

# Crabp2 Cas9-CKO Strategy

Designer: Jiaojiao Yan

Reviewer: Xiangli Bian

Design Date: 2023.8.6

# Overview

#### Target Gene Name

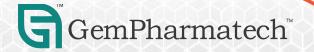
• Crabp2

# Project Type

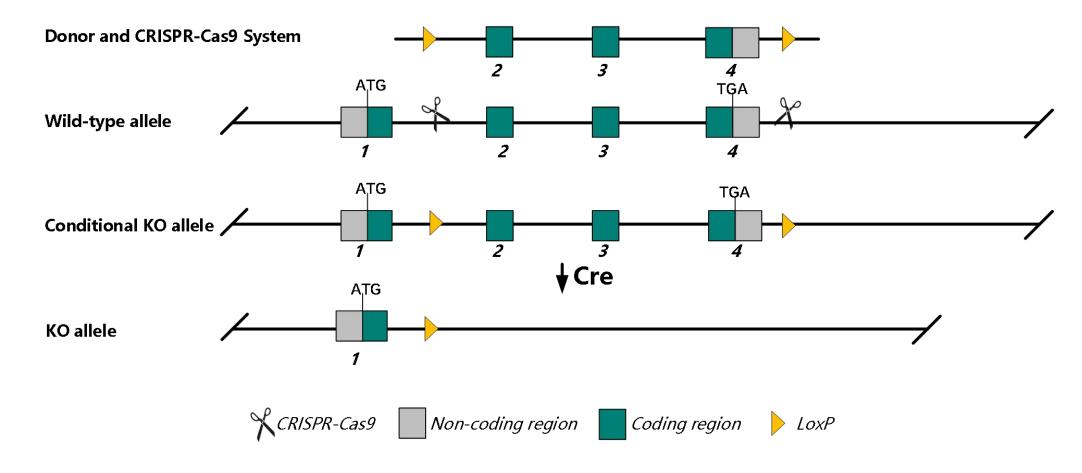
• Cas9-CKO

#### Genetic Background

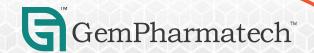
• C57BL/6JGpt



# Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the Crabp2 gene.



#### **Technical Information**

- The *Crabp2* gene has 2 transcripts. According to the structure of *Crabp2* gene, exon2-4 of *Crabp2*-201 (ENSMUST00000005019.6) transcript is recommended as the knockout region. The region contains 347bp of coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Crabp2* 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

#### Crabp2 cellular retinoic acid binding protein II [ Mus musculus (house mouse) ]



Genomic context

Location: 3 F1: 3 38.78 cM See Crabp2 in Genome Data Viewer

Exon count: 4

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

**≛** Download Datasets



# Transcript Information

The gene has 2 transcripts, all transcripts are shown below:

Transcript ID		bp 🍦	Protein   138aa	-	17.70	UniProt Match ⊕ P22935 ₽	Flags			
ENSMUST00000005019.6		931					Ensembl Canonical	GENCODE basic	APPRIS P1	TSL:1
ENSMUST00000163040.2	Crabp2-202	658	No protein	Retained intron		-	TSL:2			

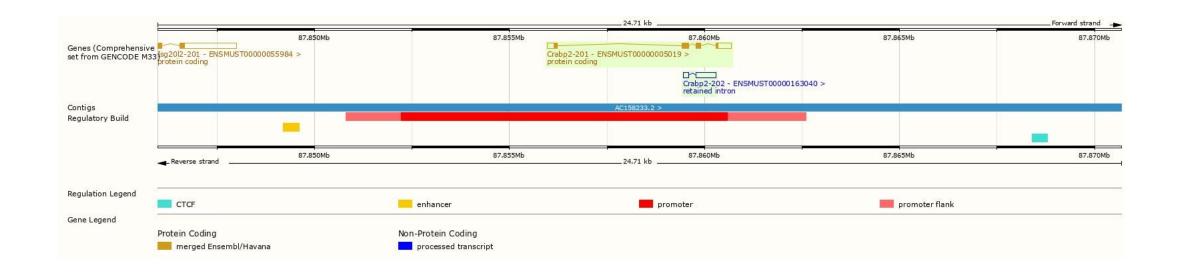
The strategy is based on the design of *Crabp2*-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org



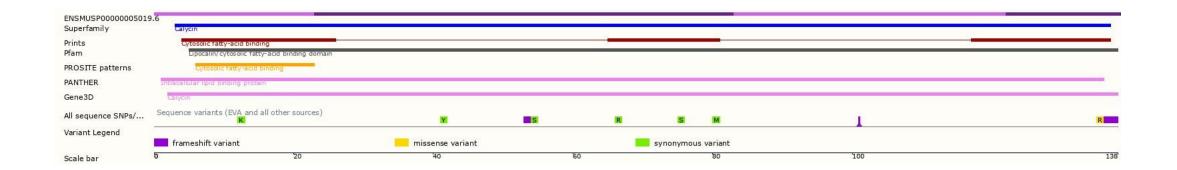
### Genomic Information





Source: https://www.ensembl.org

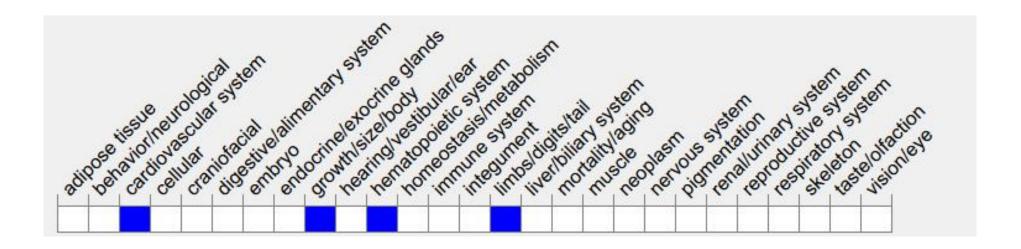
# **Protein Information**





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

# Mouse Phenotype Information (MGI)



Homozygotes for targeted null mutations may exhibit an additional postaxial digit, usually on a single forepaw. Penetrance is dependent on the genetic background.



Source: https://www.informatics.jax.org

# Important Information

- According to the existing MGI data, homozygotes for targeted null mutations may exhibit an additional postaxial digit, usually on a single forepaw. Penetrance is dependent on the genetic background.
- *Crabp2* is located on Chr3. 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.

