

Rapgef6 Cas9-CKO Strategy

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Reviewer:

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Design Date:

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Project Overview

Project Name

Rapgef6

Project type

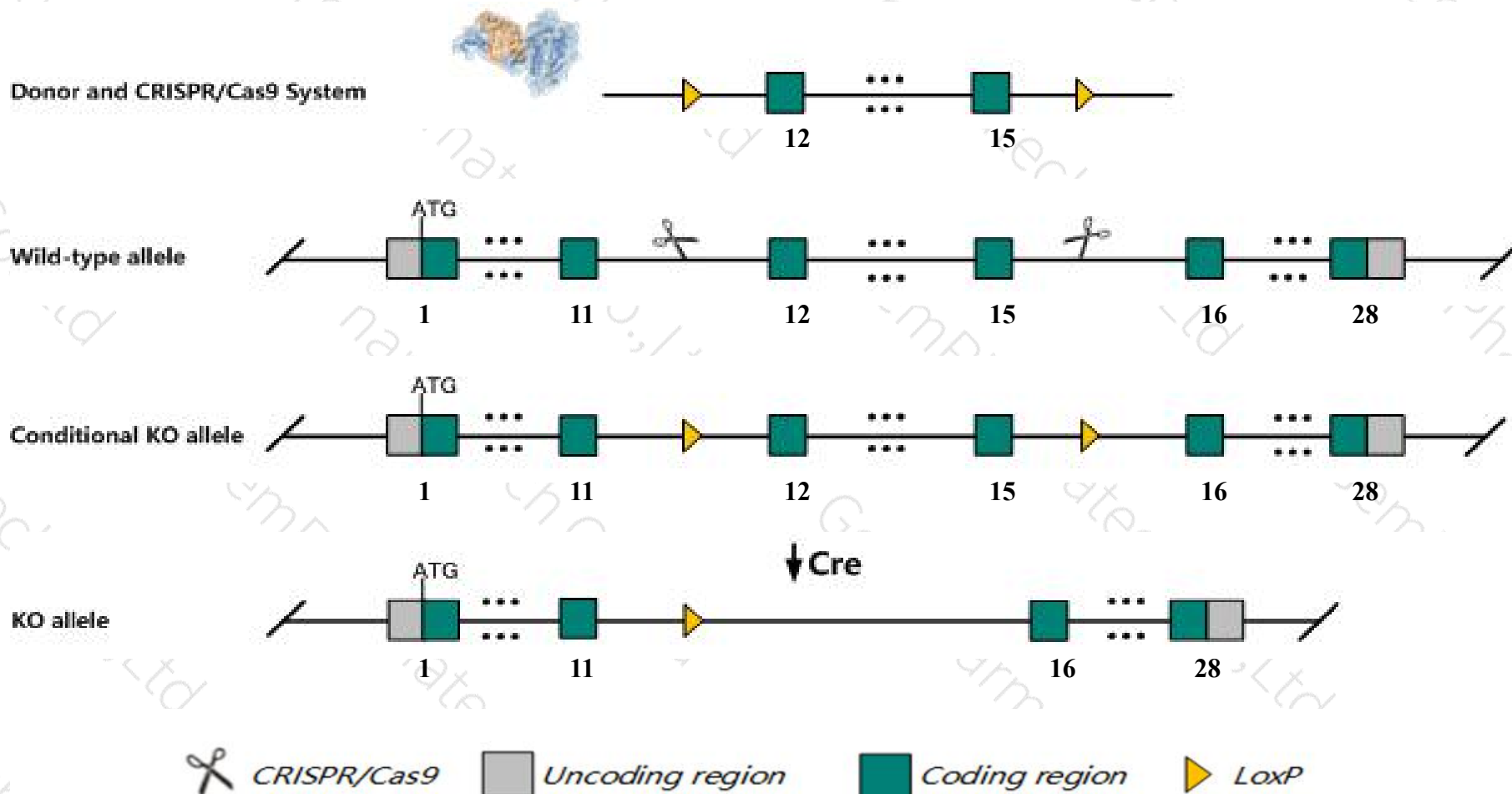
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rapgef6* gene. The schematic diagram is as follows:



Technical routes

- The *Rapgef6* gene has 12 transcripts. According to the structure of *Rapgef6* gene, exon12-exon15 of *Rapgef6*-203 (ENSMUST00000102743.9) transcript is recommended as the knockout region. The region contains 586bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rapgef6* 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 null allele exhibit an enlarged spleen, increased IgE and IgG levels and altered cytokine production.
- The *Rapgef6* gene is located on the Chr11. 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)

Rapgef6 Rap guanine nucleotide exchange factor (GEF) 6 [Mus musculus (house mouse)]

Gene ID: 192786, updated on 31-Jan-2019

Summary



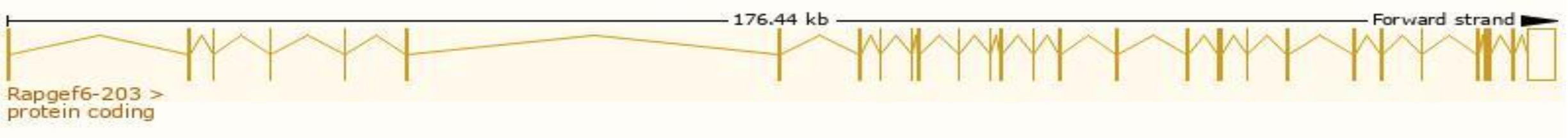
Official Symbol	Rapgef6 provided by MGI
Official Full Name	Rap guanine nucleotide exchange factor (GEF) 6 provided by MGI
Primary source	MGI:MGI:2384761
See related	Ensembl:ENSMUSG00000037533
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	A530068K01, AI844632, BB085664, C030018K18Rik, PDZ-GEF2, Pdzgef2, RA-GEF-2, Ragef2, mKIAA4052
Expression	Ubiquitous expression in thymus adult (RPKM 3.2), CNS E18 (RPKM 2.8) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

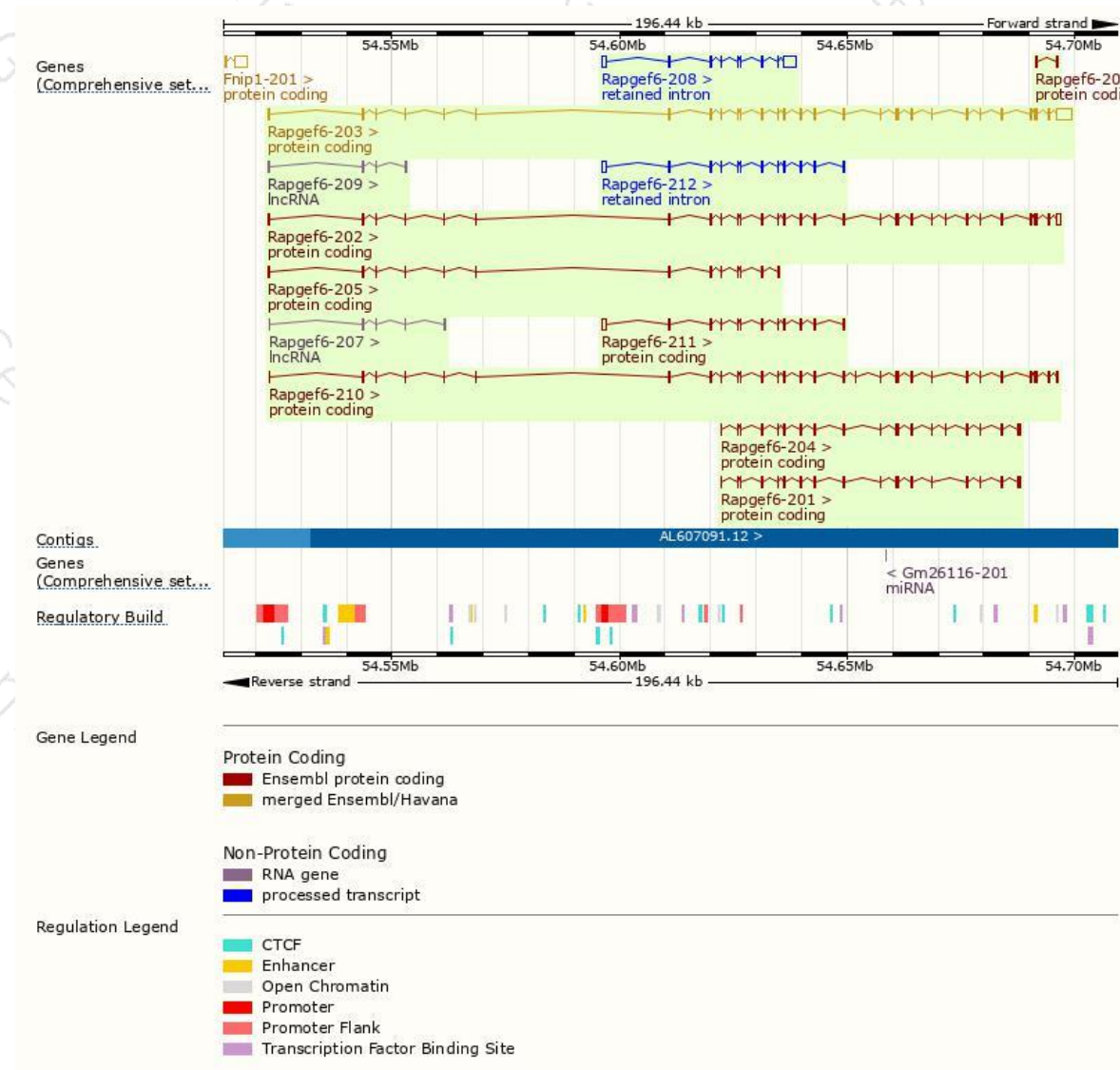
The gene has 12 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rapgef6-203	ENSMUST00000102743.9	8189	1601aa	Protein coding	CCDS24699	Q5NCJ0	TSL:5 GENCODE basic APPRIS P3
Rapgef6-210	ENSMUST00000207429.1	5164	1606aa	Protein coding	CCDS83809	B2RUJ6	TSL:1 GENCODE basic APPRIS ALT2
Rapgef6-202	ENSMUST00000101206.9	5864	1609aa	Protein coding	-	Q5NCJ1	TSL:5 GENCODE basic APPRIS ALT2
Rapgef6-204	ENSMUST00000108894.1	3475	1131aa	Protein coding	-	Q5NCJ5	TSL:5 GENCODE basic
Rapgef6-201	ENSMUST00000094536.9	3372	1123aa	Protein coding	-	Q5NCJ4	TSL:5 GENCODE basic
Rapgef6-211	ENSMUST00000218995.1	2674	599aa	Protein coding	-	A0A1W2P734	CDS 3' incomplete TSL:1
Rapgef6-205	ENSMUST00000108895.7	1829	544aa	Protein coding	-	Q5NCJ2	TSL:1 GENCODE basic
Rapgef6-206	ENSMUST00000136494.1	716	111aa	Protein coding	-	F7ACB1	CDS 5' incomplete TSL:3
Rapgef6-208	ENSMUST00000149372.1	4843	No protein	Retained intron	-	-	TSL:1
Rapgef6-212	ENSMUST00000220269.1	2797	No protein	Retained intron	-	-	TSL:1
Rapgef6-207	ENSMUST00000139360.1	692	No protein	lncRNA	-	-	TSL:3
Rapgef6-209	ENSMUST00000155132.7	628	No protein	lncRNA	-	-	TSL:2

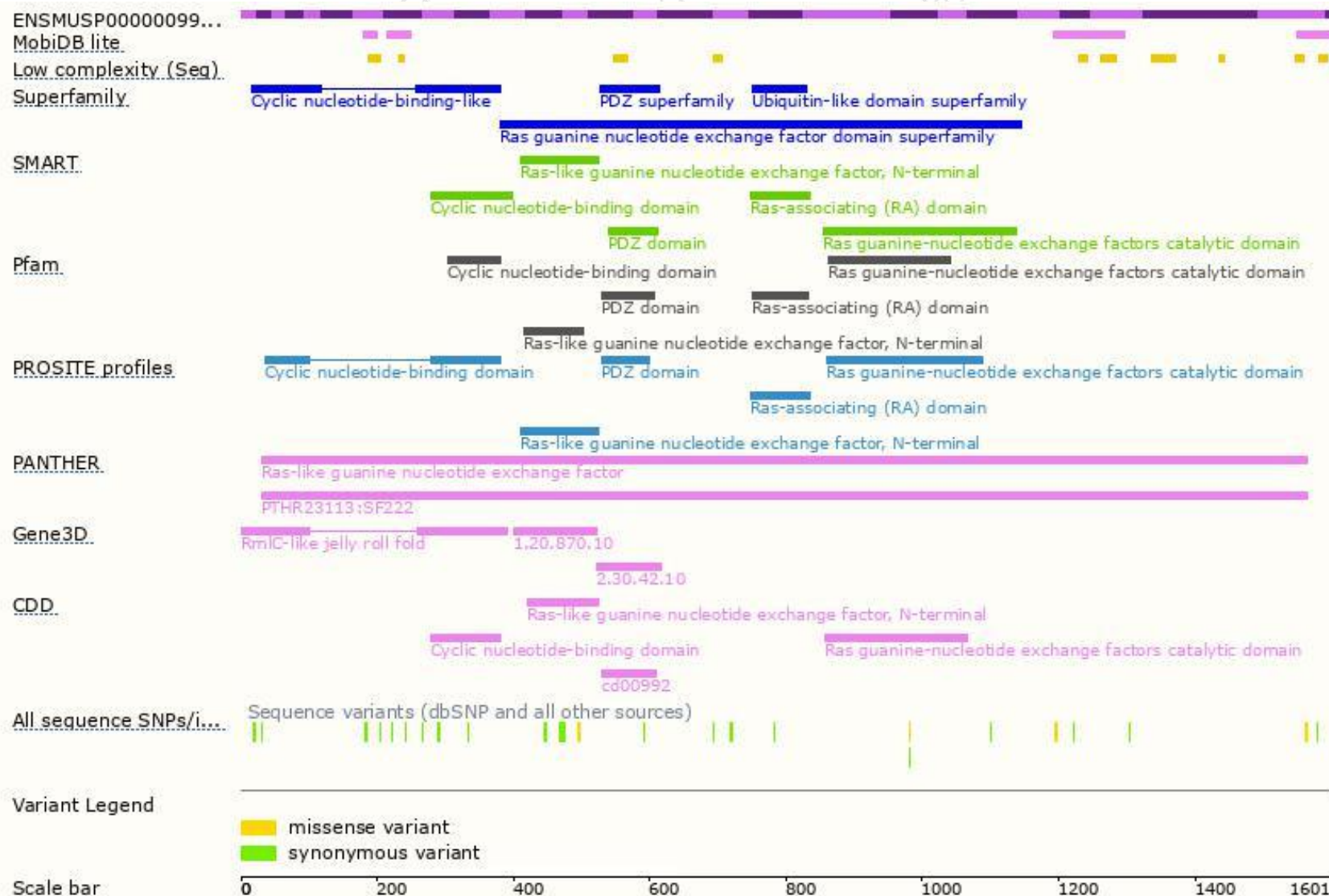
The strategy is based on the design of *Rapgef6-203* transcript,The transcription is shown below



Genomic location distribution

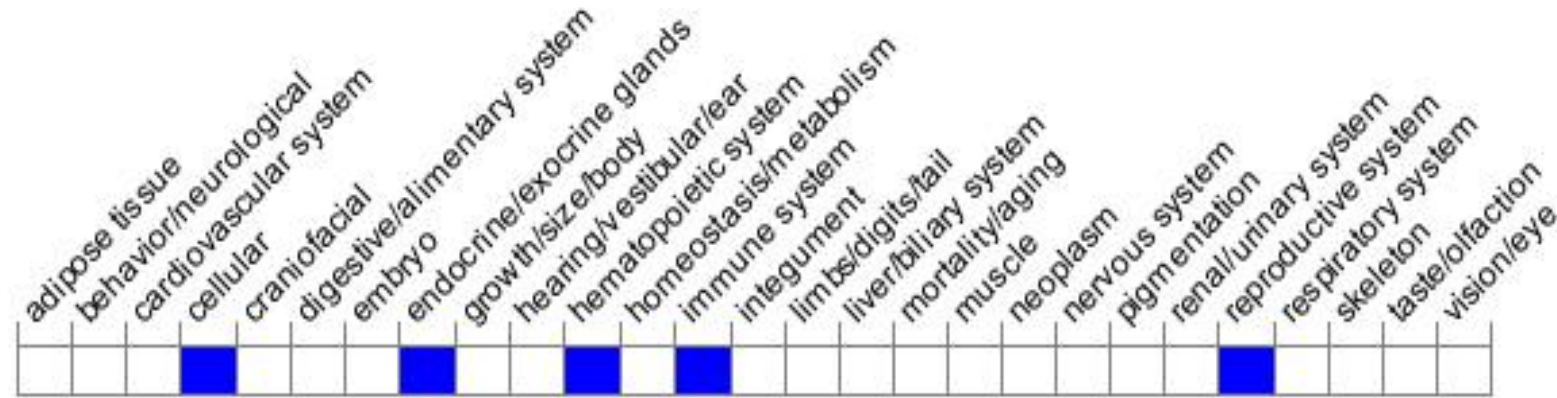


Protein domain



Mouse phenotype description(MGI)

Phenotype Overview



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 exhibit an enlarged spleen, increased IgE and IgG levels and altered cytokine production.

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

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