

***Rasal2* Cas9-CKO Strategy**

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Project Overview

Project Name

Rasal2

Project type

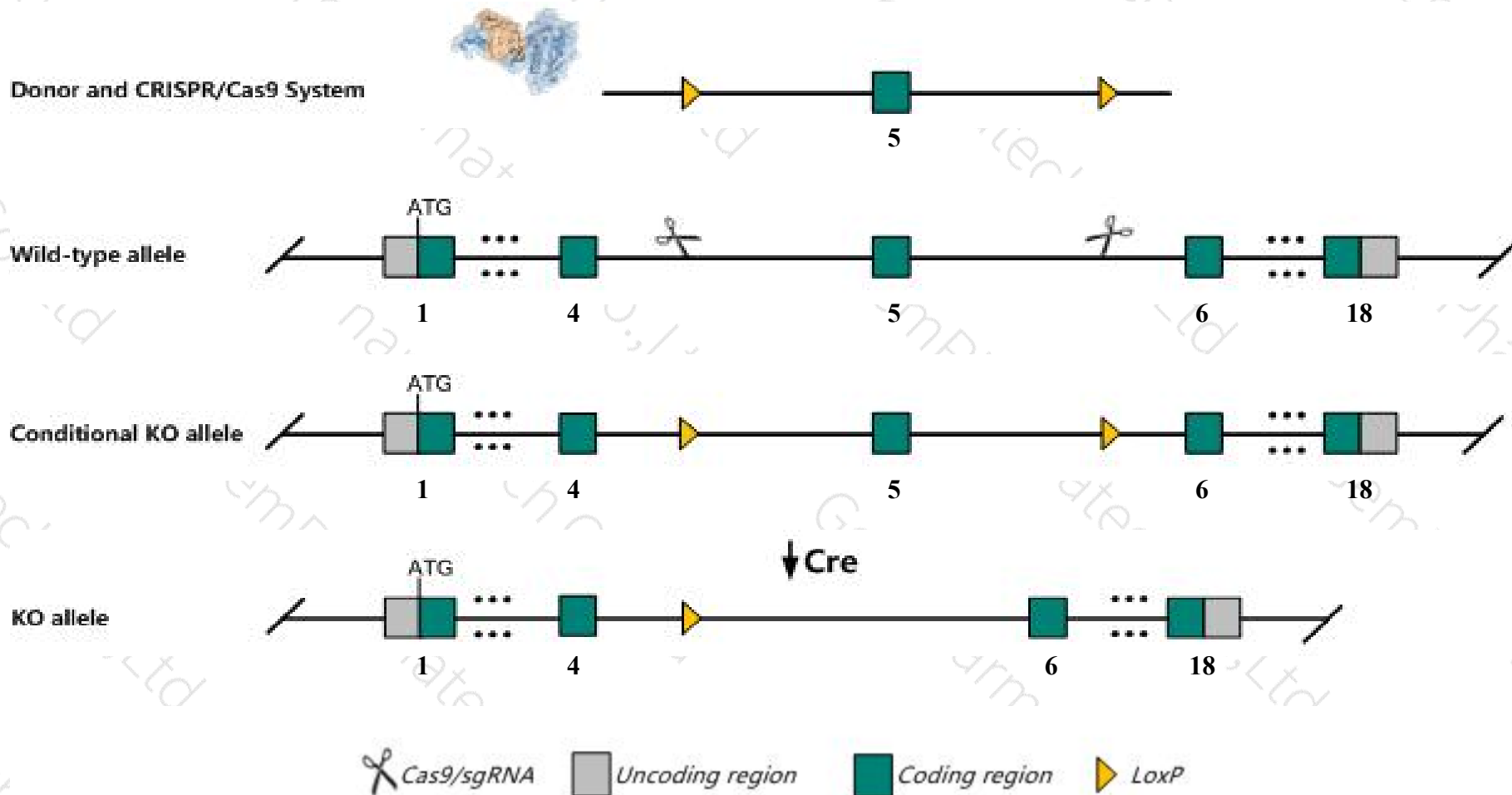
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rasal2* gene. The schematic diagram is as follows:



Technical routes

- The *Rasal2* gene has 7 transcripts. According to the structure of *Rasal2* gene, exon5 of *Rasal2*-201(ENSMUST00000078308.12) transcript is recommended as the knockout region. The region contains 110bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rasal2* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA 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 was 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 trap allele exhibit reduced survival and decreased tumor latency. In other tumorigenic models, this allele promotes increase metastatic potential.
- Some amino acids will remain at the N-terminus and some functions may be retained.
- The *Rasal2* gene is located on the Chr1. 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)

Rasal2 RAS protein activator like 2 [Mus musculus (house mouse)]

Gene ID: 226525, updated on 13-Mar-2020

Summary



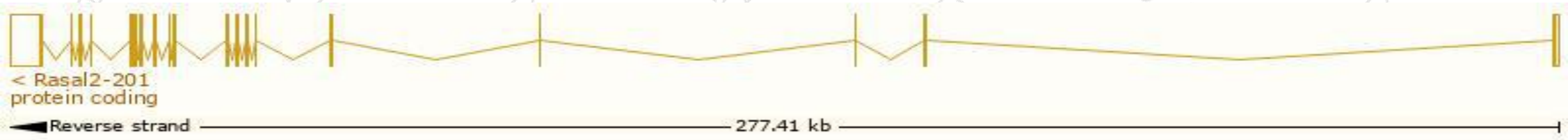
Official Symbol	Rasal2 provided by MGI
Official Full Name	RAS protein activator like 2 provided by MGI
Primary source	MGI:MGI:2443881
See related	Ensembl:ENSMUSG00000070565
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	6530401P13, A330066M24Rik
Expression	Broad expression in whole brain E14.5 (RPKM 6.2), CNS E14 (RPKM 5.6) and 26 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

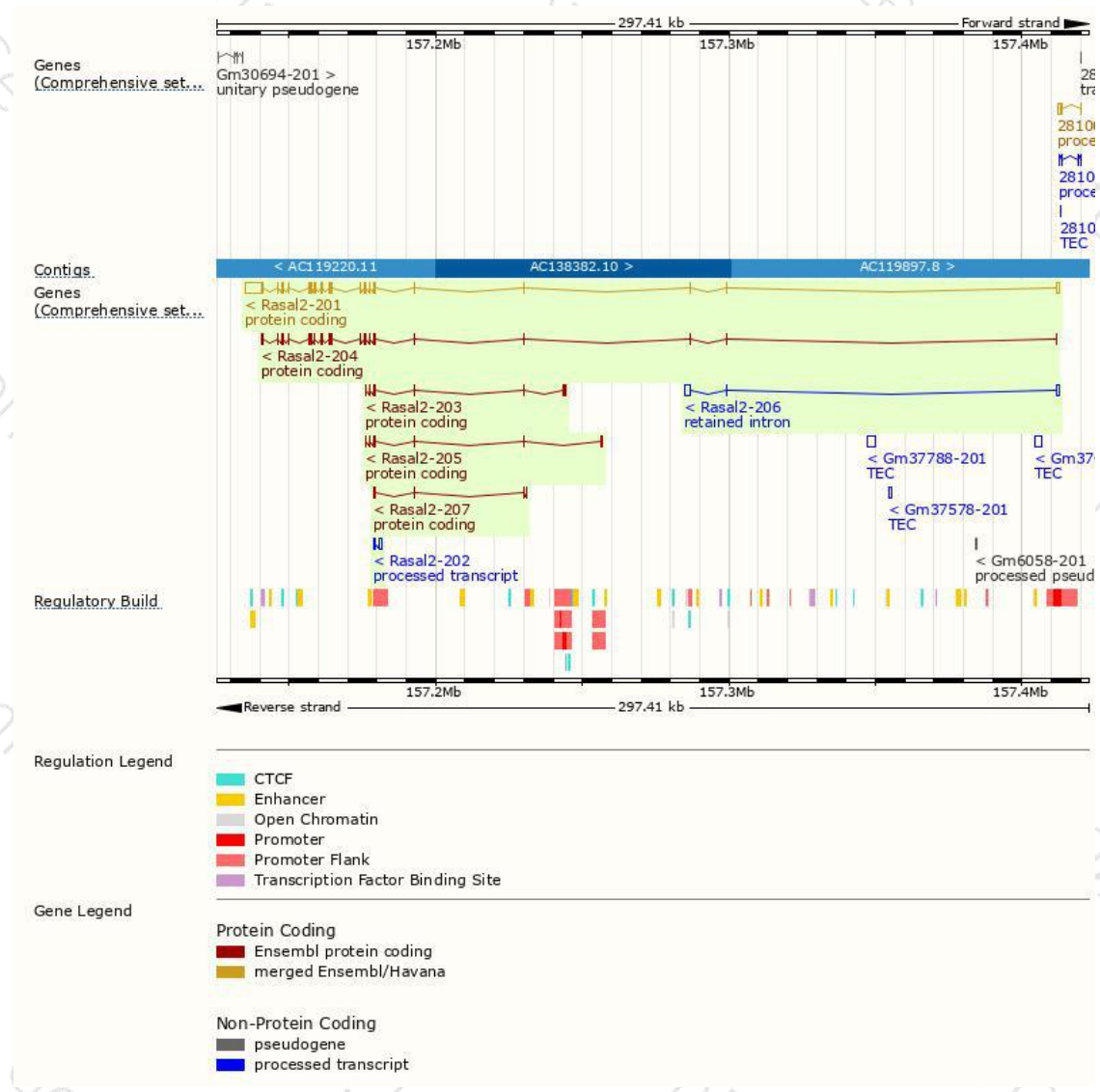
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rasal2-201	ENSMUST00000078308.12	10047	1286aa	Protein coding	CCDS48408	E9PW37	TSL:1 GENCODE basic APPRIS P1
Rasal2-204	ENSMUST00000132699.7	4013	1261aa	Protein coding	-	D3YUS5	TSL:5 GENCODE basic
Rasal2-203	ENSMUST00000129880.7	1679	323aa	Protein coding	-	D3YWZ9	CDS 3' incomplete TSL:5
Rasal2-207	ENSMUST00000143358.2	654	147aa	Protein coding	-	D3Z7S1	CDS 3' incomplete TSL:5
Rasal2-205	ENSMUST00000134543.7	641	176aa	Protein coding	-	D3Z1T6	CDS 3' incomplete TSL:3
Rasal2-202	ENSMUST00000128538.1	722	No protein	Processed transcript	-	-	TSL:2
Rasal2-206	ENSMUST00000143080.1	2858	No protein	Retained intron	-	-	TSL:1

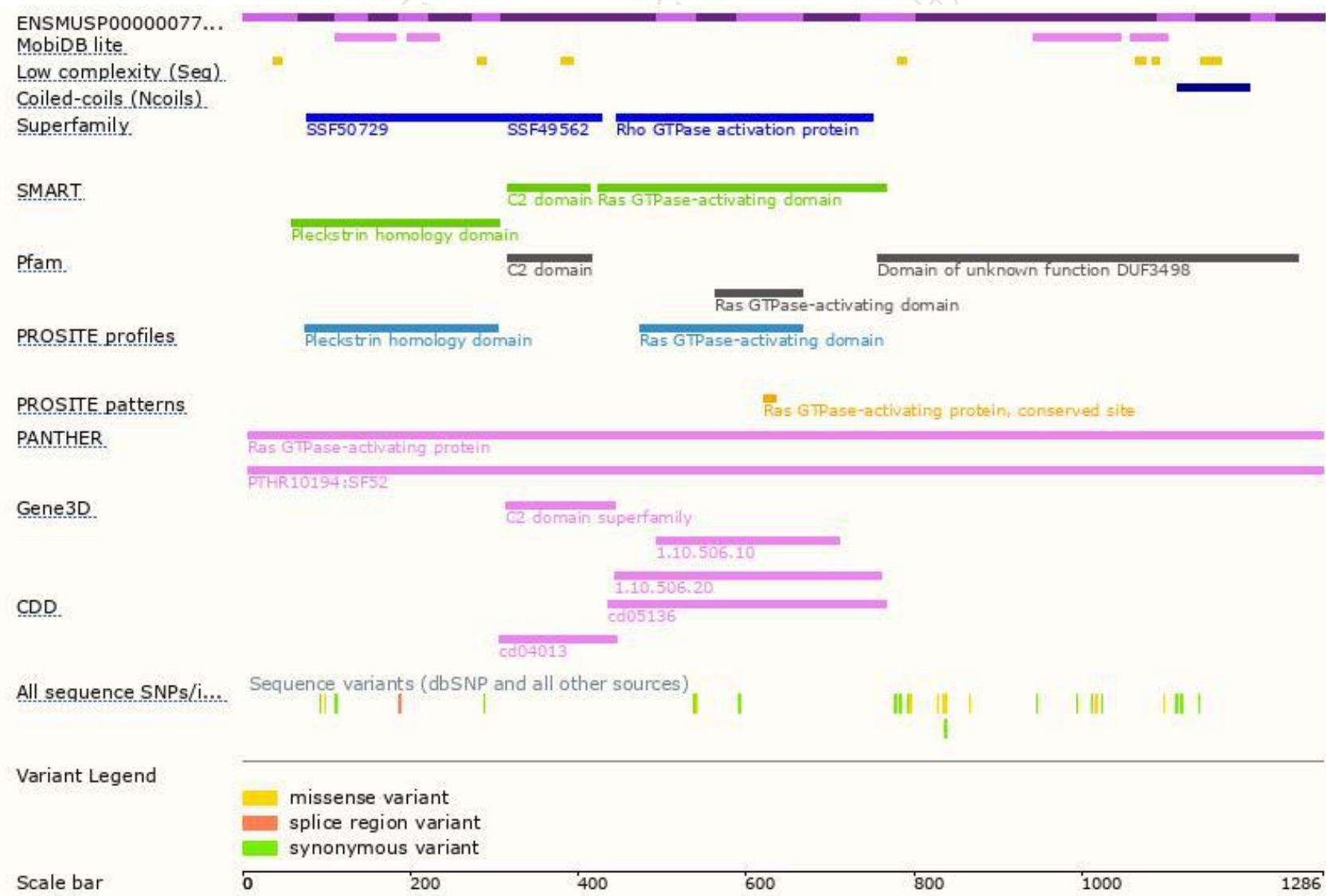
The strategy is based on the design of *Rasal2-201* transcript,the transcription is shown below:



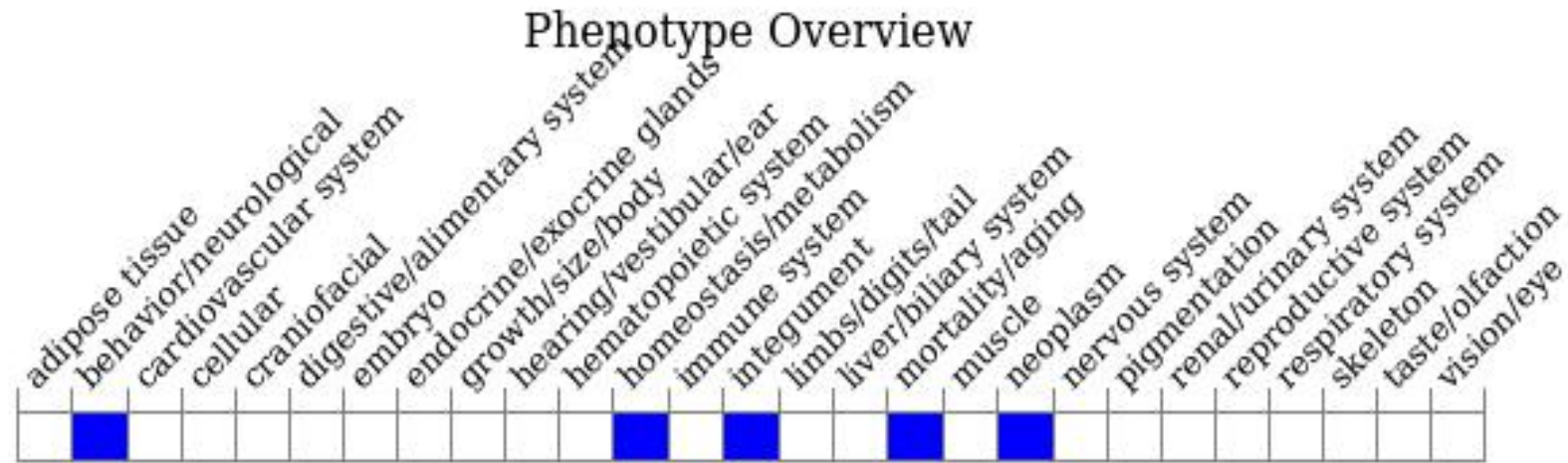
Genomic location distribution



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 gene trap allele exhibit reduced survival and decreased tumor latency. In other tumorigenic models, this allele promotes increase metastatic potential.

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

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