

# Rhof Cas9-KO Strategy

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# **Project Overview**

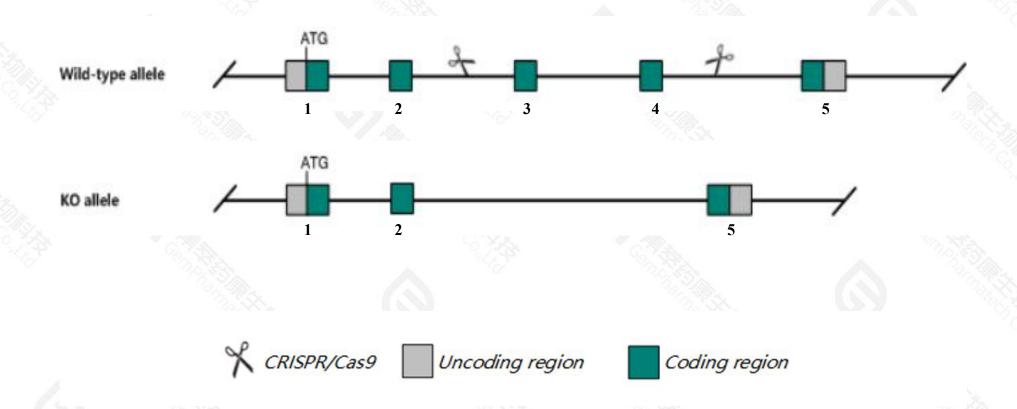


Project Name	Rhof	
Project type	Cas9-KO	
Strain background	C57BL/6JGpt	

# **Knockout strategy**



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



### **Technical routes**



- The *Rhof* gene has 4 transcripts. According to the structure of *Rhof* gene, exon3-exon4 of *Rhof-* 204(ENSMUST00000186469.6) transcript is recommended as the knockout region. The region contains 245bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Rhof* gene. The brief process is as follows: CRISPR/Cas9 system 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**



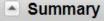
- > According to the existing MGI data, mice homozygous for a knock-out allele exhibit macrothrombocytopenia without an effect on filopodia generation.
- > The N-terminal of *Rhof* gene will remain several amino acids, it may remain the partial function of *Rhof* gene.
- ➤ Transcript *Rhof-203* may not be affected.
- > The *Rhof* gene is located on the Chr5. 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)



#### Rhof ras homolog family member F (in filopodia) [ Mus musculus (house mouse) ]

Gene ID: 23912, updated on 22-Nov-2020





Official Symbol Rhof provided by MGI

Official Full Name ras homolog family member F (in filopodia) provided by MGI

Primary source MGI:MGI:1345629

See related Ensembl: ENSMUSG00000029449

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 Ar; IfI; Rif; Arhf; IfId1; Al845056; AV026554

Expression Broad expression in spleen adult (RPKM 35.2), small intestine adult (RPKM 26.9) and 17 other tissues See more

Orthologs human all

NEW

Try the new Gene table

Try the new <u>Transcript table</u>

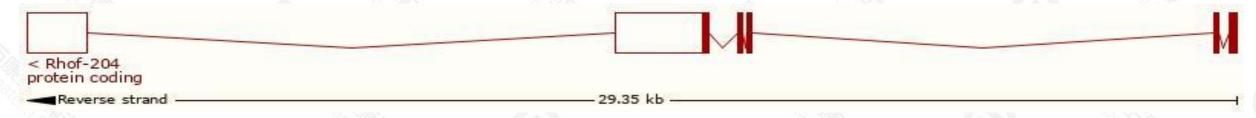
# Transcript information (Ensembl)



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

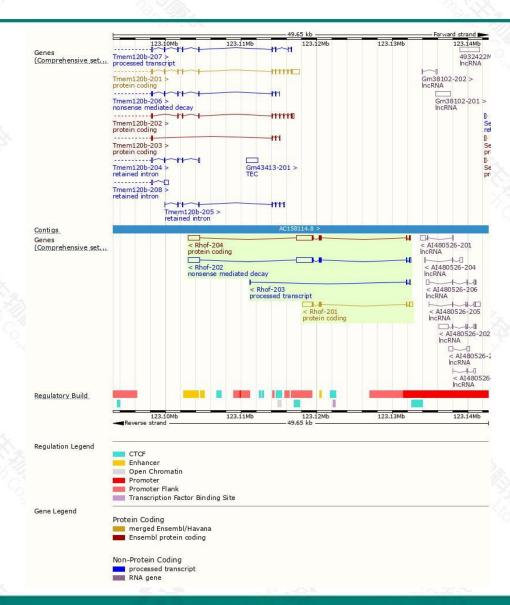
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rhof-204	ENSMUST00000186469.6	4241	211aa	Protein coding	CCDS39263	Q4VA10 Q8BYP3	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Rhof-201	ENSMUST00000031401.5	2208	211aa	Protein coding	CCDS39263	Q4VA10 Q8BYP3	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Rhof-202	ENSMUST00000160479.7	4306	211aa	Nonsense mediated decay		Q4VA10 Q8BYP3	TSL:1
Rhof-203	ENSMUST00000162208.1	348	No protein	Processed transcript	12	-	TSL:3

The strategy is based on the design of *Rhof-204* transcript, the transcription is shown below:



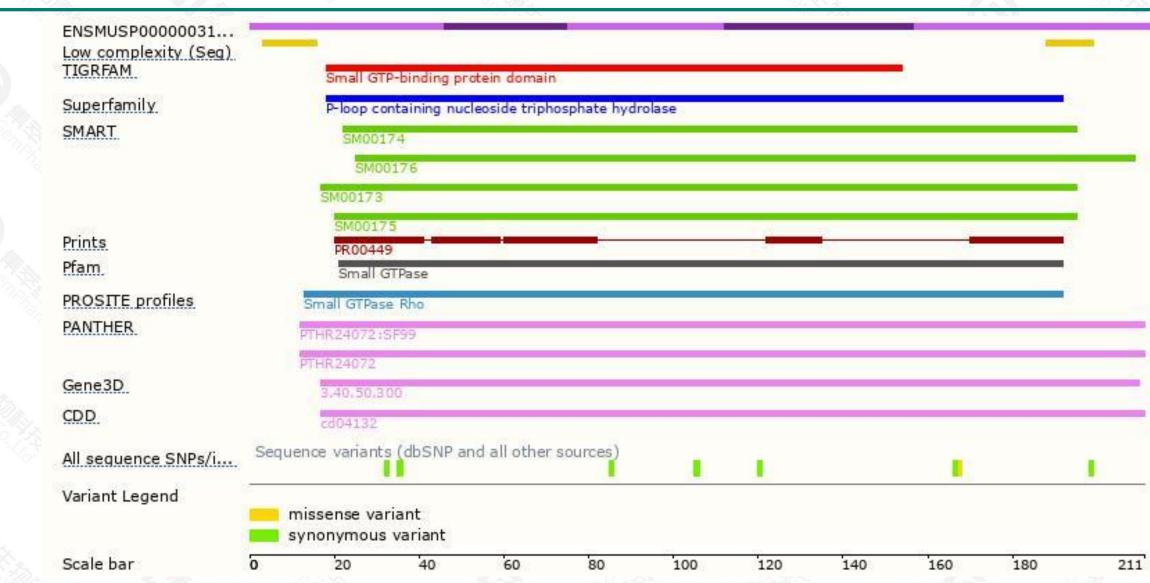
### Genomic location distribution





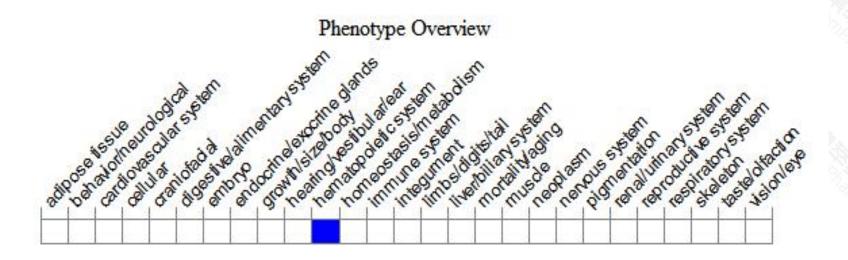
### Protein domain





# 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 macrothrombocytopenia without an effect on filopodia generation.



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

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