

Rac2 Cas9-CKO Strategy

Designer:Xueting Zhang

Reviewer: Yanhua Shen

Date:2019-10-27

Project Overview



Project Name

Rac2

Project type

Cas9-CKO

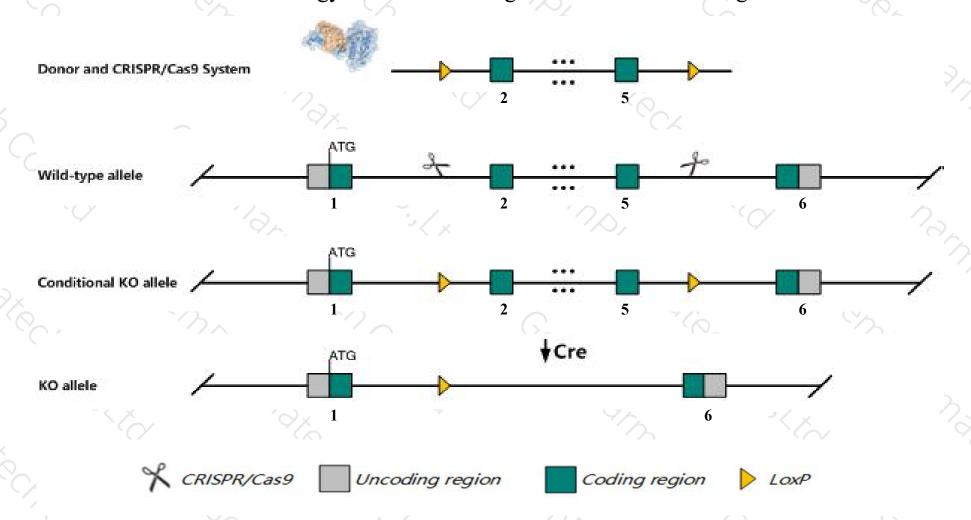
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Rac2* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Rac2* gene has 3 transcripts. According to the structure of *Rac2* gene, exon2-exon5 of *Rac2-201*(ENSMUST00000043214.7) transcript is recommended as the knockout region. The region contains 413bp coding sequence.

 Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Rac2* 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.

Notice



- > According to the existing MGI data, Homozygotes for a targeted null mutation exhibit peripheral blood lymphocytosis, reductions in peritoneal B-1a lymphocytes, marginal zone lymphocytes, and IgM-secreting plasma cells, decreased levels of serum IgM and IgA, and abnormal T cell migration.
- ➤ The effect on transcript *Rac2*-203 is unknown.
- The *Rac2* gene is located on the Chr15. 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)



Rac2 Rac family small GTPase 2 [Mus musculus (house mouse)]

Gene ID: 19354, updated on 10-Oct-2019

Summary

↑ ?

Official Symbol Rac2 provided by MGI

Official Full Name Rac family small GTPase 2 provided by MGI

Primary source MGI:MGI:97846

See related Ensembl: ENSMUSG00000033220

Gene type protein coding
RefSeq status VALIDATED
Organism <u>Mus musculus</u>

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

Muridae; Murinae; Mus; Mus

Also known as Al323801; Al452260

Expression Biased expression in spleen adult (RPKM 361.0), thymus adult (RPKM 337.1) and 8 other tissues See more

Orthologs human all

Genomic context

?

Location: 15; 15 E1

See Rac2 in Genome Data Viewer

Exon count: 7

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF 000001635.26)	15	NC_000081.6 (7855916978572783, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	15	NC_000081.5 (7838959978403213, complement)

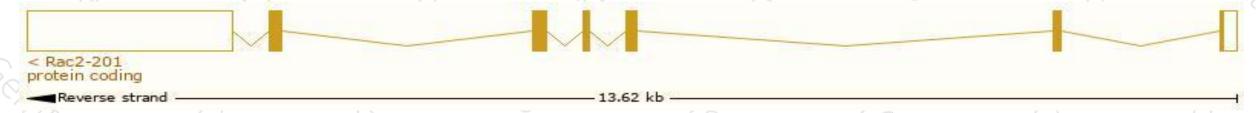
Transcript information (Ensembl)



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

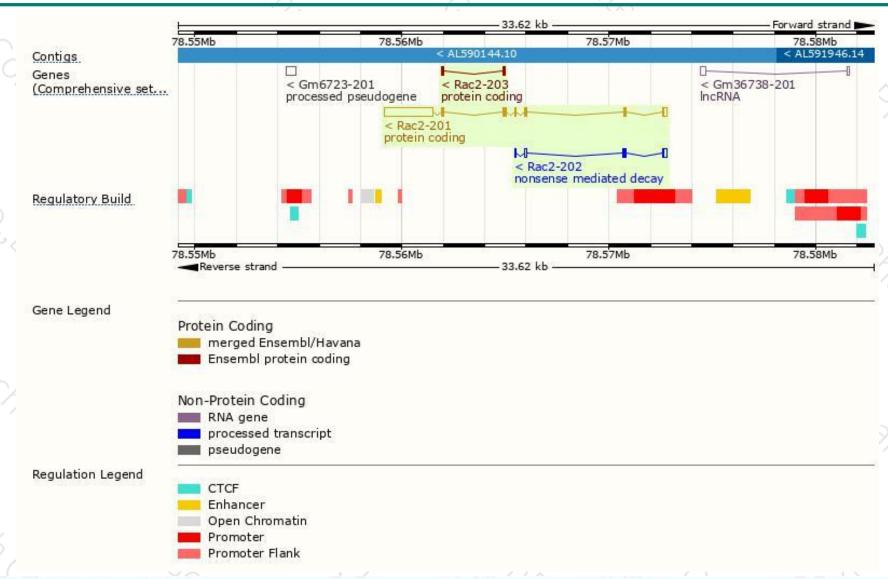
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rac2-201	ENSMUST00000043214.7	3038	<u>192aa</u>	Protein coding	CCDS27619	Q05144	TSL:1 GENCODE basic APPRIS P1
Rac2-203	ENSMUST00000230952.1	254	80aa	Protein coding	670	A0A2R8VHH0	CDS 5' incomplete
Rac2-202	ENSMUST00000229394.1	459	36aa	Nonsense mediated decay	1940	A0A2R8VH29	

The strategy is based on the design of *Rac2-201* transcript, The transcription is shown below



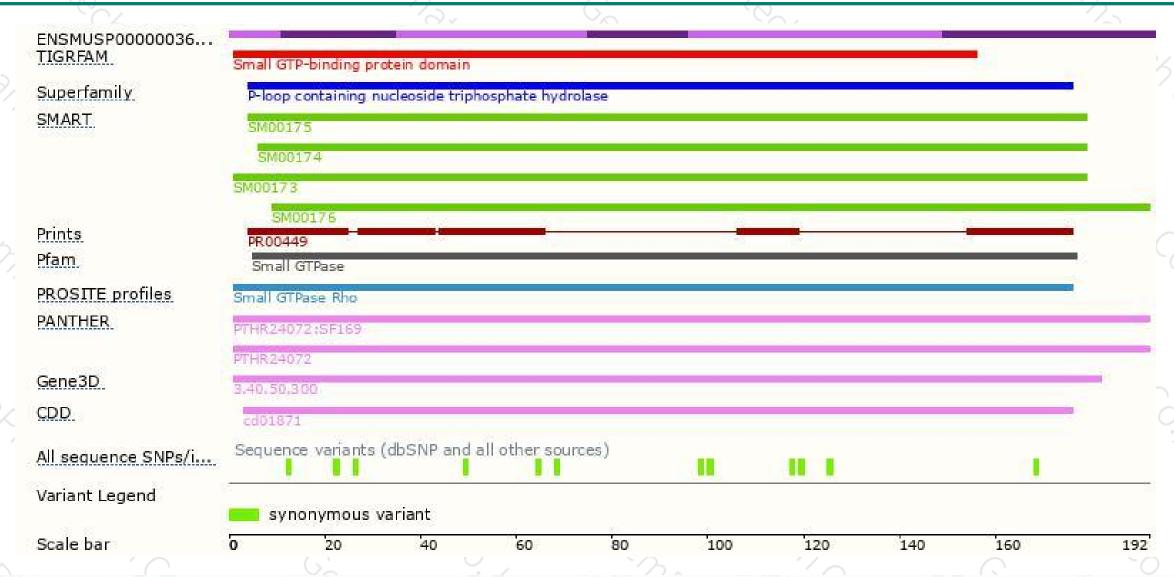
Genomic location distribution





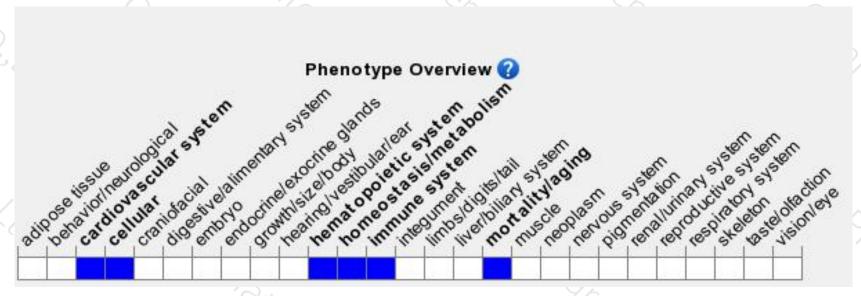
Protein domain





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, Homozygotes for a targeted null mutation exhibit peripheral blood lymphocytosis, reductions in peritoneal B-1a lymphocytes, marginal zone lymphocytes, and IgM-secreting plasma cells, decreased levels of serum IgM and IgA, and abnormal T cell migration.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





