

# *Ccl25* Cas9-KO Strategy

Designer: Xingkai Xiao

Reviewer: Xiangli Bian

Design Date: 2023-06-12

# Overview

## Target Gene Name

- *Ccl25*

## Project Type

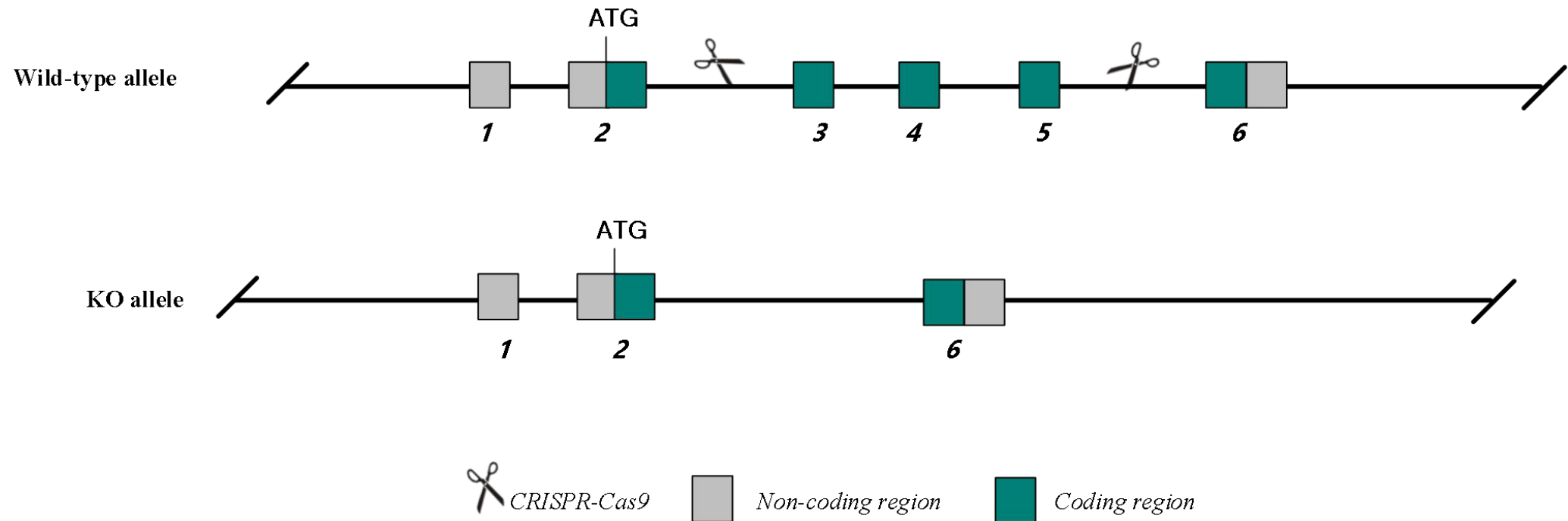
- Cas9-KO

## Genetic Background

- C57BL/6JGpt

# Strain Strategy

## Donor and CRISPR-Cas9 System



Schematic representation of CRISPR-Cas9 engineering used to edit the *Ccl25* gene.

# Technical Information

- The *Ccl25* gene has 10 transcripts. According to the structure of *Ccl25* gene, exon3-5 of *Ccl25*-201 (ENSMUST00000024004.9) transcript is recommended as the knockout region. The region contains 328 bp of coding sequences. Knocking out the region will result in disruption of the function of *Ccl25*.
- In this project we use CRISPR-Cas9 technology to modify *Ccl25* gene. The brief process is as follows: Cas9 and gRNAs 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.

# Gene Information

**Ccl25 chemokine (C-C motif) ligand 25 [ *Mus musculus* (house mouse) ]**

[Download Datasets](#)

Gene ID: 20300, updated on 12-Apr-2023

## Summary

<b>Official Symbol</b>	Ccl25 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	chemokine (C-C motif) ligand 25 provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:1099448</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000023235</a> <a href="#">AllianceGenome:MGI:1099448</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	VALIDATED
<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>	TECK; CKb15; Scya25; A130072A22Rik
<b>Summary</b>	Enables chemokine activity. Acts upstream of or within chemotaxis and leukocyte migration. Predicted to be located in extracellular region. Predicted to be active in extracellular space. Is expressed in 3rd branchial pouch; central nervous system; hemolymphoid system; retina; and thymus/parathyroid primordium. Orthologous to human CCL25 (C-C motif chemokine ligand 25). [provided by Alliance of Genome Resources, Apr 2022]
<b>Expression</b>	Biased expression in large intestine adult (RPKM 91.4), thymus adult (RPKM 40.0) and 2 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>
<b>NEW</b>	Try the new <a href="#">Gene table</a> Try the new <a href="#">Transcript table</a>

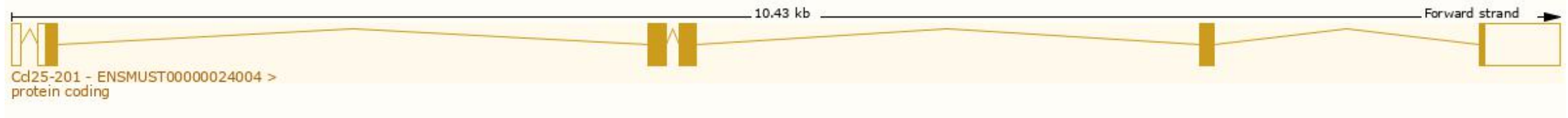
Source: <https://www.ncbi.nlm.nih.gov/>

# Transcript Information

The gene has 10 transcripts, all transcripts are shown below:

Show/hide columns (1 hidden) <span>Filter</span>							
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
<a href="#">ENSMUST00000207925.2</a>	Ccl25-210	2549	No protein	Retained intron		-	TSL:NA
<a href="#">ENSMUST00000207839.2</a>	Ccl25-209	2461	No protein	Retained intron		-	TSL:NA
<a href="#">ENSMUST00000155797.8</a>	Ccl25-208	1061	<a href="#">53aa</a>	Nonsense mediated decay		<a href="#">A0A087WRS0</a>	TSL:2
<a href="#">ENSMUST00000153254.3</a>	Ccl25-207	362	No protein	Protein coding CDS not defined		-	TSL:3
<a href="#">ENSMUST00000136191.8</a>	Ccl25-206	395	<a href="#">119aa</a>	Protein coding		<a href="#">D3Z470</a>	TSL:3 CDS 3' incomplete
<a href="#">ENSMUST00000127460.8</a>	Ccl25-205	867	<a href="#">228aa</a>	Protein coding		<a href="#">D3YWJ1</a>	Ensembl Canonical GENCODE basic APPRIS ALT2 TSL:5
<a href="#">ENSMUST00000110982.8</a>	Ccl25-204	823	<a href="#">134aa</a>	Protein coding		<a href="#">Q3V2F3</a>	GENCODE basic APPRIS P4 TSL:1
<a href="#">ENSMUST00000098949.11</a>	Ccl25-203	1109	<a href="#">89aa</a>	Nonsense mediated decay		<a href="#">Q3U4J3</a>	TSL:2
<a href="#">ENSMUST00000069762.16</a>	Ccl25-202	485	<a href="#">146aa</a>	Non stop decay		<a href="#">F7C0M8</a>	TSL:2
<a href="#">ENSMUST00000024004.9</a>	Ccl25-201	1058	<a href="#">144aa</a>	Protein coding	<a href="#">CCDS22085</a>	<a href="#">Q35903</a>	GENCODE basic APPRIS ALT2 TSL:1

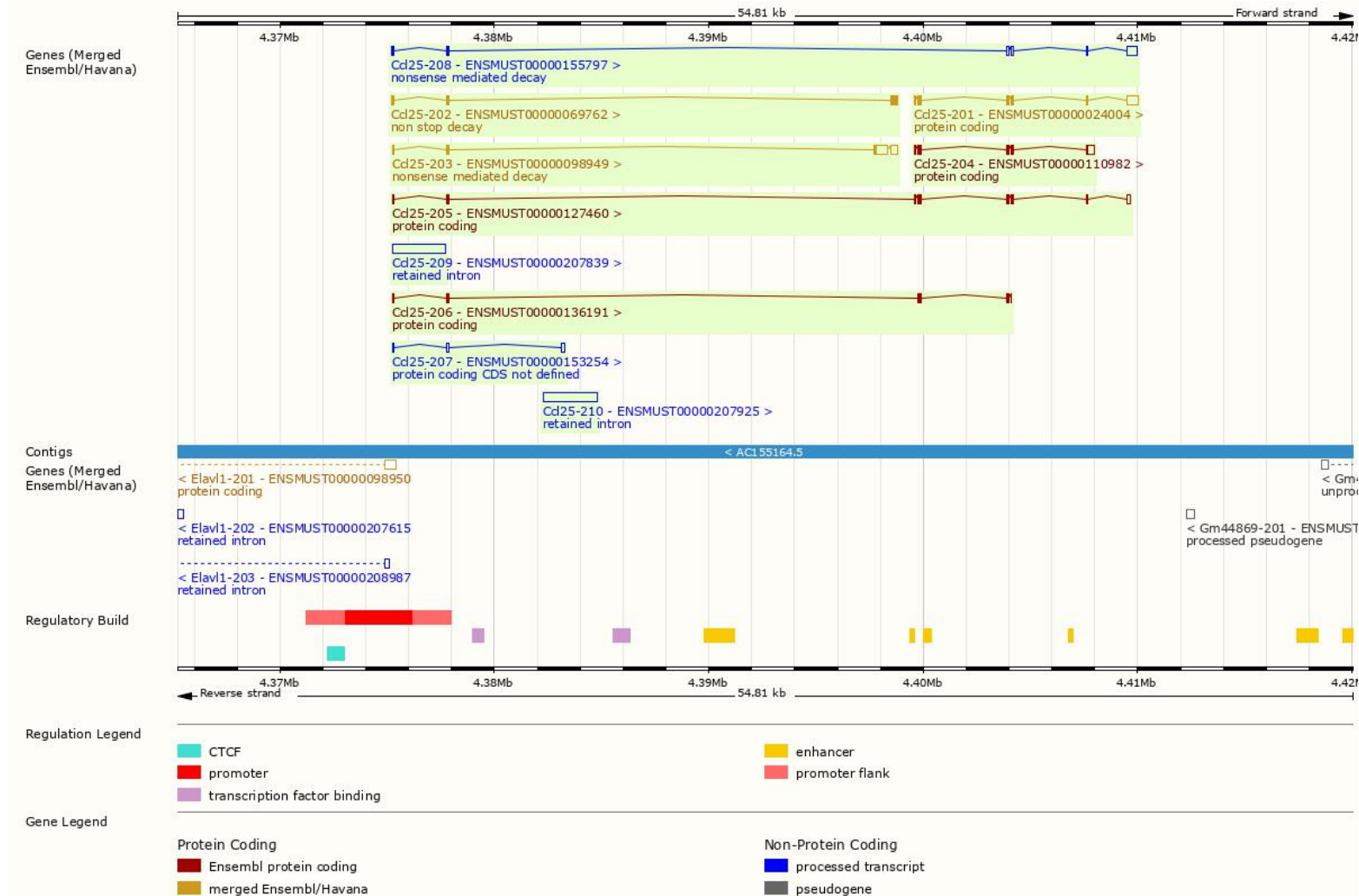
The strategy is based on the design of *Ccl25-201* transcript, the transcription is shown below:



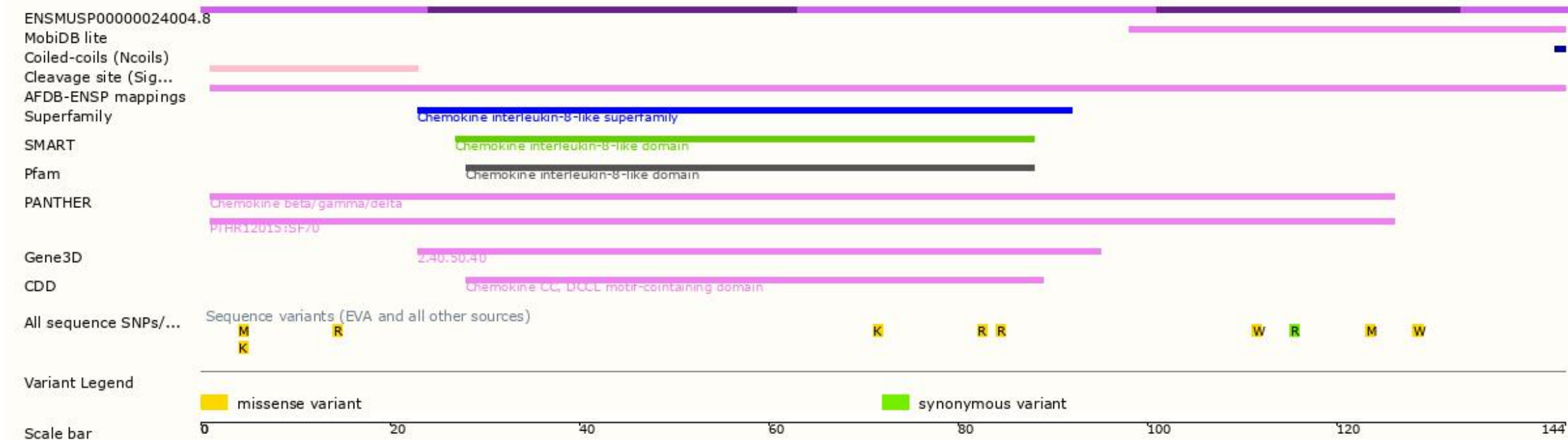
Source: <https://www.ensembl.org>



# Genomic Information



# Protein Information





# Important Information

- Mice homozygous for a knock-out allele exhibit impaired accumulation of antigen-specific CD8<sup>+</sup> T lymphocytes within both lamina propria and epithelium of the small intestine.
- This strategy don't affect transcription *Ccl25*-202, 203, 207, 208, 209 and 210, the risk is known.
- *Ccl25* is located on Chr8. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.