# Ramp1 Cas9-CKO Strategy

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Reviewer: Huimin Su

**Design Date:** 2019-8-28

## **Project Overview**



**Project Name** 

Ramp1

**Project type** 

Cas9-CKO

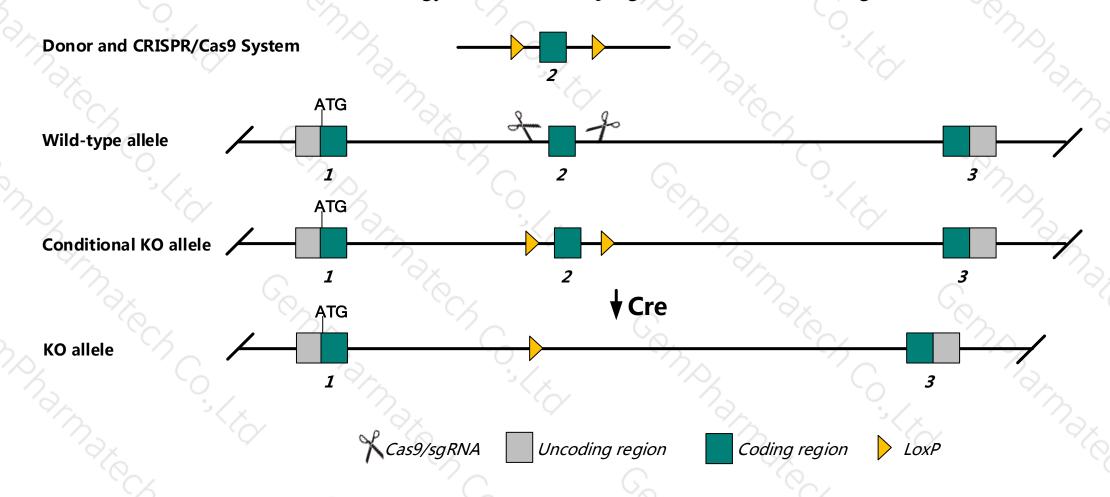
Strain background

C57BL/6JGpt

## **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the *Ramp1* gene. The schematic diagram is as follows:



### **Technical routes**



- The *Ramp1* gene has 3 transcripts. According to the structure of *Ramp1* gene, exon 2 of *Ramp1*-201 (ENSMUST00000097648.5) transcript is recommended as the knockout region. The region contains 139bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ramp1* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.
- ➤ The flox mice was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues or cell types.

### **Notice**



- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit high systolic blood pressure due to a disruption in vasodilatory regulation as well as significantly increased serum levels of proinflammatory cytokines following LPS administration.
- ➤ The *Ramp1* gene is located on the Chr1. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

## Gene information (NCBI)



#### Ramp1 receptor (calcitonin) activity modifying protein 1 [ Mus musculus (house mouse) ]

Gene ID: 51801, updated on 18-Sep-2018

#### **Summary**

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Official Symbol Ramp1 provided by MGI

Official Full Name receptor (calcitonin) activity modifying protein 1 provided by MGI

Primary source MGI:MGI:1858418

See related Ensembl: ENSMUSG00000034353 Vega: OTTMUSG00000048487

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 9130218E19Rik

Expression Broad expression in thymus adult (RPKM 23.9), colon adult (RPKM 15.0) and 16 other tissues See more

Orthologs human all

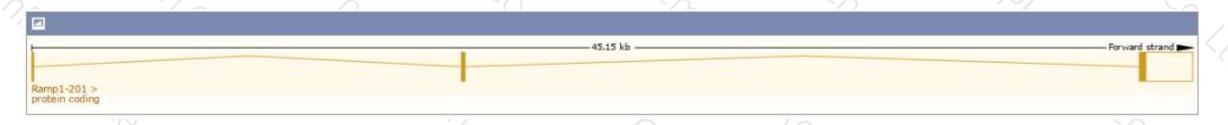
## Transcript information (Ensembl)



The gene has 3 transcripts, and all transcripts are shown below:

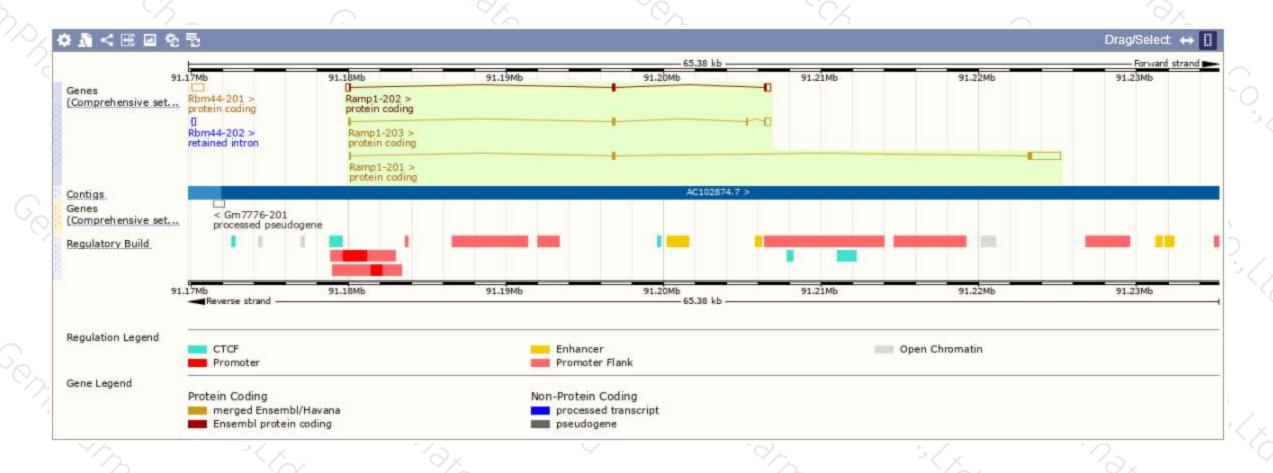
Show/hide columns (1 hidden)								
Name 🍦	Transcript ID 🍦	bp 🌲	Protein 🍦	Biotype 🍦	CCDS 🍦	UniProt	RefSeq 🍦	Flags ♣
Ramp1-201	ENSMUST00000097648.5	2325	<u>148aa</u>	Protein coding	<u>CCDS15158</u>	Q3TNJ3@ Q9WTJ5@	<u>NM_016894</u> ଜ <u>NP_058590</u> ଜ	TSL:1 GENCODE basic APPRIS P1
Ramp1-202	ENSMUST00000165855.7	852	<u>103aa</u>	Protein coding	<u>CCDS78648</u> ₽	<u>E9Q915</u> ਫ਼	NM_178401 & NP_848488 &	TSL:2 GENCODE basic
Ramp1-203	ENSMUST00000188475.6	711	<u>105aa</u>	Protein coding	CCDS48321 ₽	<u>A0A087WPC6</u> ₽	NM_001168392 ผ NP_001161864 ผ	TSL:3 GENCODE basic

The strategy is based on the design of *Ramp1*-201 transcript, The transcription is shown below



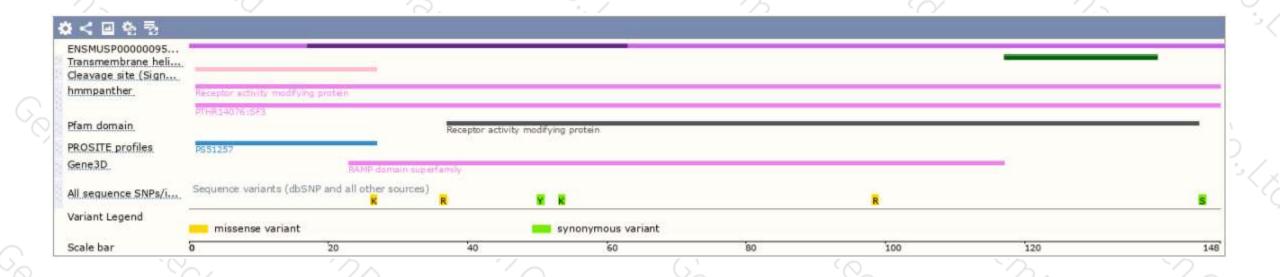
### Genomic location distribution





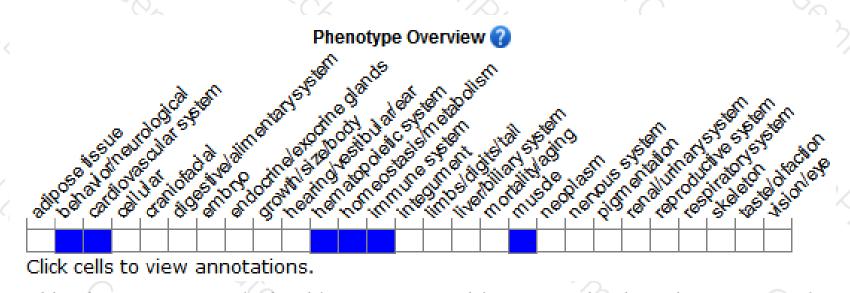
### Protein domain





### Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data, Mice homozygous for a knock-out allele exhibit high systolic blood pressure due to a disruption in vasodilatory regulation as well as significantly increased serum levels of proinflammatory cytokines following LPS administration.

If you have any questions, you are welcome to inquire. Tel: 025-5864 1534





