Rasa3 Cas9-KO Strategy

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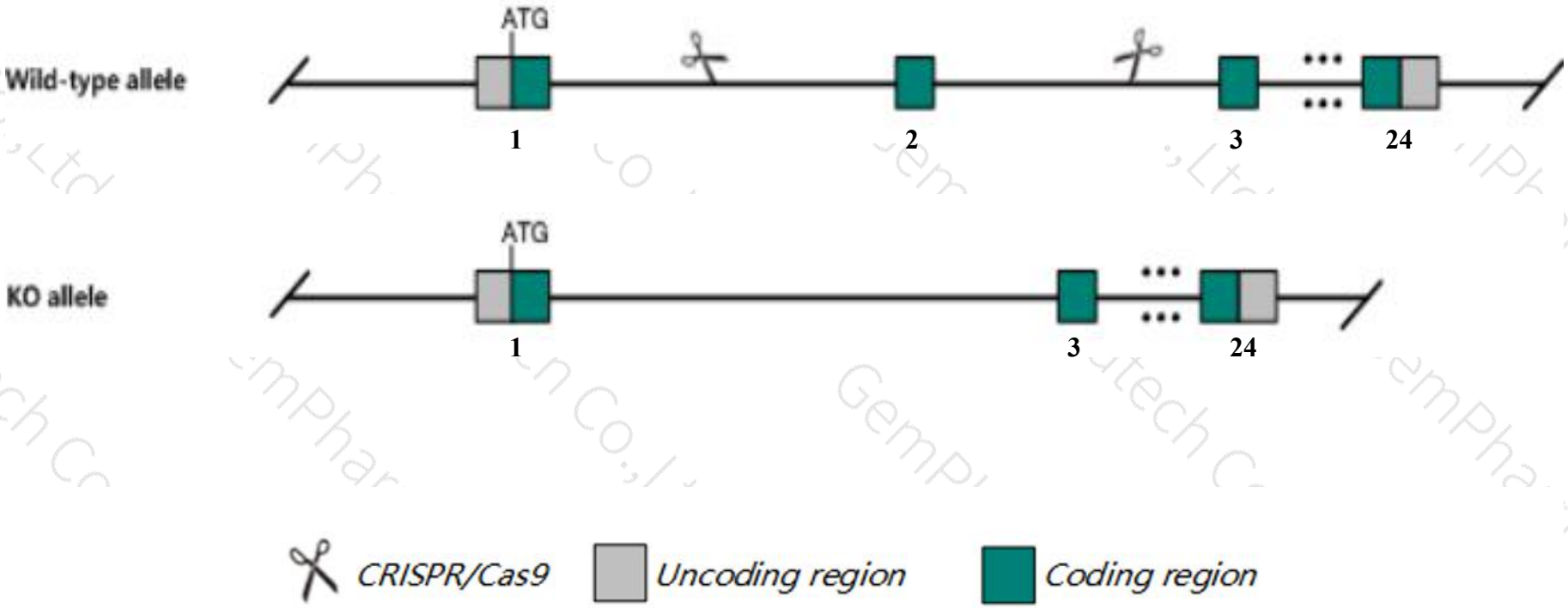
Project Overview

Project Name	***Rasa3***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

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 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 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology 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 byMGI
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, E130011G04, GAPIII, hlb381, scat
Expression	Ubiquitous expression in spleen adult (RPKM 50.1), mammary gland adult (RPKM 30.6) and 28 other tissuesSee more
Orthologs	human all

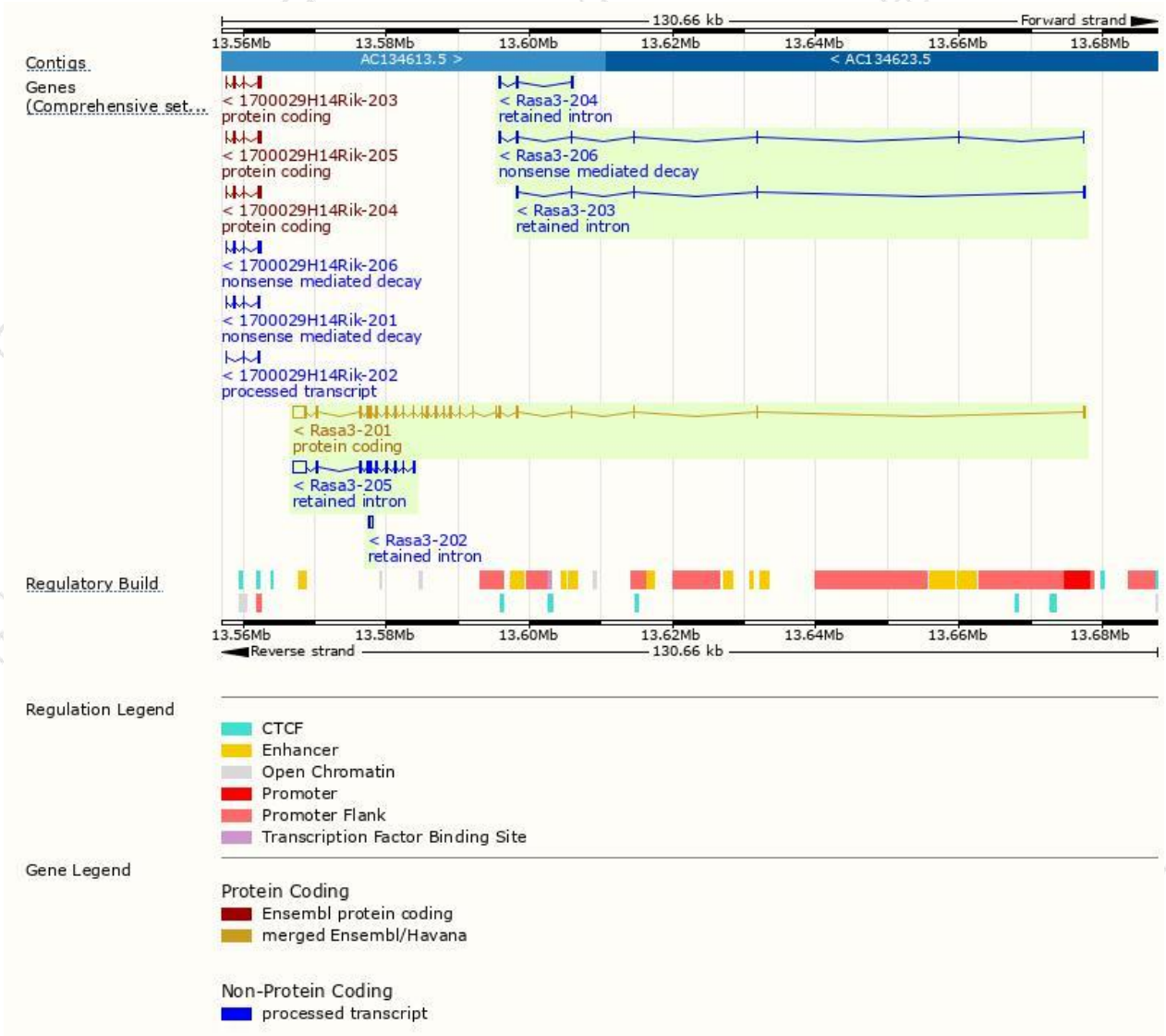
Transcript information（Ensembl）

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

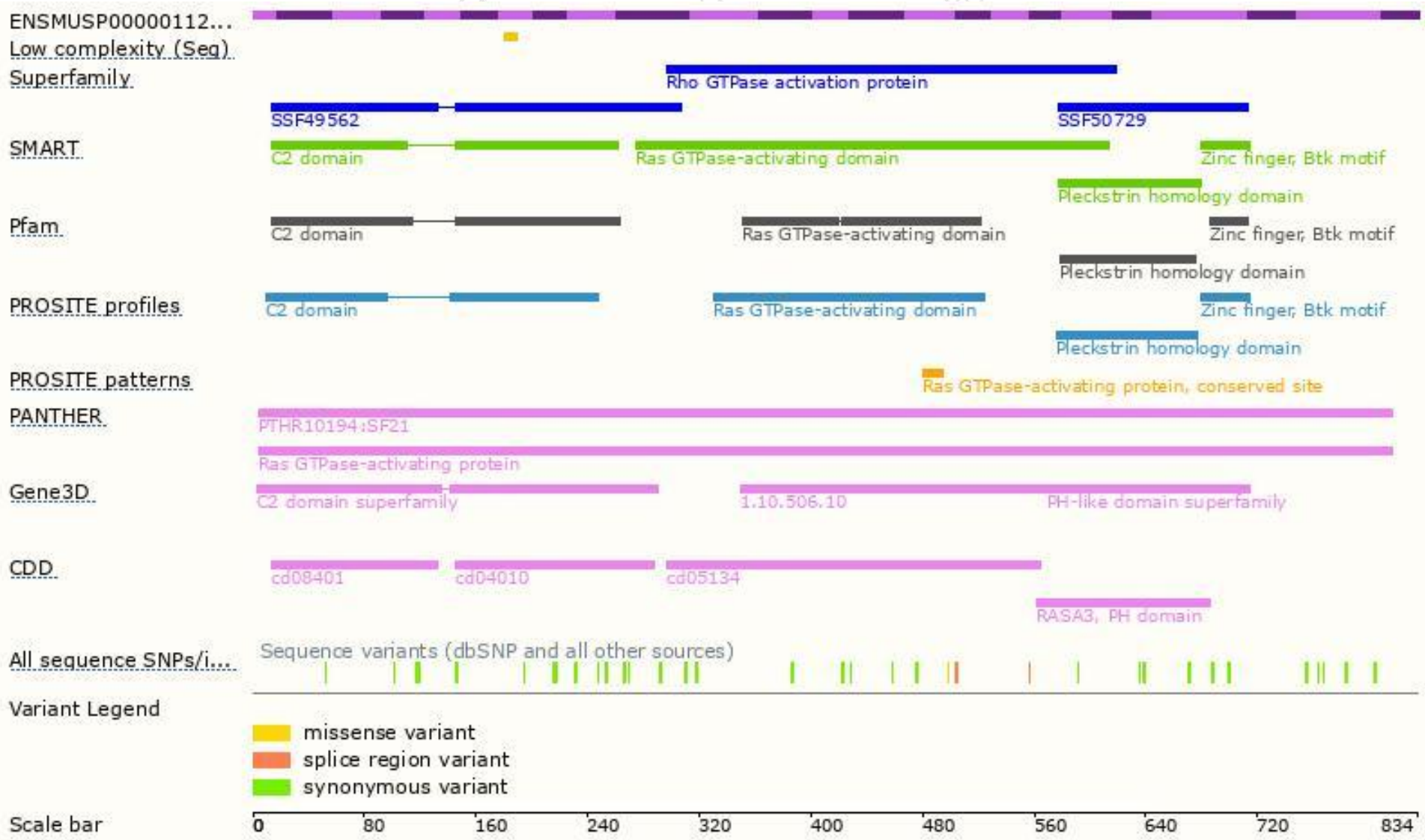
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rasa3-201	ENSMUST00000117551.3	4404	834aa	Protein coding	CCDS40234	Q60790	TSL:1 GENCODE basic APPRIS P1
Rasa3-206	ENSMUST00000154454.7	617	39aa	Nonsense mediated decay	-	D6RFE0	TSL:3
Rasa3-205	ENSMUST00000137822.1	3023	No protein	Retained intron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

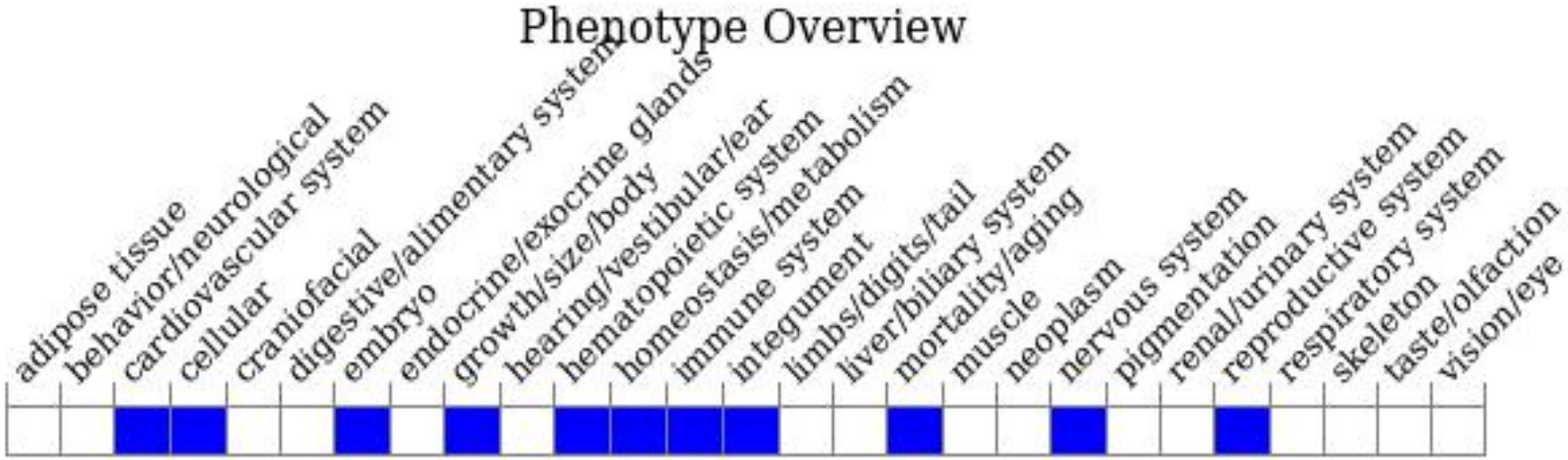
Genomic location distribution



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/).

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

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