

# Cd276 Cas9-CKO Strategy

Designer: Xueting Zhang

Reviewer: Yanhua Shen

Design Date: 2019-9-5

# Overview

## Target Gene Name

- Cd276

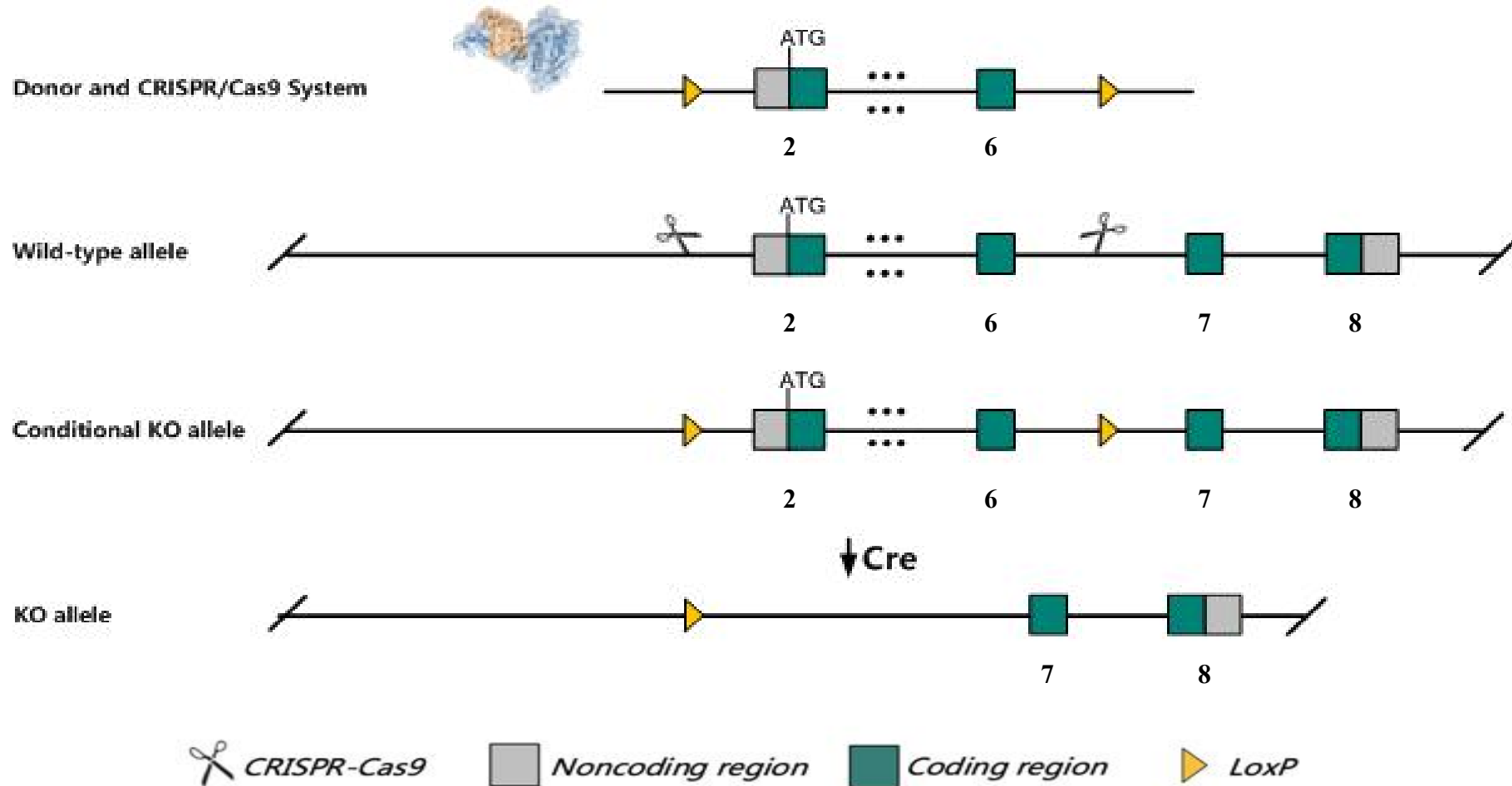
## Project Type

- Cas9-CKO

## Genetic Background

- C57BL/6JGpt

# Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Cd276* gene.

# Technical Information

- The *Cd276* gene has 4 transcripts. According to the structure of *Cd276* gene, exon2-exon6 of *Cd276*-202 (ENSMUST00000165365.3) transcript is recommended as the knockout region. The region contains start codon ATG. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Cd276* 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

## Cd276 CD276 antigen [Mus musculus (house mouse)]

Gene ID: 102657, updated on 13-Mar-2020

### Summary

<b>Official Symbol</b>	Cd276 <small>provided by <a href="#">MGI</a></small>
<b>Official Full Name</b>	CD276 antigen <small>provided by <a href="#">MGI</a></small>
<b>Primary source</b>	<a href="#">MGI:MGI:2183926</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000035914</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	PROVISIONAL
<b>Organism</b>	<a href="#">Mus musculus</a>
<b>Lineage</b>	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
<b>Also known as</b>	6030411F23Rik, AU016588, B7RP-2, B7h3
<b>Expression</b>	Broad expression in limb E14.5 (RPKM 25.4), CNS E14 (RPKM 11.9) and 22 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

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

# Transcript Information

The gene has 4 transcripts, all transcripts are shown below:

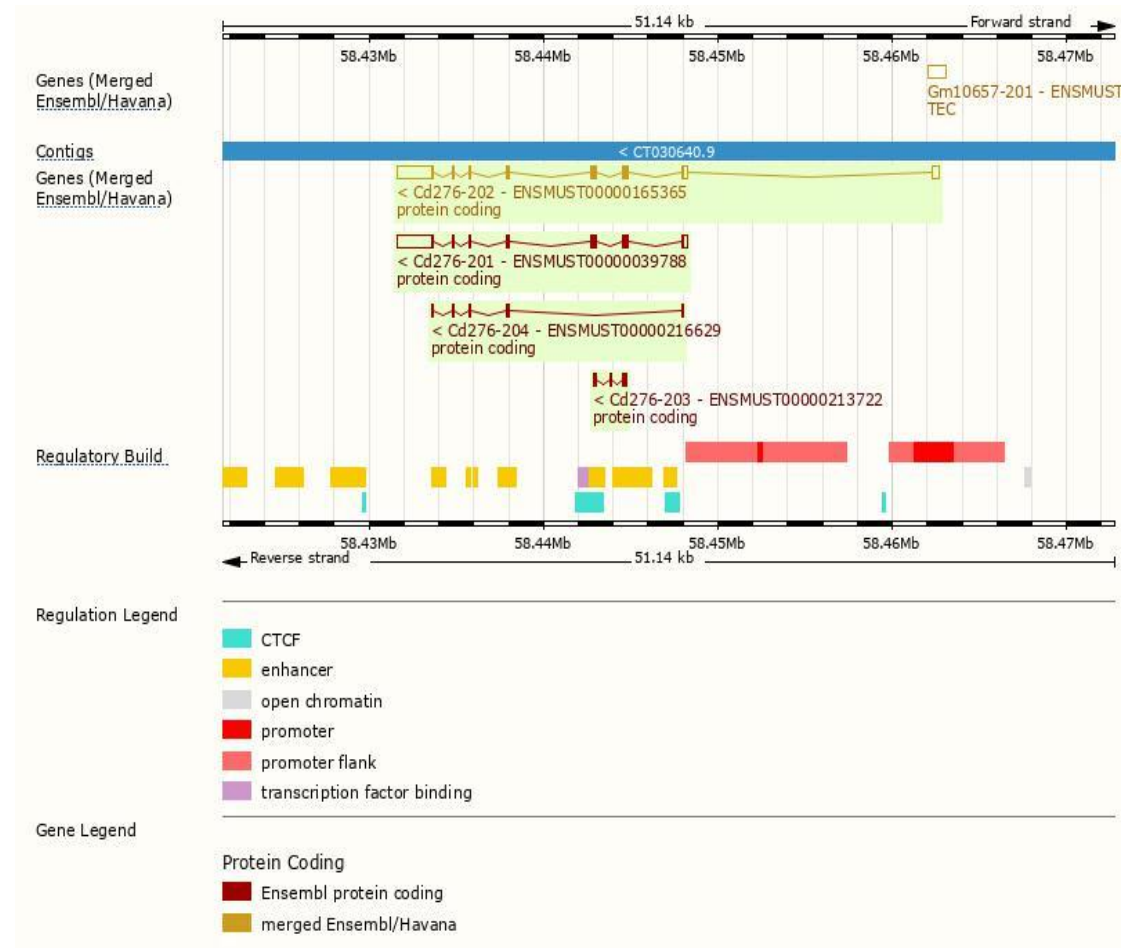
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cd276-202	<a href="#">ENSMUST00000165365.2</a>	3603	<a href="#">316aa</a>	Protein coding	<a href="#">CCDS23244</a>	<a href="#">A6MDC5 Q8VE98</a>	TSL1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Cd276-201	<a href="#">ENSMUST00000039788.10</a>	3186	<a href="#">316aa</a>	Protein coding	<a href="#">CCDS23244</a>	<a href="#">A6MDC5 Q8VE98</a>	TSL1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Cd276-203	<a href="#">ENSMUST00000213722.1</a>	434	<a href="#">145aa</a>	Protein coding	.	<a href="#">A0A1L1SUD4</a>	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Cd276-204	<a href="#">ENSMUST00000216629.1</a>	346	<a href="#">94aa</a>	Protein coding	.	<a href="#">A0A1L1SV51</a>	CDS 5' incomplete TSL:3

The strategy is based on the design of *Cd276-202* transcript, the transcription is shown below:

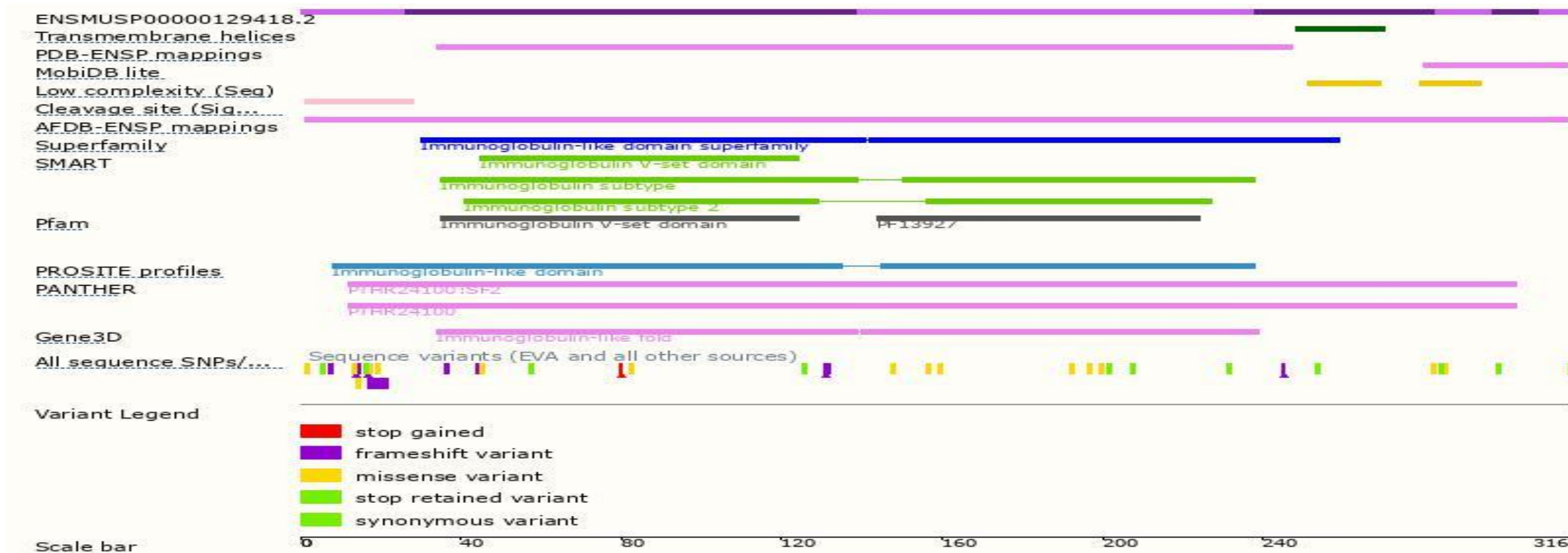


Source: <https://www.ensembl.org>

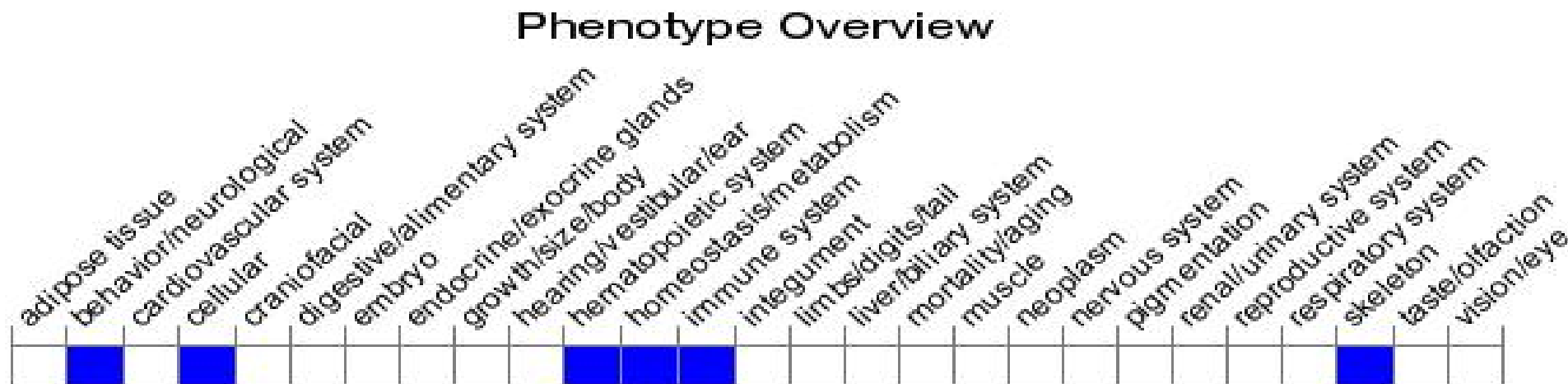
# Genomic Information



# Protein Information



# Mouse Phenotype Information (MGI)



- Inactivation of this locus results in abnormal T helper 1 physiology. Mutant mice have an increased susceptibility to inflammation and autoimmunity.

# Important Information

- According to the existing MGI data, Inactivation of this locus results in abnormal T helper 1 physiology. Mutant mice have an increased susceptibility to inflammation and autoimmunity.
- The effect on transcript *Cd276-203* is unknown.
- *Cd276* is located on Chr9. 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.