

# Ccl25 Cas9-CKO Strategy

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## Overview

#### **Target Gene Name**

• Ccl25

#### **Project Type**

• Cas9-CKO

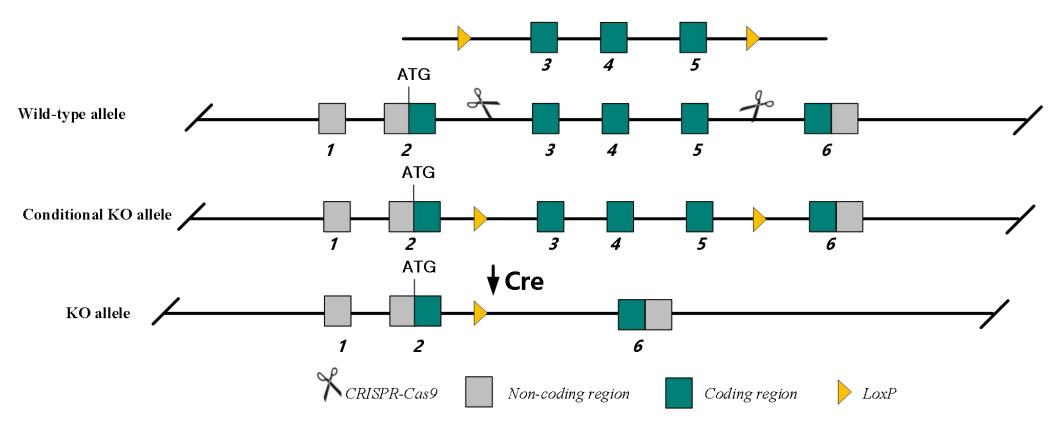
### Genetic Background

• C57BL/6JGpt

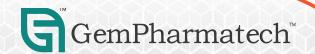


# Strain Strategy

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: 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

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

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

Summary



Official Full Name chemokine (C-C motif) ligand 25 provided by MGI

Primary source MGI:MGI:1099448

Official Symbol Ccl25 provided by MGI

See related Ensembl:ENSMUSG00000023235 AllianceGenome:MGI:1099448

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 TECK; CKb15; Scya25; A130072A22Rik

Summary 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]

Expression Biased expression in large intestine adult (RPKM 91.4), thymus adult (RPKM 40.0) and 2 other tissues See more

Orthologs <u>human</u> all

W Try the new Gene table

Try the new Transcript table

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

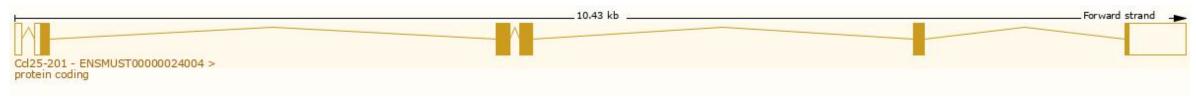


# Transcript Information

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

Transcript ID ▼	Name	bp 🌲	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000207925.2	Ccl25-210	2549	No protein	Retained intron			TSL:NA
ENSMUST00000207839.2	Ccl25-209	2461	No protein	Retained intron		-	TSL:NA
ENSMUST00000155797.8	Ccl25-208	1061	<u>53aa</u>	Nonsense mediated decay		A0A087WRS0₺	TSL:2
ENSMUST00000153254.3	Ccl25-207	362	No protein	Protein coding CDS not defined		929	TSL:3
ENSMUST00000136191.8	Ccl25-206	395	<u>119aa</u>	Protein coding		<u>D3Z470</u> ₽	TSL:3 CDS 3' incomplete
ENSMUST00000127460.8	Ccl25-205	867	228aa	Protein coding		D3YWJ1₽	Ensembl Canonical GENCODE basic APPRIS ALT2 TSL:
ENSMUST00000110982.8	Ccl25-204	823	<u>134aa</u>	Protein coding		Q3V2F3₺	GENCODE basic APPRIS P4 TSL:1
ENSMUST00000098949.11	Ccl25-203	1109	89aa	Nonsense mediated decay		Q3U4J3&	TSL:2
ENSMUST00000069762.16	Ccl25-202	485	<u>146aa</u>	Non stop decay		F7C0M8 ₺	TSL:2
ENSMUST00000024004.9	Ccl25-201	1058	144aa	Protein coding	CCDS22085 ₺	O35903₽	GENCODE basic APPRIS ALT2 TSL:1

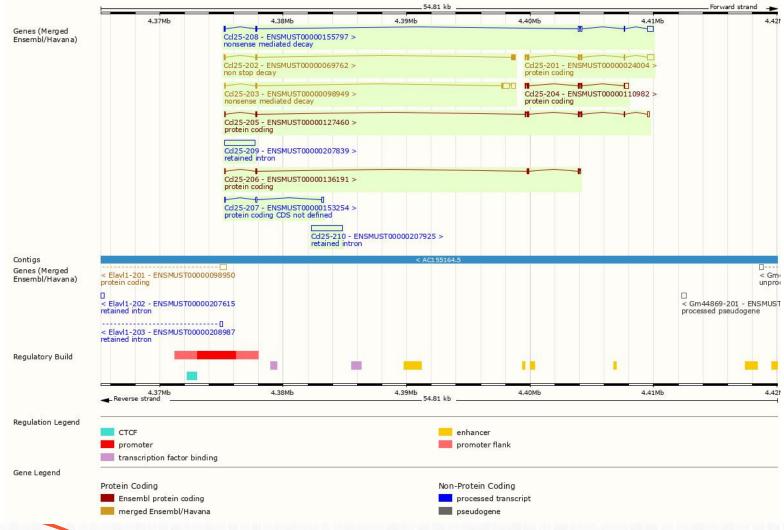
The strategy is based on the design of *Ccl25*-201 transcript, the transcription is shown below:

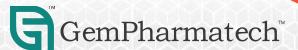


Source: https://www.ensembl.org



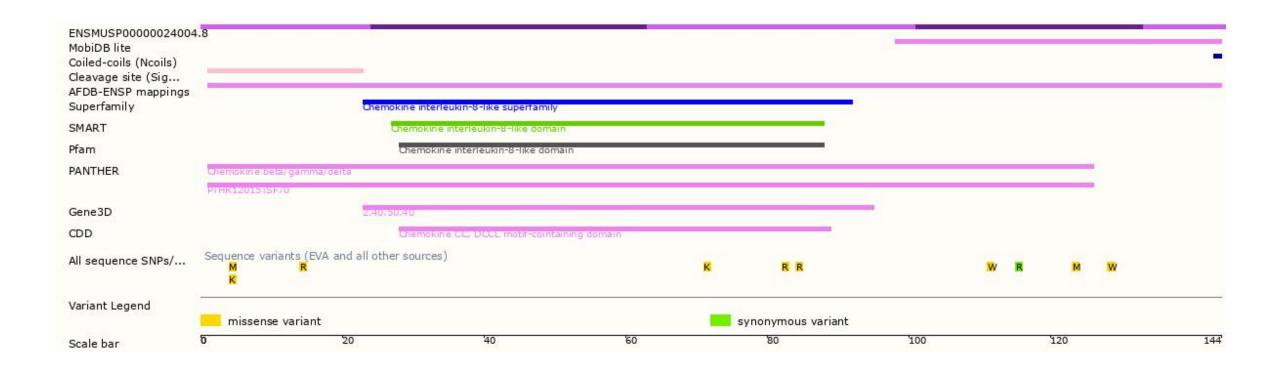
# Genomic Information





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

## Protein Information





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

# Important Information

- Mice homozygous for a knock-out allele exhibit impaired accumulation of antigenspecific CD8<sup>+</sup> T lymphocytes within both lamina propria and epithelium of the small intestine.
- This stratergy 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

