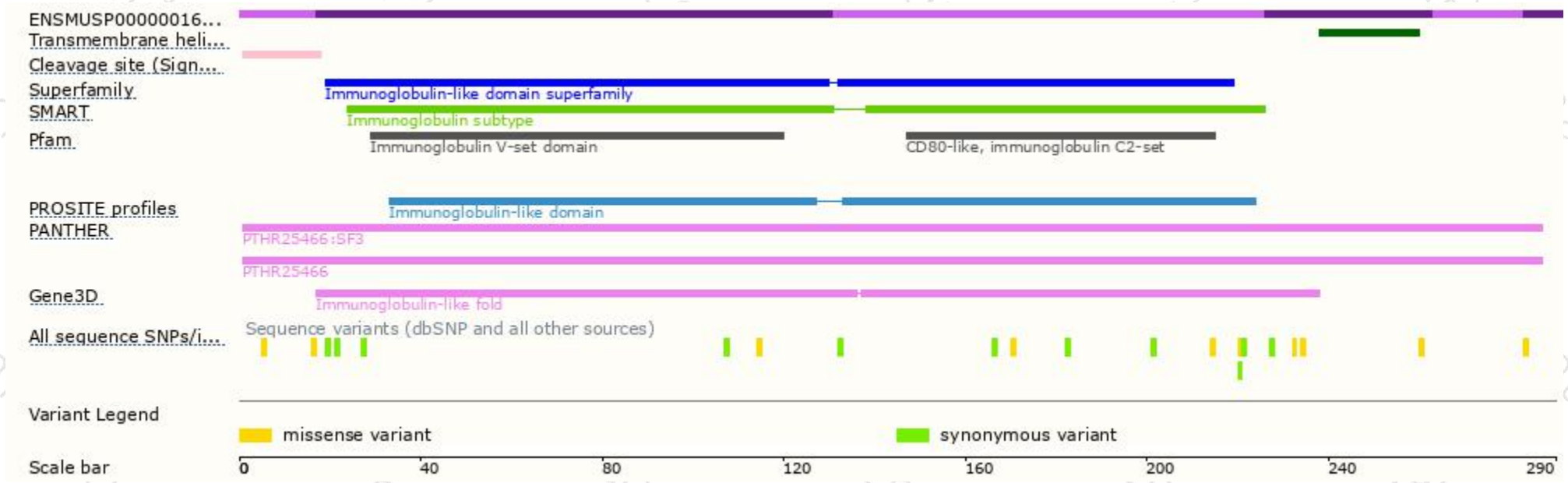
The strategy is based on the design of *Cd274-201* transcript,The transcription is shown below



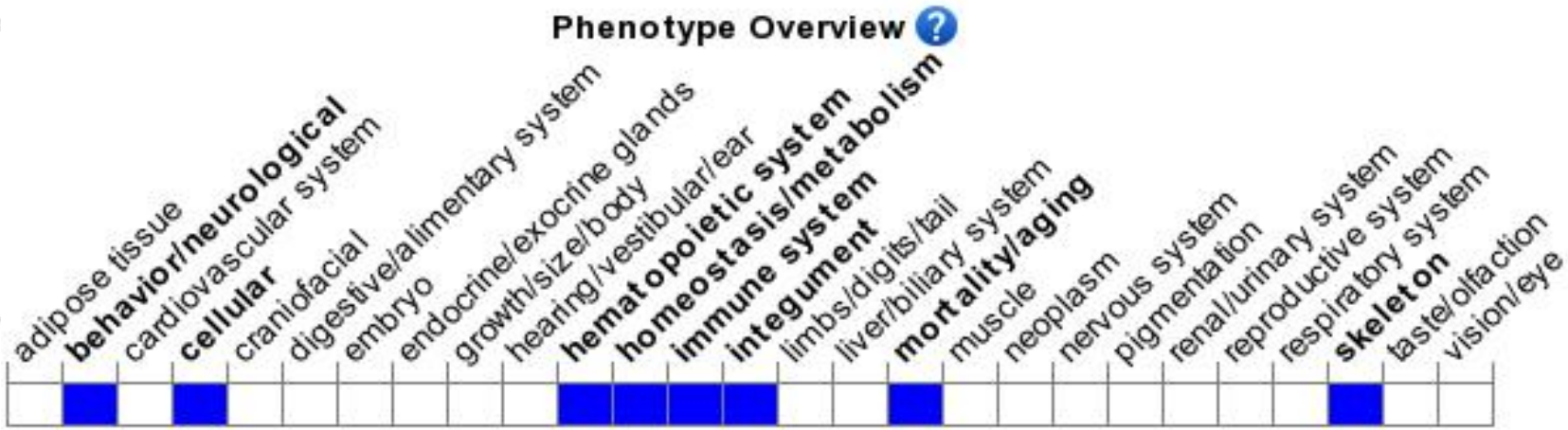
# Genomic location distribution



# 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/).*

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

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