Rasgrf1 Cas9-CKO Strategy

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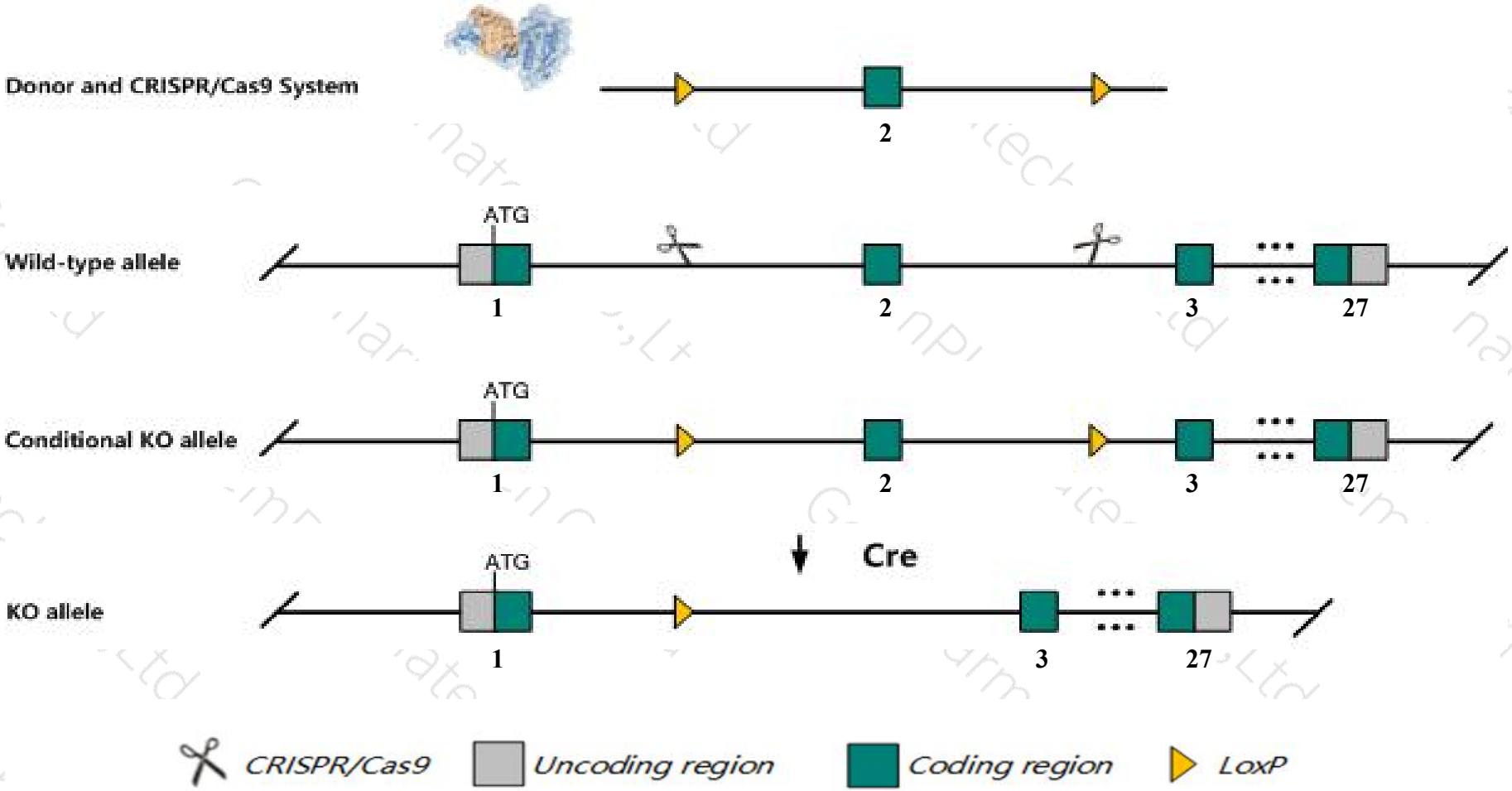
Project Overview

Project Name	***Rasgrf1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rasgrf1* gene. The schematic diagram is as follows:



Technical routes

- The *Rasgrf1* gene has 4 transcripts. According to the structure of *Rasgrf1* gene, exon2 of *Rasgrf1-202* (ENSMUST00000034912.5) 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 *Rasgrf1* 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.

Notice

➢ According to the existing MGI data,Homozygotes for null mutations (and heterozygotes with a paternally inherited mutant allele) exhibit reduced postnatal growth, low insulin and IGF I levels, glucose intolerance, beta-cell hypoplasia, impaired long-term synaptic plasticity, and impaired hippocampal-dependent learning.

➢Transcript *Rasgrf1*-201&203 may not be affected.

➢ The *Rasgrf1* gene is located on the Chr9. 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）

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Rasgrf1 RAS protein-specific guanine nucleotide-releasing factor 1 [*Mus musculus* (house mouse)]

Gene ID: 19417, updated on 13-Aug-2019

Summary

Official Symbol	Rasgrf1 provided by MGI
Official Full Name	RAS protein-specific guanine nucleotide-releasing factor 1 provided by MGI
Primary source	MGI:MGI:99694
See related	Ensembl:ENSMUSG00000032356
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	Gnrp; Grf1; p190; CDC25; P190-A; CDC25Mm; Grfbeta; AI844718; Ras-GRF1; p190RhoGEF
Expression	Biased expression in cerebellum adult (RPKM 26.4), frontal lobe adult (RPKM 25.1) and 6 other tissues See more
Orthologs	human all

Genomic context

Location: 9 E3.1; 9 47.31 cM

See Rasgrf1 in Genome Data Viewer

Exon count: 29

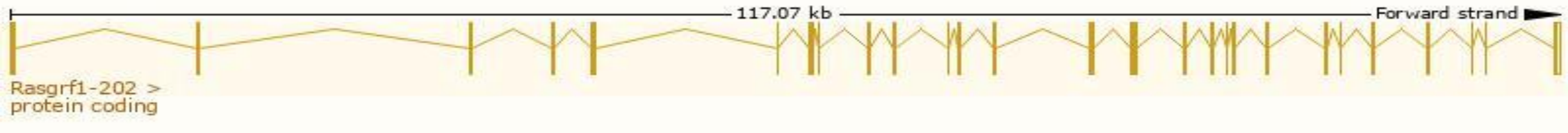
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	9	NC_000075.6 (89909508..90026979)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	9	NC_000075.5 (89804613..89921817)

Transcript information（Ensembl）

The gene has 4 transcripts,all transcripts are shown below:

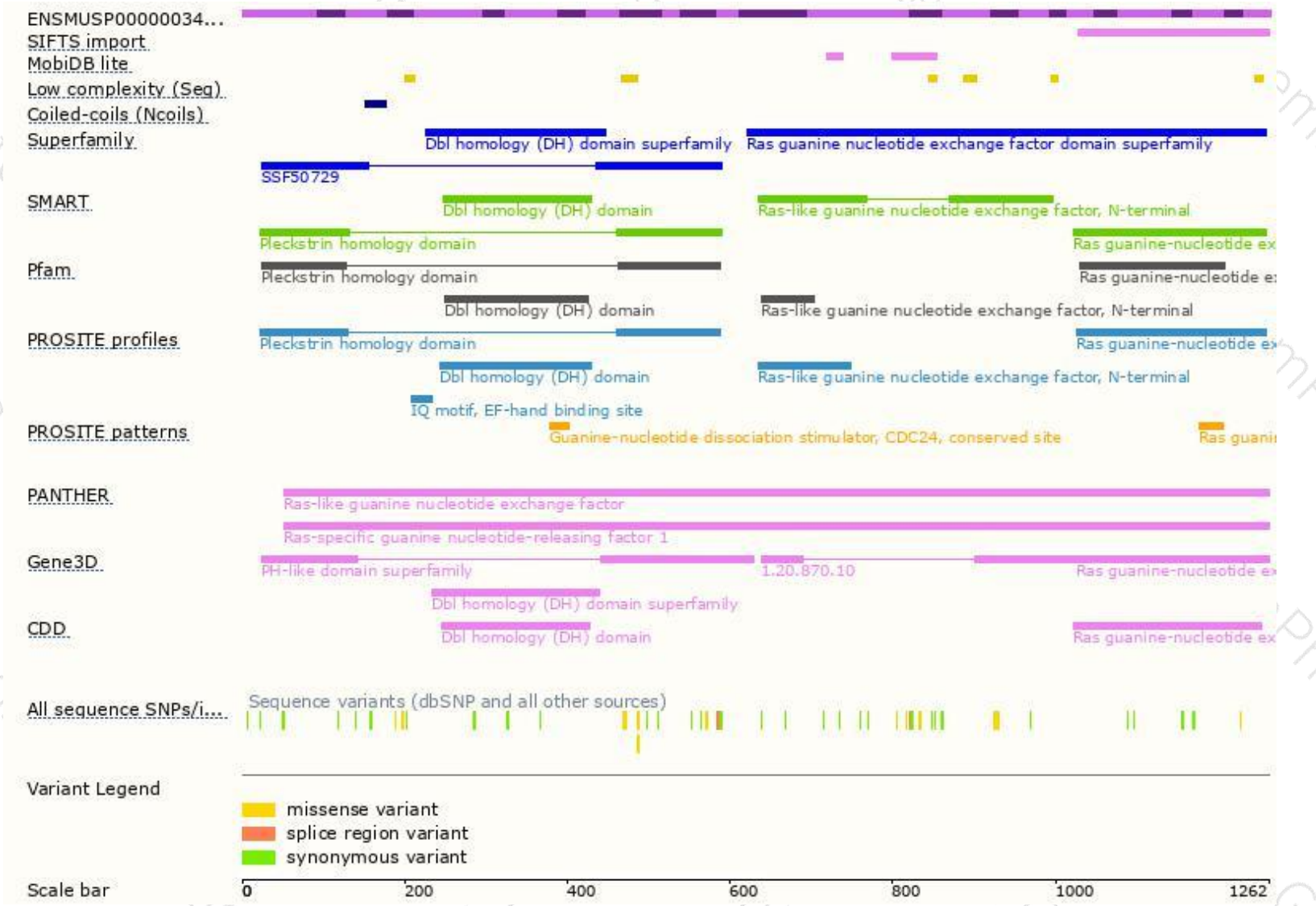
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rasgrf1-202	ENSMUST00000034912.5	4092	1262aa	Protein coding	CCDS40722	B2RS27 P27671	TSL:1 GENCODE basic APPRIS P1
Rasgrf1-201	ENSMUST00000034909.10	853	178aa	Protein coding	CCDS40723	Q9QZR7	TSL:1 GENCODE basic
Rasgrf1-203	ENSMUST00000189545.1	4766	153aa	Protein coding	-	A0A087WS68	TSL:1 GENCODE basic
Rasgrf1-204	ENSMUST00000190073.1	3042	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Rasgrf1-202* transcript,The transcription is shown below

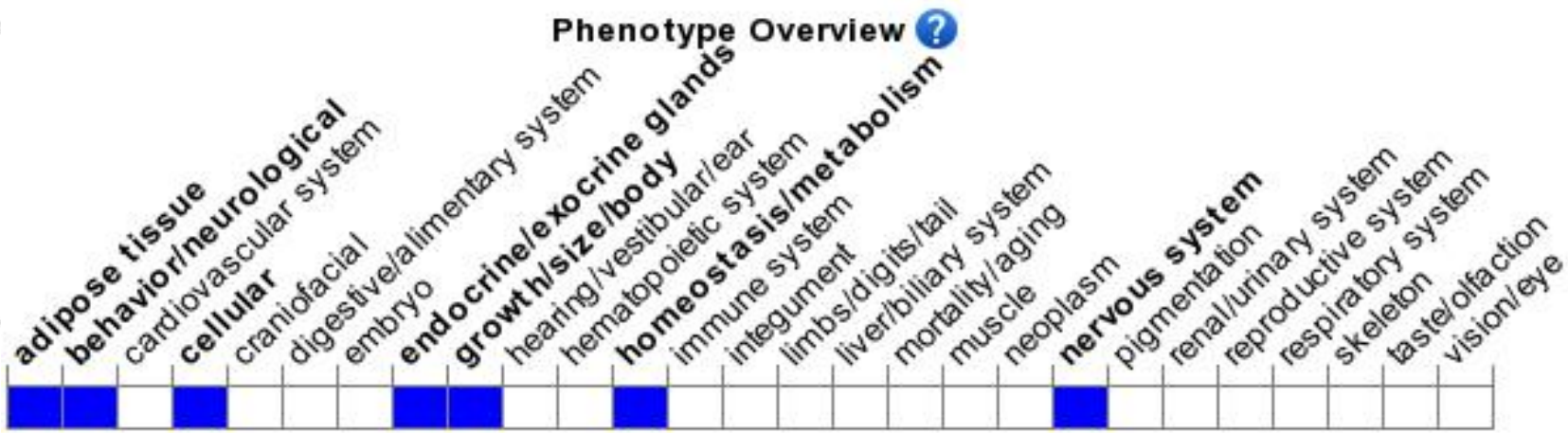


Genomic location distribution

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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