

# Rap1a Cas9-KO Strategy

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**Reviewer:** Yanhua Shen

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### **Project Overview**



Project Name Rap1a

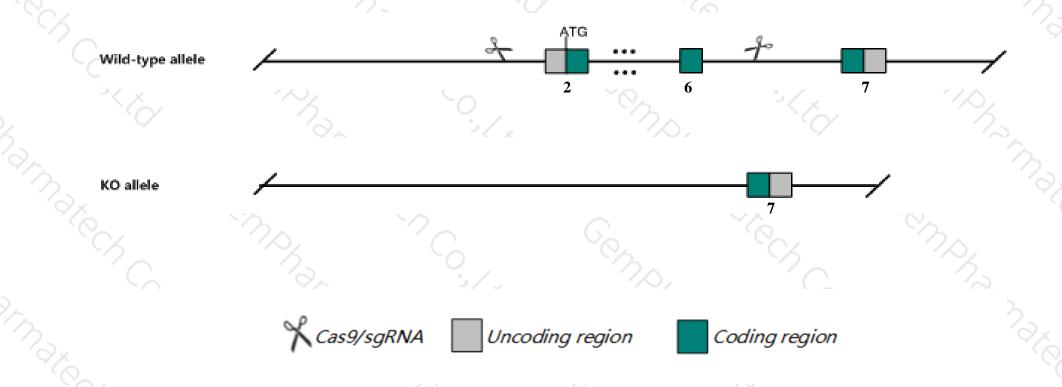
Project type Cas9-KO

Strain background C57BL/6JGpt

### **Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the *Rap1a* gene. The schematic diagram is as follows:



### **Technical routes**



- ➤ The *Rap1a* gene has 4 transcripts. According to the structure of *Rap1a* gene, exon2-exon6 of *Rap1a-201* (ENSMUST00000090678.10) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Rap1a* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

### **Notice**



- ➤ According to the existing MGI data, Mice homozygous for a null allele exhibit impaired leukocyte migration and decreased angiogenesis.
- ➤ The *Rap1a* gene is located on the Chr3. 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 gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

### Gene information (NCBI)



#### Rap1a RAS-related protein 1a [ Mus musculus (house mouse) ]

Gene ID: 109905, updated on 25-Oct-2019

#### Summary

**☆?** ‡

Official Symbol Rap1a provided by MGI

Official Full Name RAS-related protein 1a provided by MGI

Primary source MGI:MGI:97852

See related Ensembl: ENSMUSG00000068798

Gene type protein coding
RefSeq status VALIDATED
Organism Mus musculus

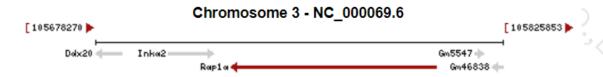
Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;

Muroidea; Muridae; Murinae; Mus; Mus

Also known as Rap1; G-22K; Krev-1; Al848598

**Expression** Broad expression in bladder adult (RPKM 24.1), liver E14 (RPKM 21.9) and 21 other tissues See more

Orthologs <u>human</u> all



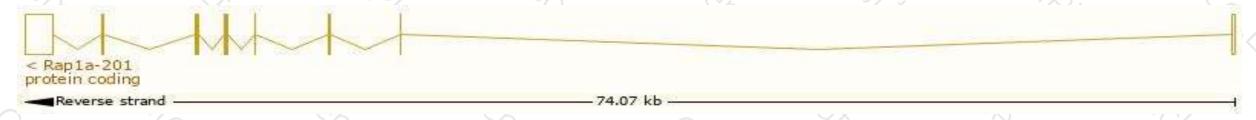
# Transcript information (Ensembl)



The gene has 4 transcripts, all transcripts are shown below:

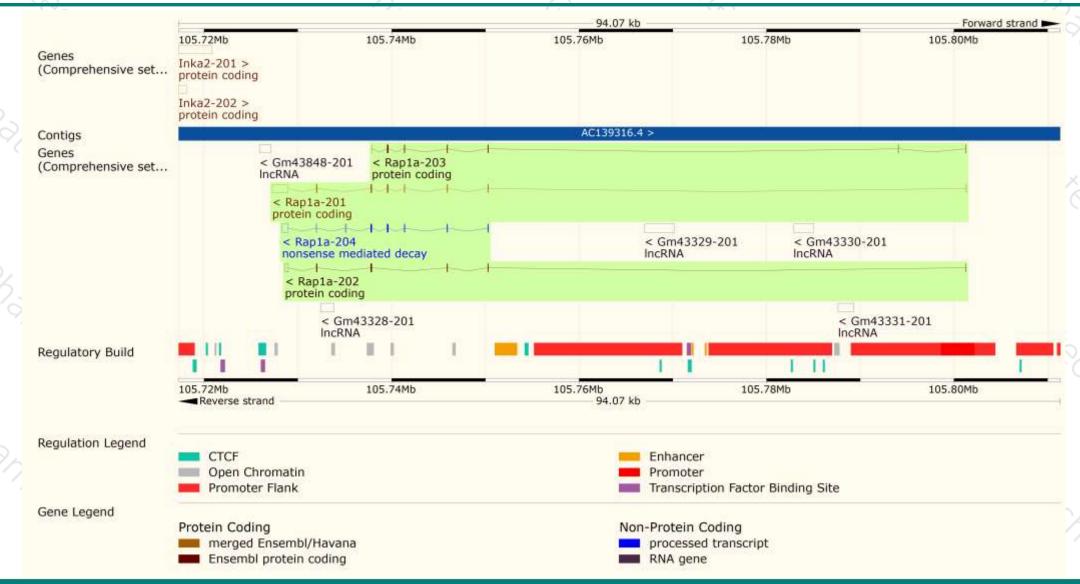
Name 🍦	Transcript ID 🗼	bp 🌲	Protein 🍦	Translation ID 🗼	Biotype	CCDS	UniProt 🍦	Flags 🝦
Rap1a-201	ENSMUST00000090678.10	2407	<u>184aa</u>	ENSMUSP00000088174.6	Protein coding	CCDS17711 ₺	<u>P62835</u> ₽	TSL:1 GENCODE basic APPRIS P1
Rap1a-202	ENSMUST00000197094.4	782	<u>118aa</u>	ENSMUSP00000142419.1	Protein coding	-	A0A0G2JDL9₺	TSL:3 GENCODE basic
Rap1a-203	ENSMUST00000198004.1	511	<u>109aa</u>	ENSMUSP00000142733.1	Protein coding	-	A0A0G2JED9₺	CDS 3' incomplete TSL:5
Rap1a-204	ENSMUST00000199969.4	1377	<u>159aa</u>	ENSMUSP00000142634.1	Nonsense mediated decay	-	A0A0G2JE52₺	TSL:5

The strategy is based on the design of Rap1a-201 transcript, The transcription is shown below



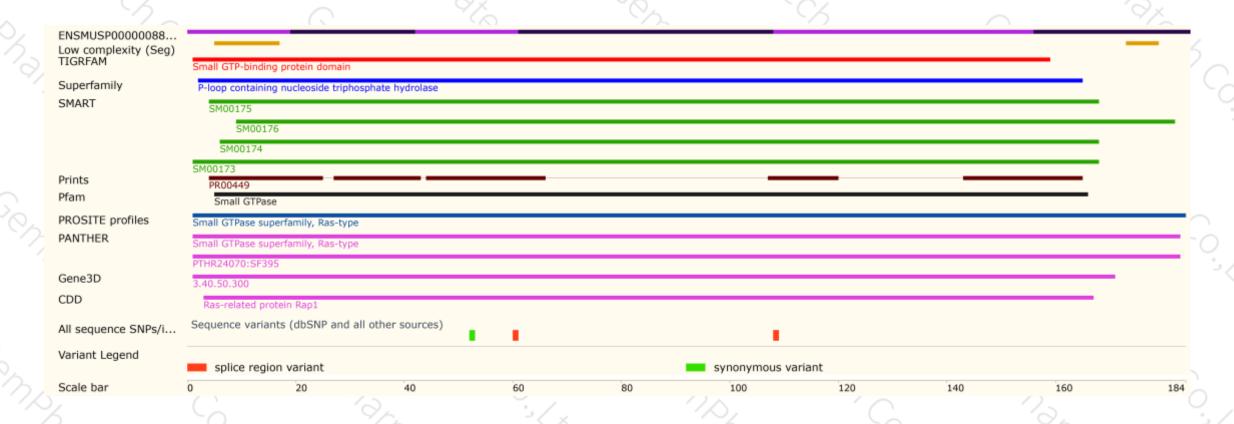
### Genomic location distribution





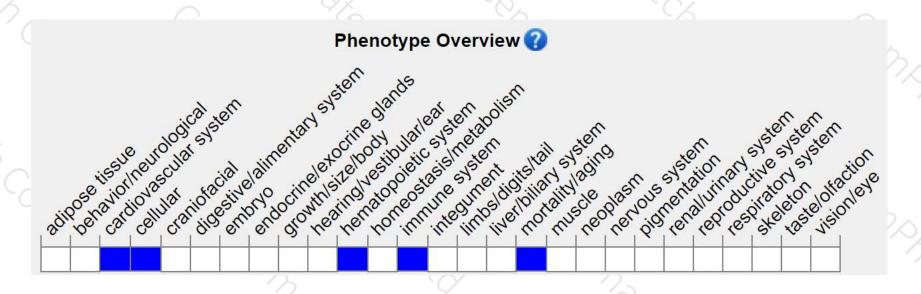
### Protein domain





## 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 null allele exhibit impaired leukocyte migration and decreased angiogenesis.



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

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