

Rala Cas9-CKO Strategy

Designer:

Ruirui Zhang

Reviewer:

Huimin Su

Design Date:

2020-2-27

Project Overview

Project Name

Rala

Project type

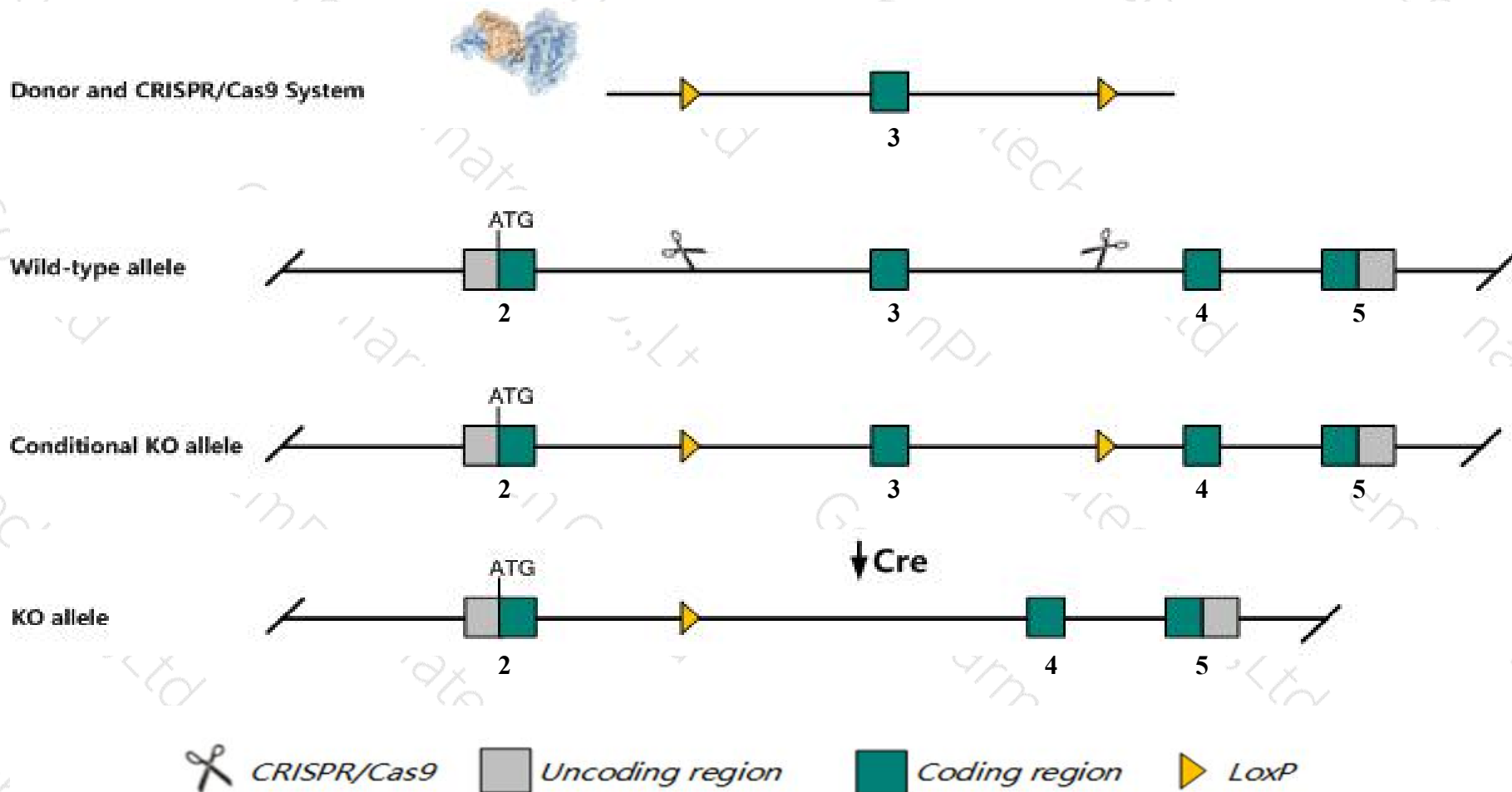
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



Technical routes

- The *Rala* gene has 2 transcripts. According to the structure of *Rala* gene, exon3 of *Rala-201* (ENSMUST00000009003.8) transcript is recommended as the knockout region. The region contains 209bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rala* 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.
- 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.

- According to the existing MGI data, mice homozygous for a knock-out allele exhibit complete preweaning lethality and exencephaly.
- The *Rala* gene is located on the Chr13. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)

Rala v-ral simian leukemia viral oncogene A (ras related) [*Mus musculus* (house mouse)]

Gene ID: 56044, updated on 8-Dec-2019

Summary

Official Symbol Rala provided by [MGI](#)

Official Full Name v-ral simian leukemia viral oncogene A (ras related) provided by [MGI](#)

Primary source [MGI:MGI:1927243](#)

See related [Ensembl:ENSMUSG00000008859](#)

Gene type protein coding

RefSeq status PROVISIONAL

Organism [Mus musculus](#)

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus

Also known as Ral; Rasl1; AW322615; 3010001O15Rik

Expression Ubiquitous expression in CNS E18 (RPKM 28.4), CNS E14 (RPKM 21.1) and 28 other tissues [See more](#)

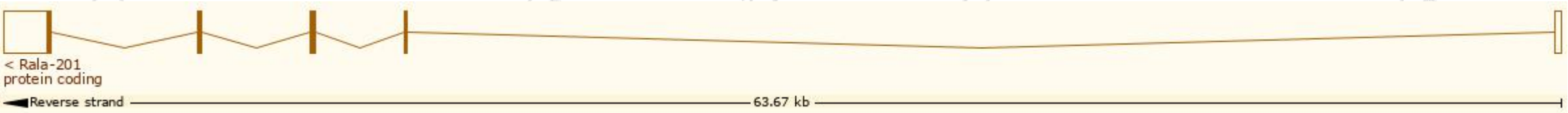
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

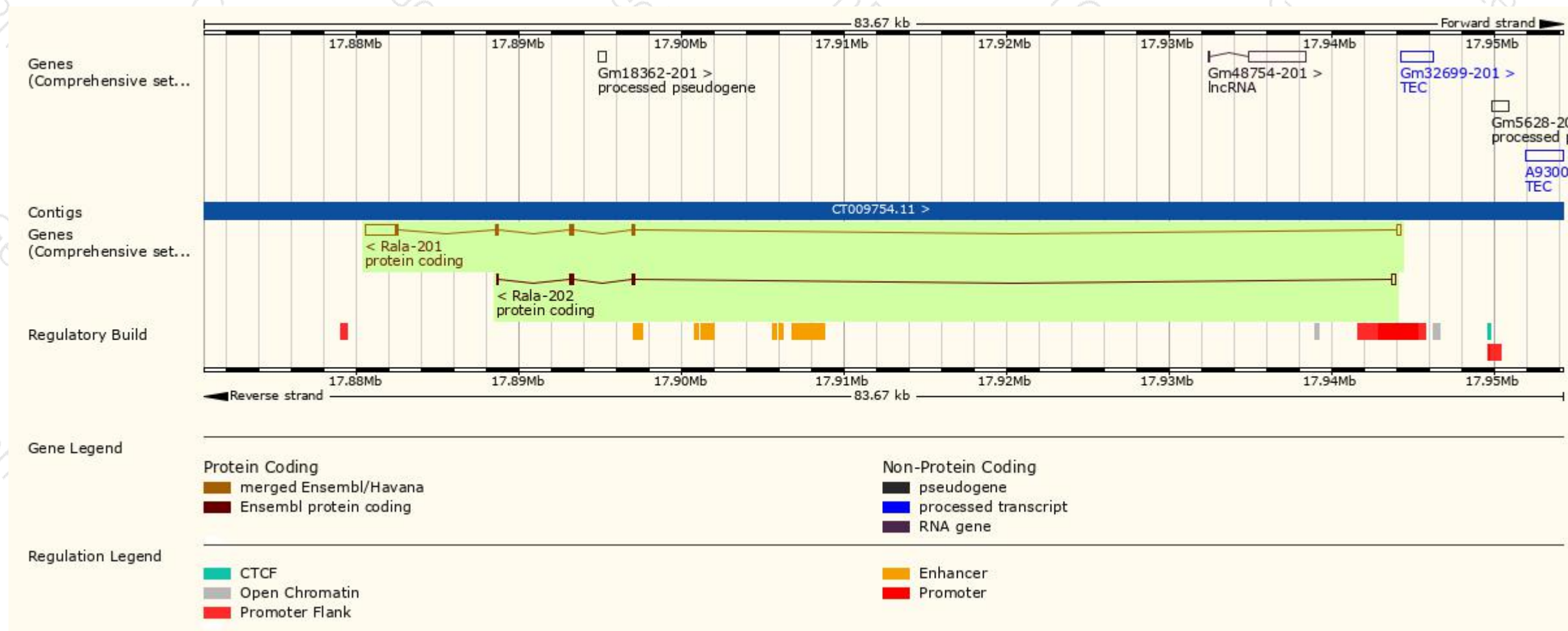
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rala-201	ENSMUST00000009003.8	2688	206aa	Protein coding	CCDS26255	P63321	TSL:1 GENCODE basic APPRIS P1
Rala-202	ENSMUST00000223147.1	659	137aa	Protein coding	-	A0A1Y7VL93	CDS 3' incomplete TSL:5

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



Genomic location distribution



Protein domain

ENSMUSP00000009...

Low complexity (Seg)

Coiled-coils (Ncoils)

TIGRFAM

Superfamily

SMART

Prints

Pfam

PROSITE profiles

PANTHER

Gene3D

CDD

All sequence SNPs/i...

Variant Legend

Scale bar

Small GTP-binding protein domain

P-loop containing nucleoside triphosphate hydrolase

SM00174

SM00175

SM00176

SM00173

PR00449

Small GTPase

Small GTPase superfamily, Ras-type

Ras-related protein Ral

Small GTPase superfamily, Ras-type

3.40.50.300

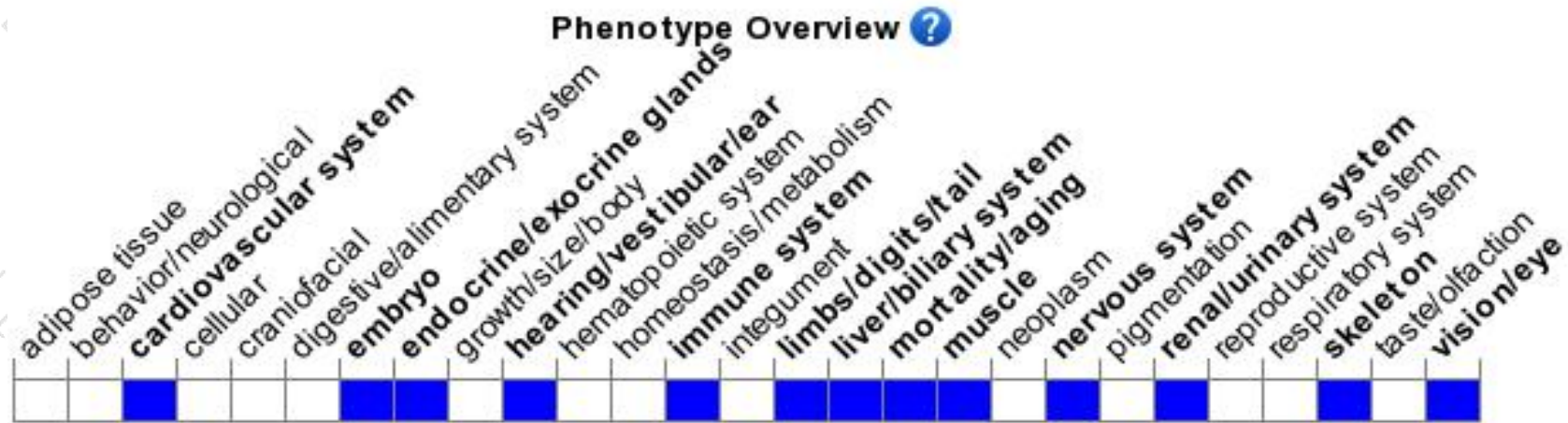
cd04139

Sequence variants (dbSNP and all other sources)

synonymous variant

0 20 40 60 80 100 120 140 160 180 206

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 complete preweaning lethality and exencephaly.

If you have any questions, you are welcome to inquire.

Tel: 400-9660890

