

***Rasa3* Cas9-CKO Strategy**

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Project Overview

Project Name

Rasa3

Project type

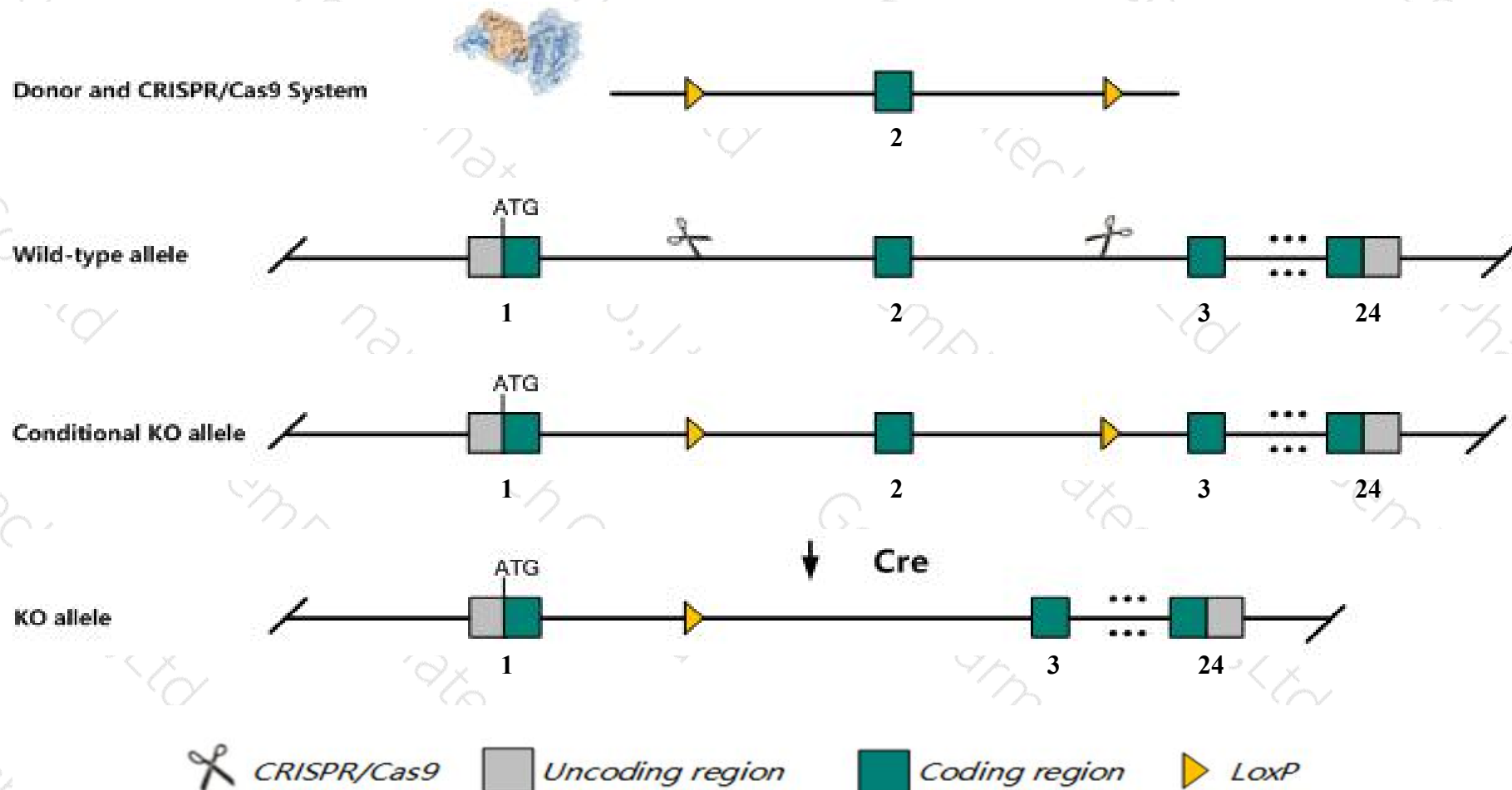
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rasa3* gene. The schematic diagram is as follows:



Technical routes

- The *Rasa3* gene has 6 transcripts. According to the structure of *Rasa3* gene, exon2 of *Rasa3-201*(ENSMUST00000117551.3) transcript is recommended as the knockout region. The region contains 118bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rasa3* 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 targeted null mutation die at E12.5-13.5 of massive subcutaneous and intraparenchymal hemorrhage, probably due to underdeveloped adherens junctions between capillary endothelial cells. At E12.5, edema and severe hemorrhaging is frequently observed in the brain and/or rump.
- The *Rasa3* gene is located on the Chr8. 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)

Rasa3 RAS p21 protein activator 3 [Mus musculus (house mouse)]

Gene ID: 19414, updated on 13-Mar-2020

Summary



Official Symbol [Rasa3](#) provided by [MGI](#)

Official Full Name [RAS p21 protein activator 3](#) provided by [MGI](#)

Primary source [MGI:MGI:1197013](#)

See related [Ensembl:ENSMUSG00000031453](#)

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 [AI326412](#), [C86362](#), [E130011604](#), [GAPIII](#), [h1b381](#), [scat](#)

Expression Ubiquitous expression in spleen adult (RPKM 50.1), mammary gland adult (RPKM 30.6) and 28 other tissues [See more](#)

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

Transcript information (Ensembl)

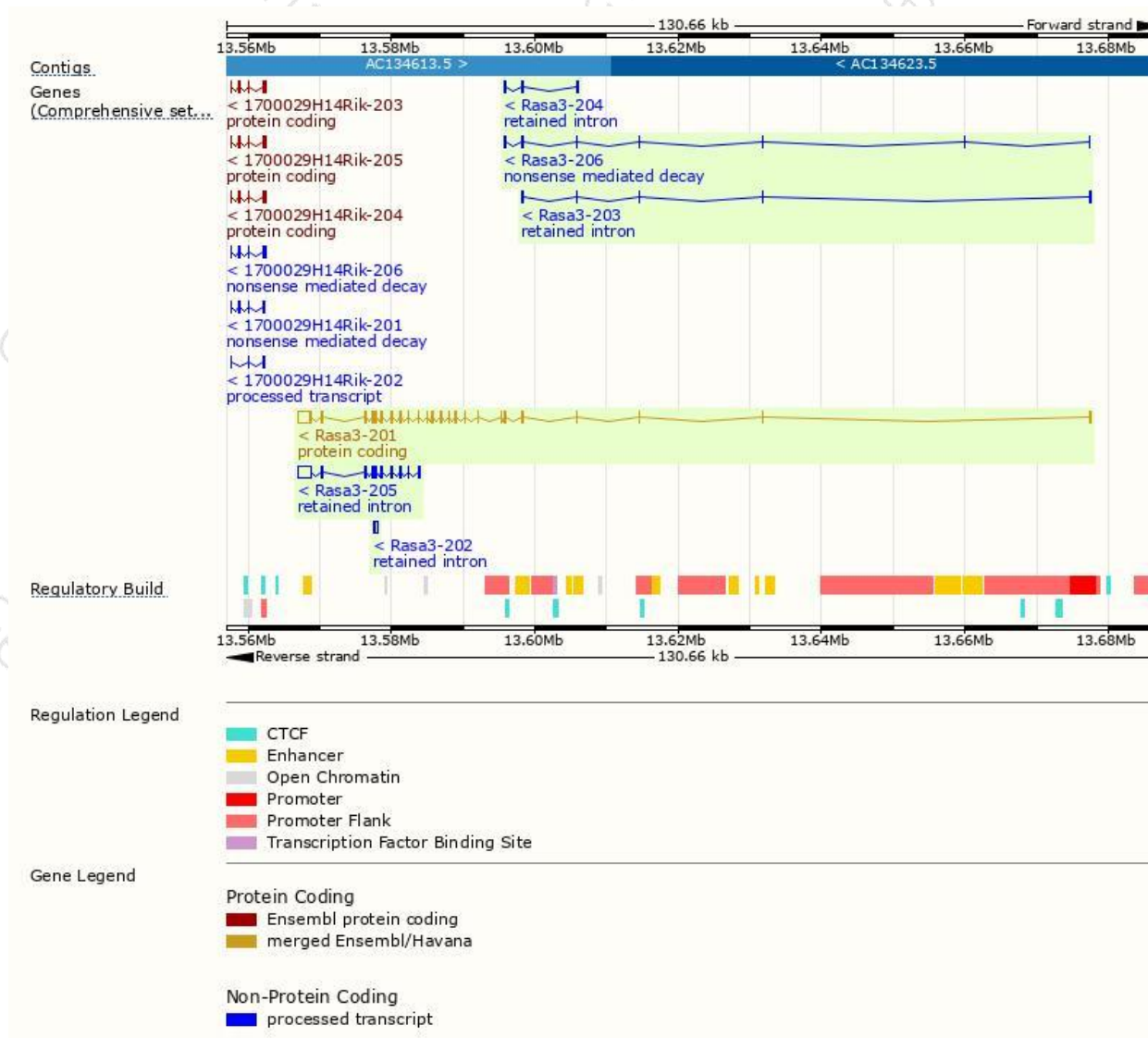
The gene has 6 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|-----------|--------------------------------------|------|-----------------------|-------------------------|---------------------------|------------------------|-------------------------------|
| Rasa3-201 | ENSMUST00000117551.3 | 4404 | 834aa | Protein coding | CCDS40234 | Q60790 | TSL:1 GENCODE basic APPRIS P1 |
| Rasa3-206 | ENSMUST00000154454.7 | 617 | 39aa | Nonsense mediated decay | - | D6RFE0 | TSL:3 |
| Rasa3-205 | ENSMUST00000137822.1 | 3023 | No protein | Retained intron | - | - | TSL:1 |
| Rasa3-203 | ENSMUST00000132439.2 | 604 | No protein | Retained intron | - | - | TSL:2 |
| Rasa3-204 | ENSMUST00000132637.7 | 405 | No protein | Retained intron | - | - | TSL:3 |
| Rasa3-202 | ENSMUST00000127418.1 | 336 | No protein | Retained intron | - | - | TSL:2 |

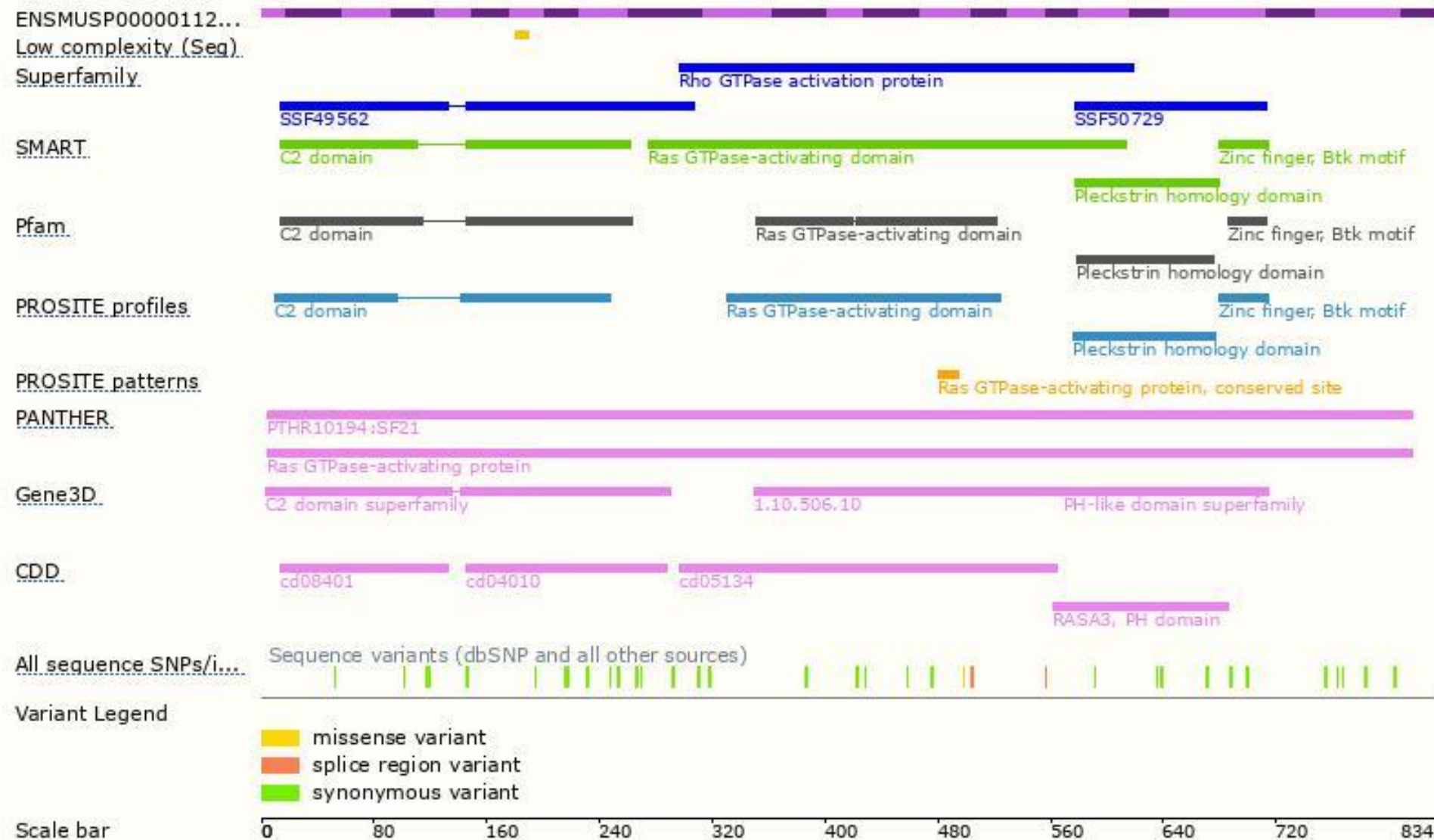
The strategy is based on the design of *Rasa3-201* transcript,the transcription is shown below:



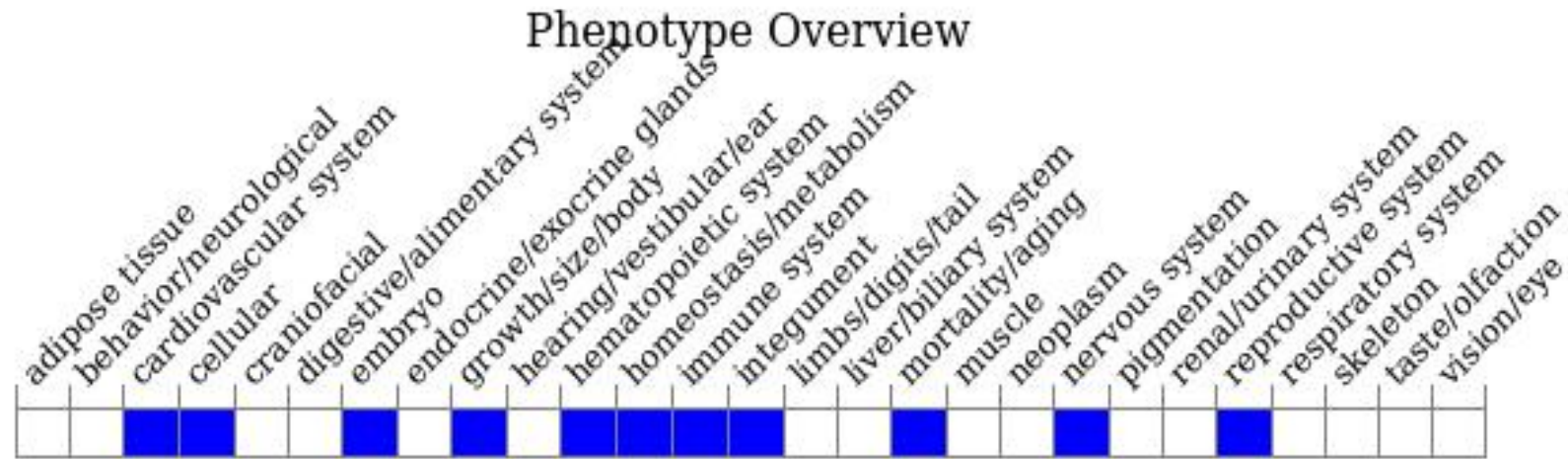
Genomic location distribution



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 targeted null mutation die at E12.5-13.5 of massive subcutaneous and intraparenchymal hemorrhage, probably due to underdeveloped adherens junctions between capillary endothelial cells. At E12.5, edema and severe hemorrhaging is frequently observed in the brain and/or rump.

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

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