Rasgrf2 Cas9-CKO Strategy

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Project Overview

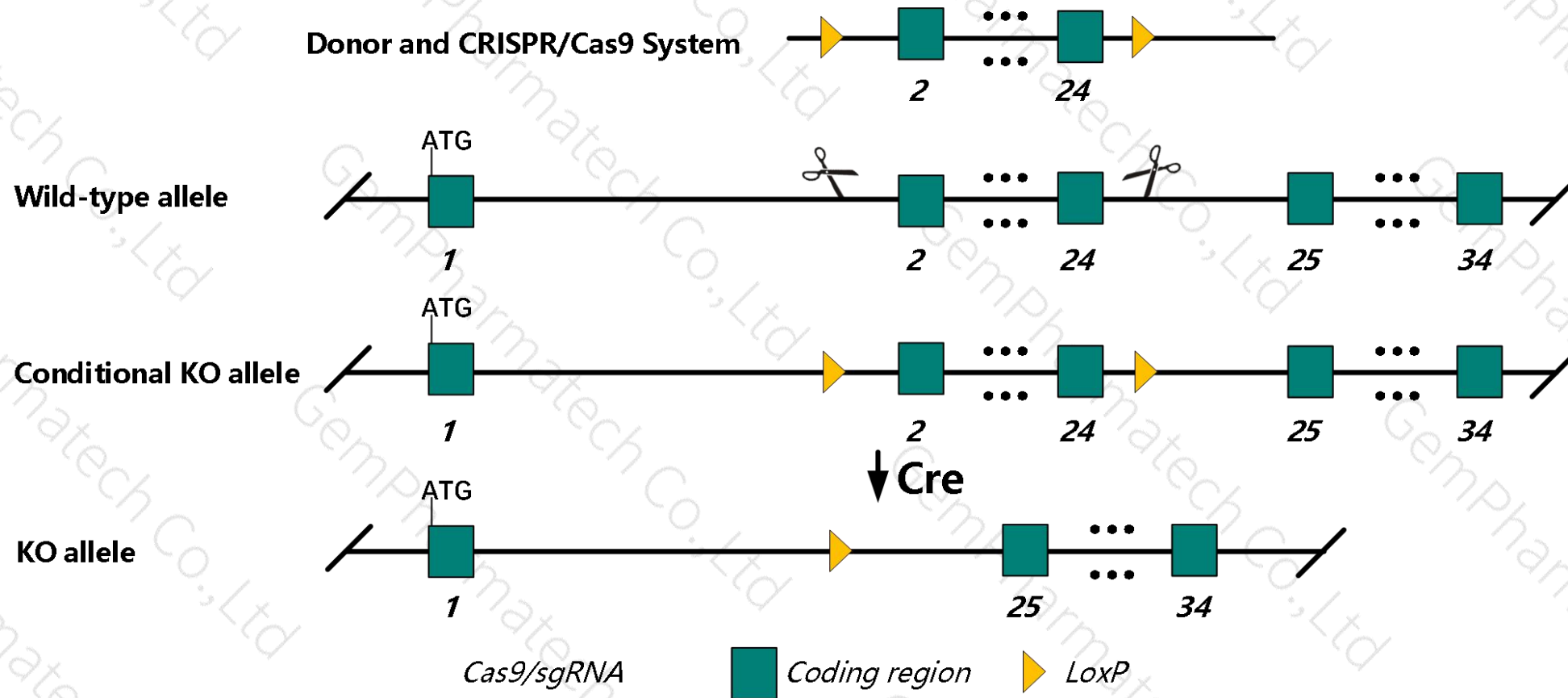|	
Project Name	***Rasgrf2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rasgrf2* gene. The schematic diagram is as follows:

➤ The *Rasgrf2* gene has 6 transcripts. According to the structure of *Rasgrf2* gene, exon2-exon24 of *Rasgrf2-201* (ENSMUST00000099326.9) transcript is recommended as the knockout region.The region contains Ens 2254bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Rasgrf2* 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 exhibit decreased Il2 and TNF-alpha production in stimulated T cells. Mice homozygous for mutations in both *Rasgrf1* and *Rasgrf2* exhibit no additional abnormalities than those observed in the *Rasgrf1* mutant mice.

➢ The effect on transcript *Rasgrf2*-202&203&204&205 is unknown.

➢ The *Rasgrf2* 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.

➢ 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）

Rasgrf2 RAS protein-specific guanine nucleotide-releasing factor 2 [*Mus musculus* (house mouse)]

Gene ID: 19418, updated on 5-Nov-2019

Summary

Official Symbol	Rasgrf2 provided by MGI
Official Full Name	RAS protein-specific guanine nucleotide-releasing factor 2 provided by MGI
Primary source	MGI:MGI:109137
See related	Ensembl:ENSMUSG00000021708
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	Grf2; AW048350; Ras-GRF2; 6330417G04Rik
Annotation information	Annotation category: suggests misassembly
	Annotation category: partial on reference assembly
Expression	Biased expression in frontal lobe adult (RPKM 11.9), cortex adult (RPKM 11.9) and 14 other tissues See more
Orthologs	human all

Genomic context

Location: 13 C3; 13 47.43 cM

See Rasgrf2 in Genome Data Viewer

Exon count: 26

| Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	13	NC_000079.6 (91880407..92131828, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	13	NC_000079.5 (92792695..92901449, complement) , (92020012..92127647, complement)

Transcript information（Ensembl）

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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rasgrf2-205	ENSMUST00000151408.7	5797	588aa	Protein coding	-	F7B9R2	CDS 5' incomplete TSL:1
Rasgrf2-201	ENSMUST00000099326.9	3567	1188aa	Protein coding	-	D3Z6K8	TSL:5 GENCODE basic APPRIS P1
Rasgrf2-206	ENSMUST00000216219.1	2115	505aa	Protein coding	-	A0A1L1SS23	TSL:5 GENCODE basic
Rasgrf2-202	ENSMUST00000142378.1	1789	252aa	Protein coding	-	F6TYF8	CDS 5' incomplete TSL:1
Rasgrf2-203	ENSMUST00000146492.2	1323	387aa	Protein coding	-	D3Z685	CDS 3' incomplete TSL:5
Rasgrf2-204	ENSMUST00000149630.7	502	168aa	Protein coding	-	F6TCD3	CDS 5' and 3' incomplete TSL:5

The strategy is based on the design of *Rasgrf2-201* transcript,The transcription is shown below
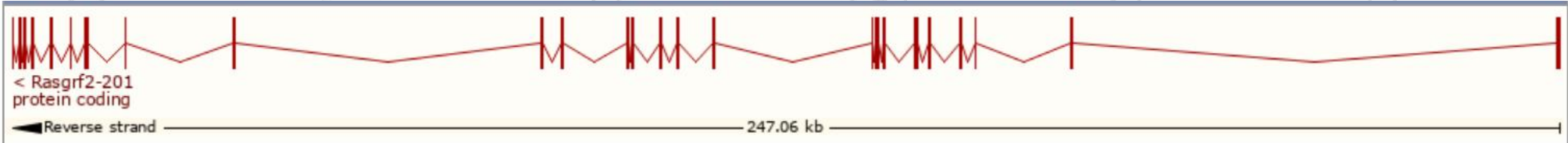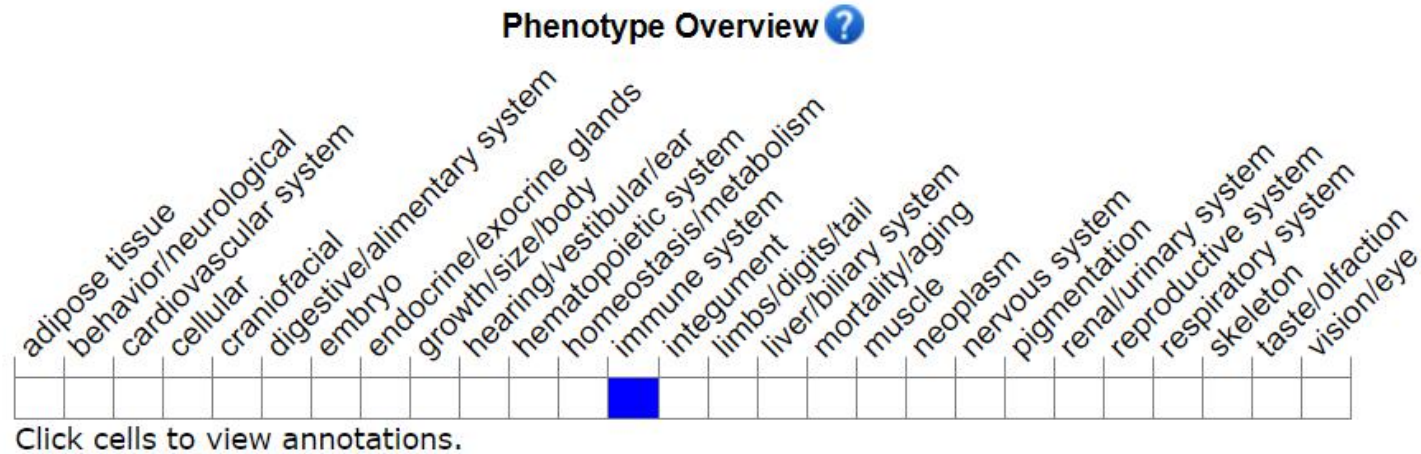
Genomic location distribution
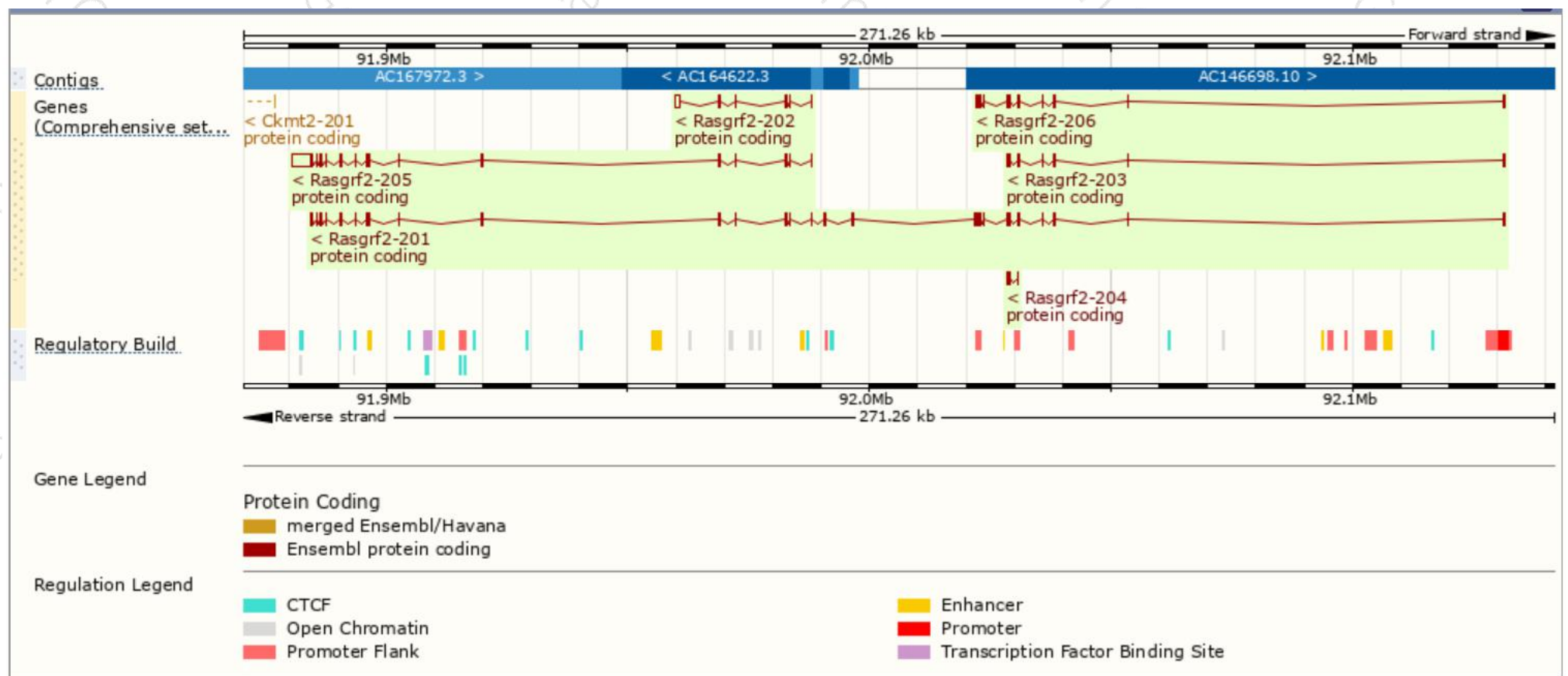
Protein domain

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 exhibit decreased Il2 and TNF-alpha production in stimulated T cells. Mice homozygous for mutations in both Rasgrf1 and Rasgrf2 exhibit no additional abnormalities than those observed in the Rasgrf1 mutant mice.

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