

# ***Rras Cas9-CKO Strategy***

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# Project Overview

**Project Name**

***Rras***

**Project type**

**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

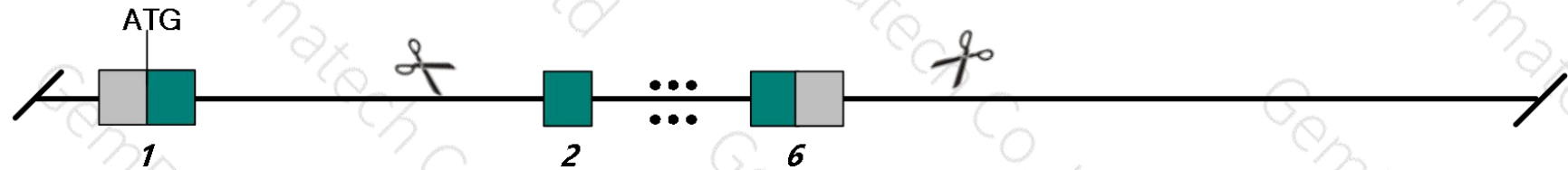
# Conditional Knockout strategy

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

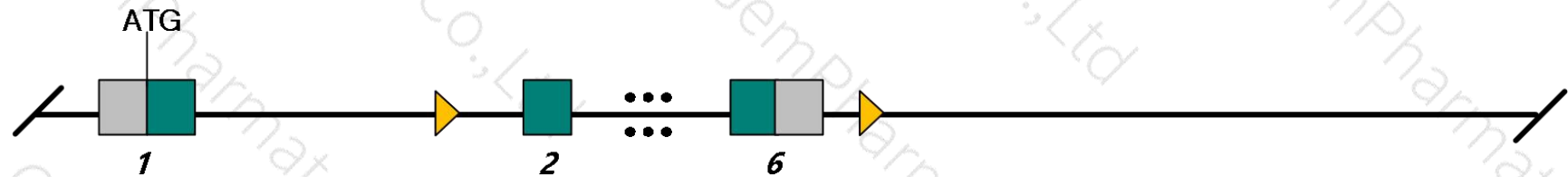
Donor and CRISPR/Cas9 System



Wild-type allele



Conditional KO allele



KO allele



Cas9/sgRNA



Uncoding region



Coding region



LoxP

# Technical routes

- The *Rras* gene has 3 transcripts. According to the structure of *Rras* gene, exon2-exon6 of *Rras*-201 (ENSMUST00000044111.9) transcript is recommended as the knockout region. The region contains most of coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rras* 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 gene-trapped allele exhibit enhanced neointimal thickening in response to arterial injury, increased angiogenesis in matrigel plugs and aortic ring cultures, and increased neovascularization of tumor implants, and decreased B-cell and increased T-cell population percentages.
- The knockout region is about 3.5kb from the 5-terminal of *scaf1* gene, which may affect its 5-terminal regulation.
- The *Rras* gene is located on the Chr7. 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)

## Rras related RAS viral (r-ras) oncogene [ *Mus musculus* (house mouse) ]

Gene ID: 20130, updated on 12-Aug-2019

### Summary

Official Symbol	Rras provided by <a href="#">MGI</a>
Official Full Name	related RAS viral (r-ras) oncogene provided by <a href="#">MGI</a>
Primary source	<a href="#">MGI:MGI:98179</a>
See related	<a href="#">Ensembl:ENSMUSG00000038387</a>
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<a href="#">Mus musculus</a>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI573426
Expression	Broad expression in subcutaneous fat pad adult (RPKM 109.9), bladder adult (RPKM 109.8) and 25 other tissues <a href="#">See more</a>
Orthologs	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

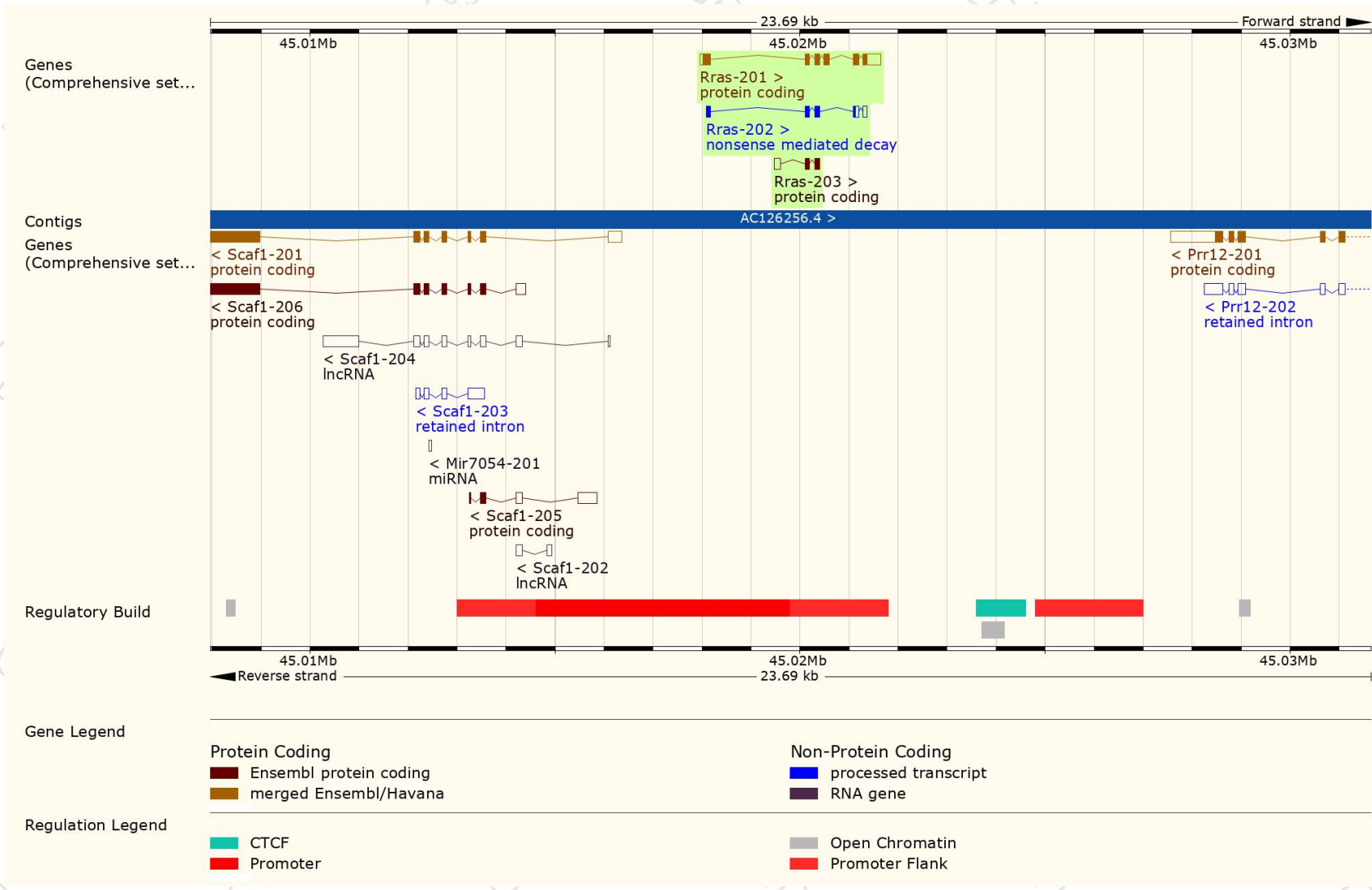
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rras-203	<a href="#">ENSMUST00000210895.1</a>	317	<a href="#">57aa</a>	Protein coding	-	<a href="#">A0A1B0GSL0</a>	CDS 3' incomplete TSL:5
Rras-202	<a href="#">ENSMUST00000210397.1</a>	485	<a href="#">107aa</a>	Nonsense mediated decay	-	<a href="#">A0A1B0GRG1</a>	CDS 5' incomplete TSL:5
Rras-201	<a href="#">ENSMUST00000044111.9</a>	995	<a href="#">218aa</a>	Protein coding	<a href="#">CCDS21226</a>	<a href="#">P10833</a> <a href="#">Q3U1N3</a>	TSL:1 GENCODE basic APPRIS P1

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

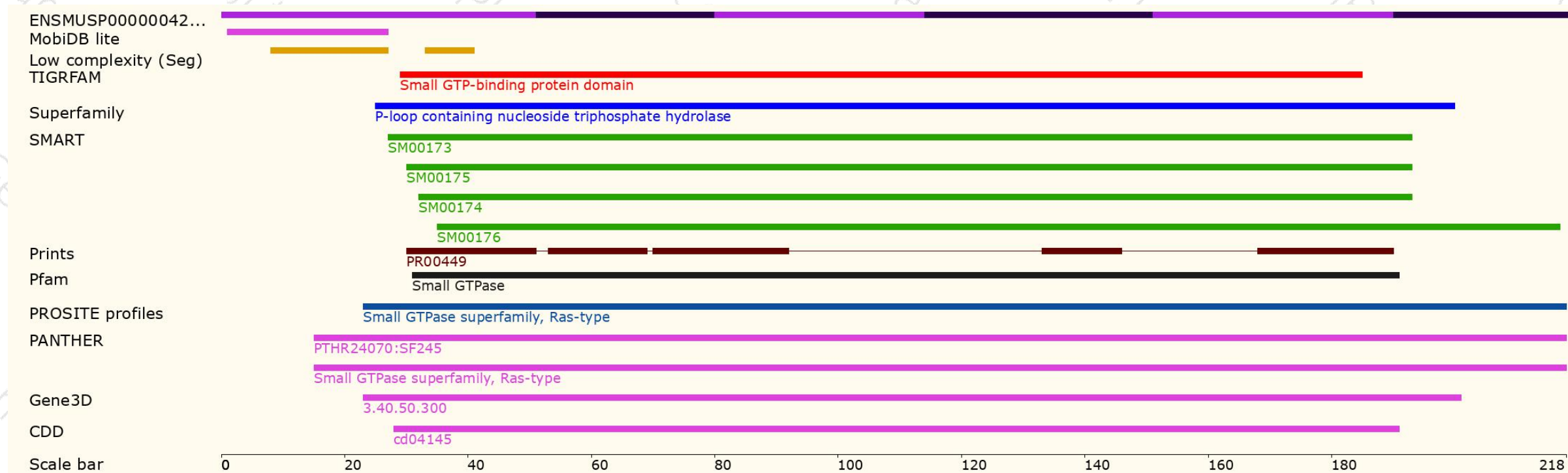


# Genomic location distribution

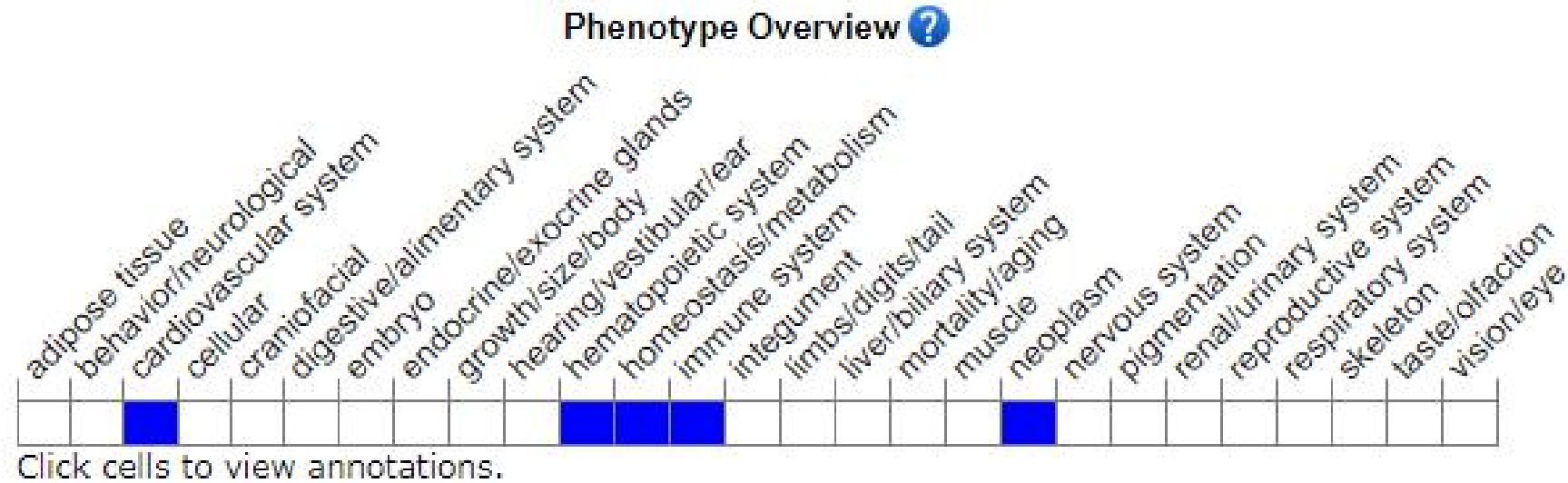




# Protein domain



# Mouse phenotype description(MGI)



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

Mice homozygous for a gene-trapped allele exhibit enhanced neointimal thickening in response to arterial injury, increased angiogenesis in matrigel plugs and aortic ring cultures, and increased neovascularization of tumor implants, and decreased B-cell and increased T-cell population percentages..

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

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