Rapgef1 Cas9-CKO Strategy

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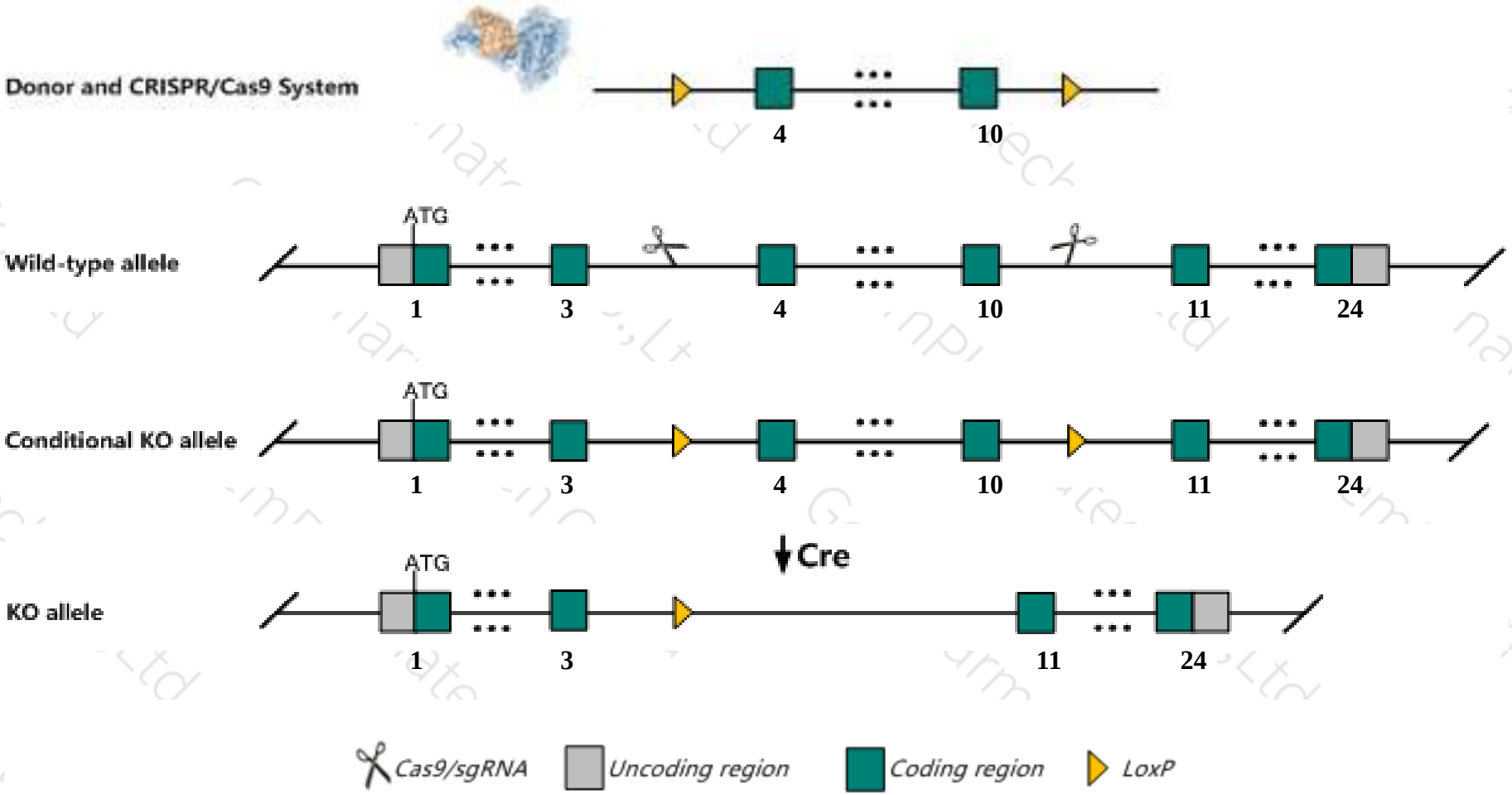
Project Overview

Project Name	***Rapgef1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rapgef1* gene. The schematic diagram is as follows:



Technical routes

- The *Rapgef1* gene has 7 transcripts. According to the structure of *Rapgef1* gene, exon4-exon10 of *Rapgef1-203* (ENSMUST00000102872.10) transcript is recommended as the knockout region.The region contains 1369bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Rapgef1* 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.

Notice

➢ According to the existing MGI data,Mice homozygous for a null allele die before E7.5. Mice homozygous for a hypomorphic gene trap allele show embryonic lethality during organogenesis, altered neuroepithelium morphology, vascular maturation defects, hemorrhage, and reduced cell migration and adhesion.

➢Transcript *Rapgef1*-205 lncRNA may not be affected.

➢ The *Rapgef1* gene is located on the Chr2. 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.

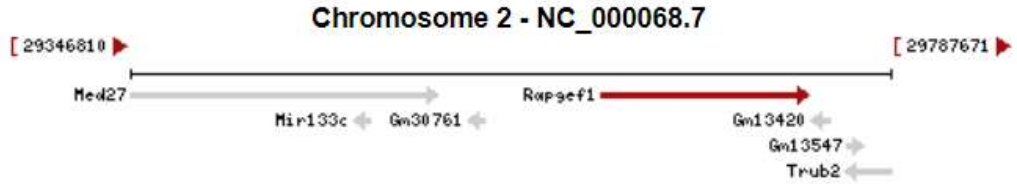
Gene information （NCBI）

Rapgef1 Rap guanine nucleotide exchange factor (GEF) 1 [*Mus musculus* (house mouse)]

Gene ID: 107746, updated on 24-Oct-2019

Summary

Official Symbol	Rapgef1 provided by MGI
Official Full Name	Rap guanine nucleotide exchange factor (GEF) 1 provided by MGI
Primary source	MGI:MGI:104580
See related	Ensembl:ENSMUSG00000039844
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	C3G; Grf2; 4932418O06Rik
Expression	Ubiquitous expression in thymus adult (RPKM 38.9), spleen adult (RPKM 34.8) and 28 other tissues See more
Orthologs	human all

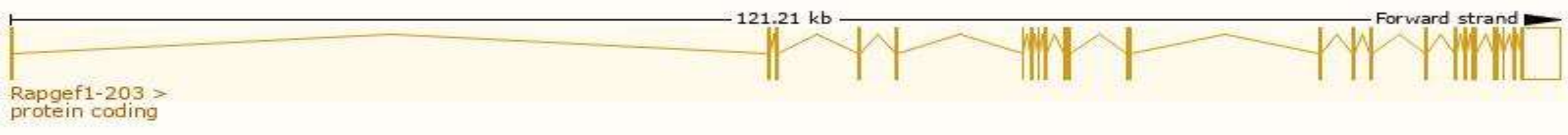


Transcript information（Ensembl）

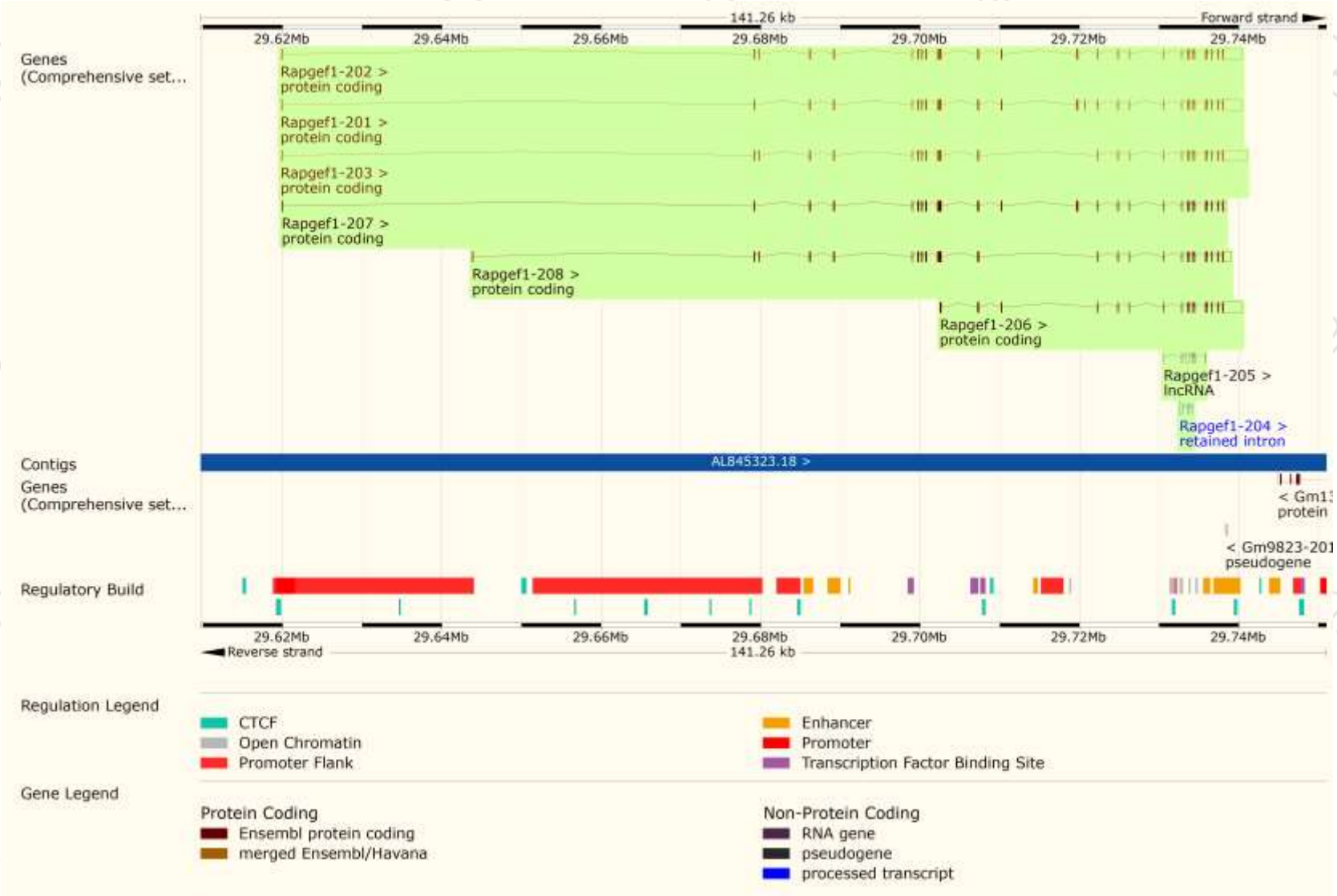
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Rapgef1-203	ENSMUST00000102872.10	6402	1086aa	ENSMUSP00000099936.4	Protein coding	CCDS15854	Q91ZZ2	TSL:1 GENCODE basic APPRIS P3
Rapgef1-202	ENSMUST00000095087.10	6241	1224aa	ENSMUSP00000092703.4	Protein coding	CCDS38091	Q3UHC1	TSL:1 GENCODE basic APPRIS ALT2
Rapgef1-201	ENSMUST00000091146.11	6212	1218aa	ENSMUSP00000088680.5	Protein coding	CCDS38092	Q3UGX8	TSL:1 GENCODE basic APPRIS ALT2
Rapgef1-206	ENSMUST00000147488.1	4223	643aa	ENSMUSP00000117631.1	Protein coding	-	F6ZDE5	CDS 5' incomplete TSL:1
Rapgef1-208	ENSMUST00000238899.1	4205	1087aa	ENSMUSP00000158995.1	Protein coding	-	-	GENCODE basic APPRIS ALT2
Rapgef1-207	ENSMUST00000147755.9	3908	1185aa	ENSMUSP00000121615.3	Protein coding	-	A1L338 E0CXV1	TSL:1 GENCODE basic APPRIS ALT2
Rapgef1-204	ENSMUST00000123953.1	571	No protein	-	Retained intron	-	-	TSL:3
Rapgef1-205	ENSMUST00000137719.1	683	No protein	-	lncRNA	-	-	TSL:5

The strategy is based on the design of *Rapgef1-203* transcript,The transcription is shown below

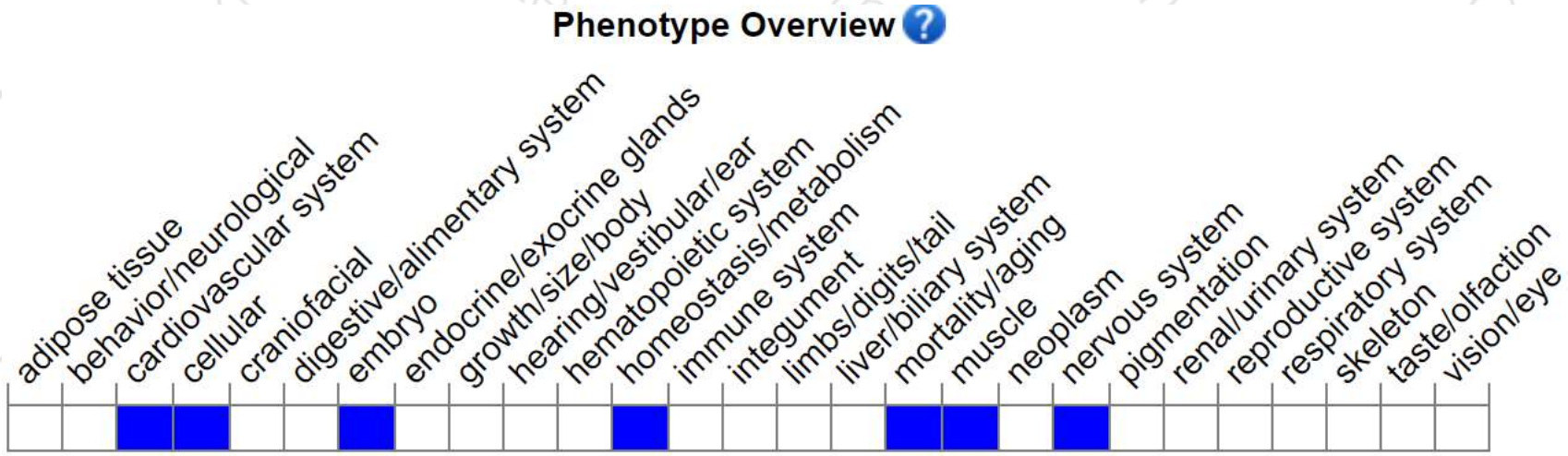


Genomic location distribution



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 null allele die before E7.5. Mice homozygous for a hypomorphic gene trap allele show embryonic lethality during organogenesis, altered neuroepithelium morphology, vascular maturation defects, hemorrhage, and reduced cell migration and adhesion.

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