GemPharmatech™

Rasa1 Cas9-CKO Strategy

Designer: Hui Bao

Reviewer: Yun Li

Design Date: 2023-04-18

Overview

Target Gene Name

- *Rasa1*

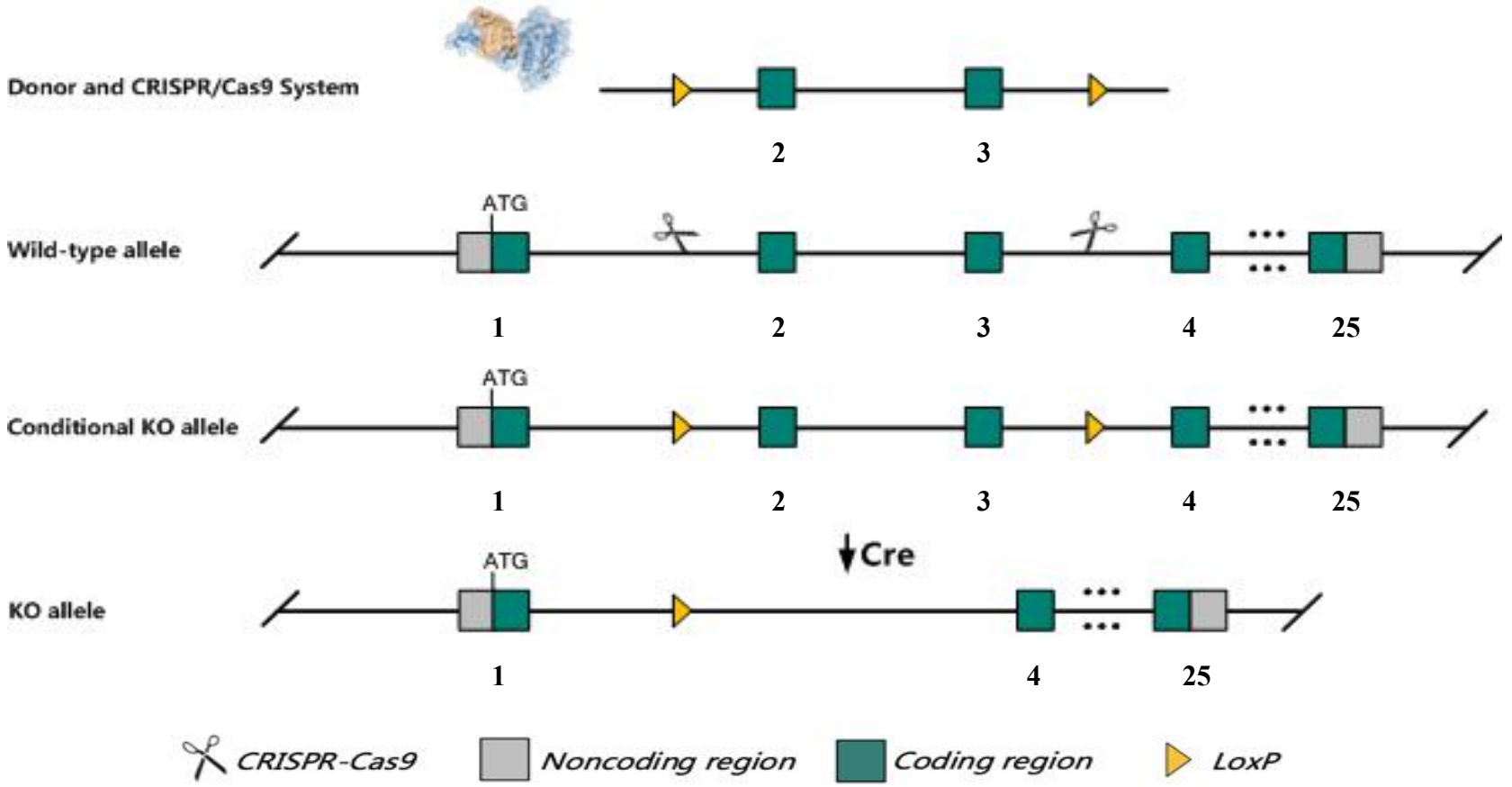
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Rasa1* gene.

Technical Information

- The *Rasa1* gene has 10 transcripts. According to the structure of *Rasa1* gene, exon2-exon3 of *Rasa1-201*(ENSMUST00000109552.3) transcript is recommended as the knockout region.The region contains 289bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Rasa1* 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.

Gene Information

Rasa1 RAS p21 protein activator 1 [*Mus musculus* (house mouse)]

Gene ID: 218397, updated on 4-Oct-2022

Summary

Official Symbol	Rasa1 provided by MGI
Official Full Name	RAS p21 protein activator 1 provided by MGI
Primary source	MGI:MGI:97860
See related	Ensembl:ENSMUSG00000021549 AllianceGenome:MGI:97860
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	Gap; Rasa; RasGAP
Summary	Predicted to enable several functions, including GTPase activator activity; enzyme binding activity; and phosphotyrosine residue binding activity. Predicted to be involved in several processes, including activation of GTPase activity; cellular response to growth factor stimulus; and positive regulation of glucose import. Located in plasma membrane and ruffle. Is expressed in several structures, including alimentary system; brain; cardiovascular system; genitourinary system; and hemolymphoid system gland. Human ortholog(s) of this gene implicated in arteriovenous malformation and basal cell carcinoma. Orthologous to human RASA1 (RAS p21 protein activator 1). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Ubiquitous expression in CNS E14 (RPKM 8.0), CNS E11.5 (RPKM 7.1) and 28 other tissues See more
Orthologs	human all

Source: https://www.ncbi.nlm.nih.gov/

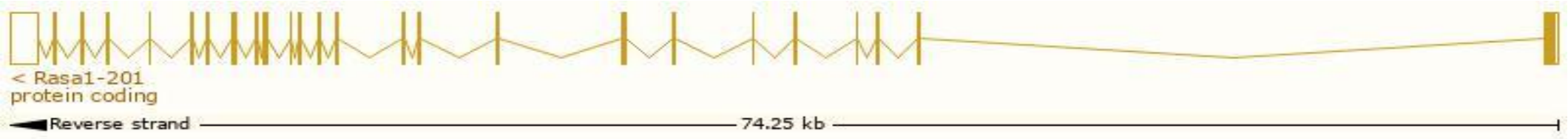
Transcript Information

The gene has 10 transcripts,all transcripts are shown below:

Show/hide columns (1 hidden) Filter

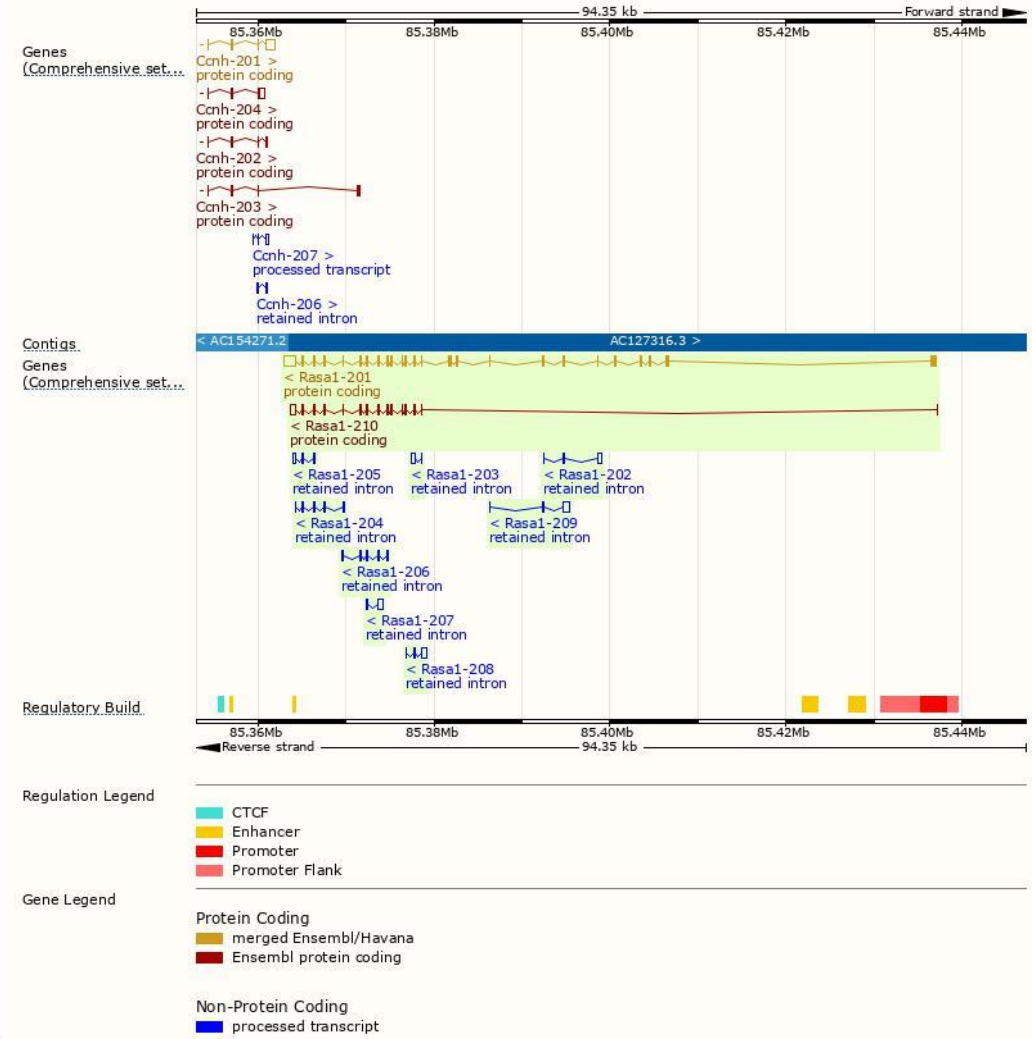
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000109552.3	Rasa1-201	4563	1038aa	Protein coding	CCDS26667	E9PYG6	Ensembl Canonical, GENCODE basic, APPRIS P1, TSL:1
ENSMUST00000223598.2	Rasa1-210	2102	497aa	Protein coding		A0A286YDU0	CDS 5' incomplete
ENSMUST00000153231.2	Rasa1-209	854	No protein	Retained intron		-	TSL:3
ENSMUST00000152466.2	Rasa1-208	831	No protein	Retained intron		-	TSL:2
ENSMUST00000146792.8	Rasa1-205	678	No protein	Retained intron		-	TSL:2
ENSMUST00000148014.2	Rasa1-206	676	No protein	Retained intron		-	TSL:2
ENSMUST00000142285.2	Rasa1-204	669	No protein	Retained intron		-	TSL:2
ENSMUST00000149799.2	Rasa1-207	641	No protein	Retained intron		-	TSL:2
ENSMUST00000132711.2	Rasa1-202	585	No protein	Retained intron		-	TSL:3
ENSMUST00000141879.2	Rasa1-203	483	No protein	Retained intron		-	TSL:2

The strategy is based on the design of *Rasa1-201* transcript,the transcription is shown below:



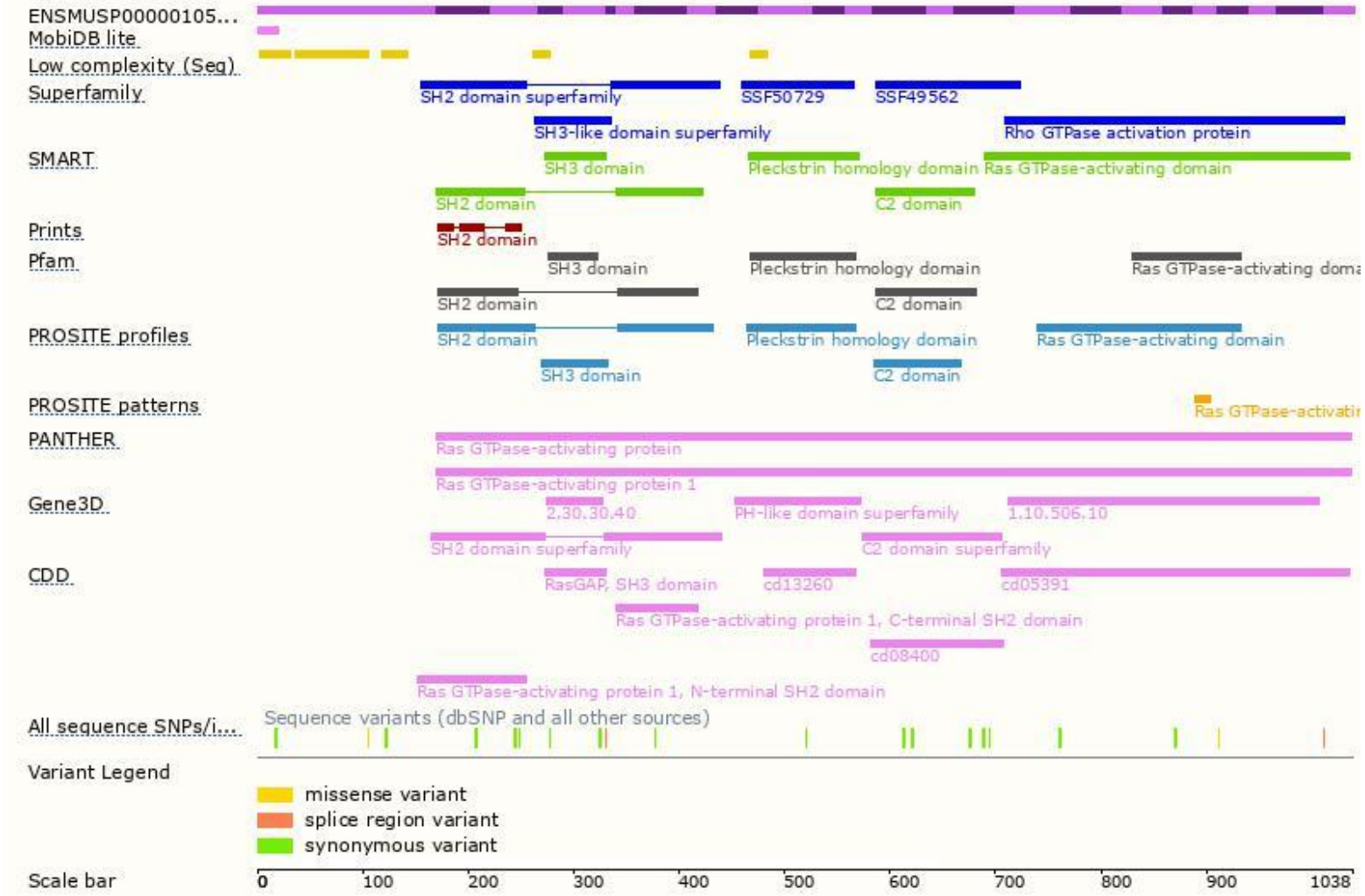
Source: https://www.ensembl.org

Genomic Information



Source: : https://www.ensembl.org

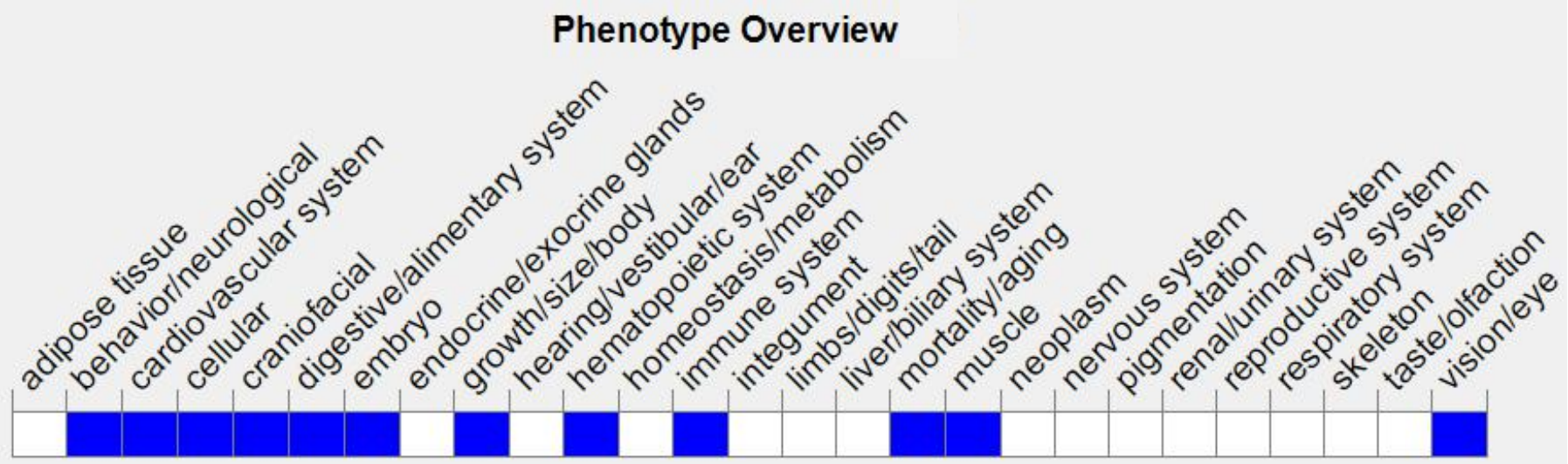
Protein Information



GemPharmatech™

Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



- Phenotypes affected by the mutations of *Rasa1* gene are marked in blue. According to the existing MGI data,homozygotes for a targeted null mutation exhibit reduced embryonic growth associated with defects of both yolk sac and embryonic vascular systems resulting in lethality by embryonic day 10.5. Mice homozygous for a knock-in allele exhibit increased sensitivity to induced cell death and colitis.

Source: https://www.informatics.jax.org

Important Information

- The *Rasa1* gene is located on the Chr13. 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.
- Transcript *Rasa1-202*、*Rasa1-203*、*Rasa1-204*、*Rasa1-205*、*Rasa1-206*、*Rasa1-207*、*Rasa1-208*、*Rasa1-209*、*Rasa1-210* may not be affected.
- 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.