

***Raph1* Cas9-CKO Strategy**

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Project Overview

Project Name

Raph1

Project type

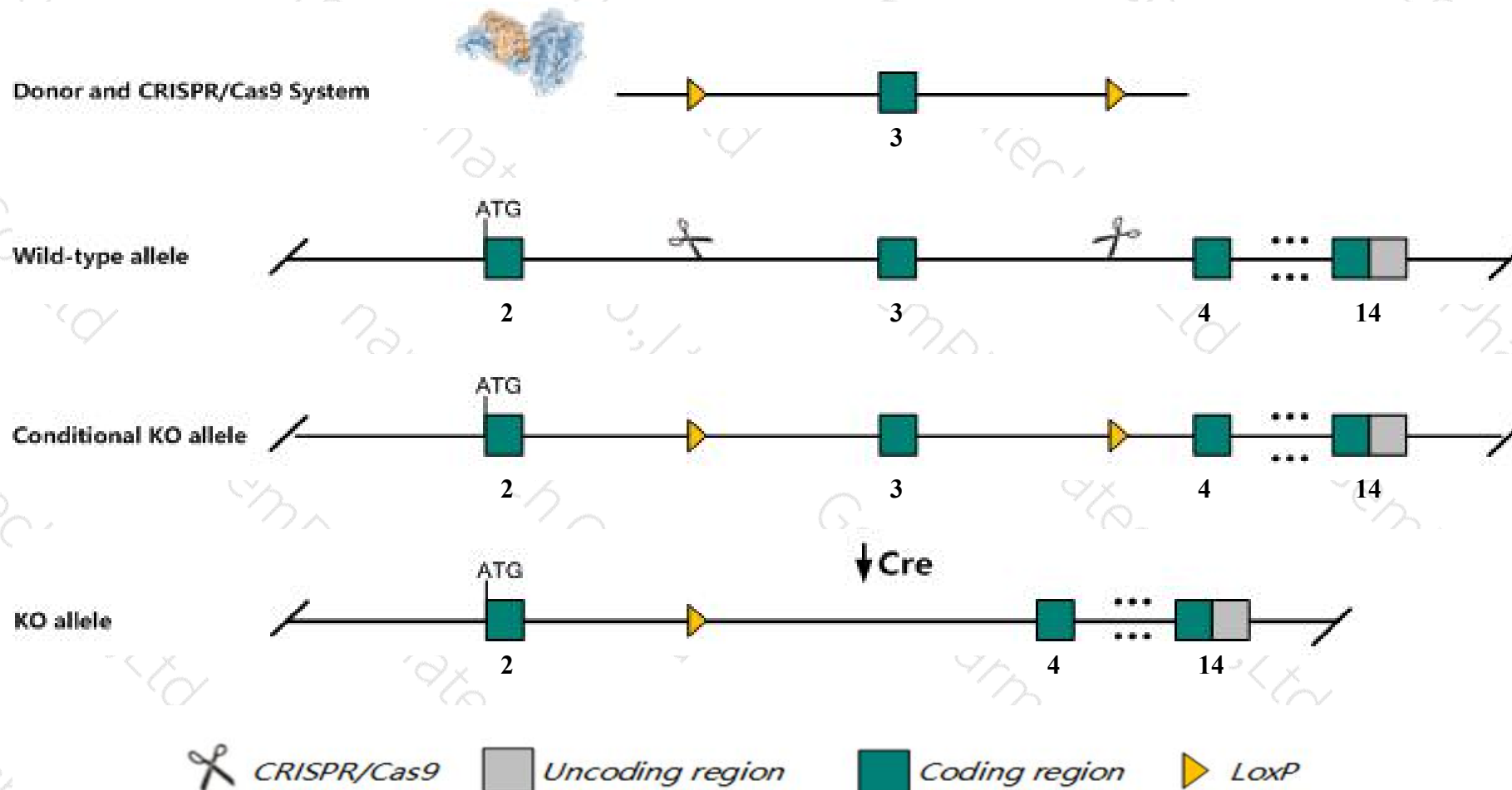
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Raph1* gene. The schematic diagram is as follows:



Technical routes

- The *Raph1* gene has 8 transcripts. According to the structure of *Raph1* gene, exon3 of *Raph1*-204(ENSMUST00000140485.7) transcript is recommended as the knockout region. The region contains 106bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Raph1* 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 conditional allele activated in all cells exhibit background sensitive neonatal or postnatal lethality, decreased body size, belly spotting and decreased melanocyte numbers in the trunk.
- Transcript 206 CDS 5' incomplete the influences is unknown.
- The *Raph1* gene is located on the Chr1. 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)

Raph1 Ras association (RalGDS/AF-6) and pleckstrin homology domains 1 [Mus musculus (house mouse)]

Gene ID: 77300, updated on 13-Mar-2020

Summary



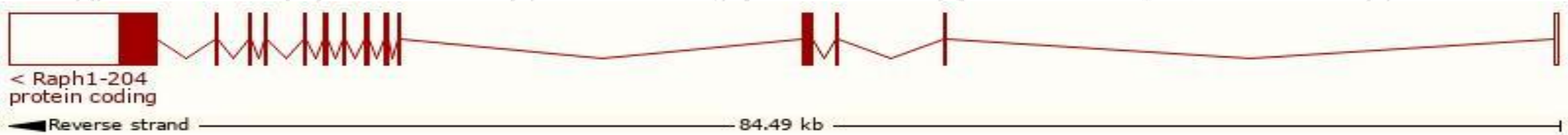
Official Symbol	Raph1 provided by MGI
Official Full Name	Ras association (RalGDS/AF-6) and pleckstrin homology domains 1 provided by MGI
Primary source	MGI:MGI:1924550
See related	Ensembl:ENSMUSG00000026014
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	9430025M21Rik, C730009O10Rik, Lpd
Expression	Ubiquitous expression in whole brain E14.5 (RPKM 6.0), CNS E18 (RPKM 5.8) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

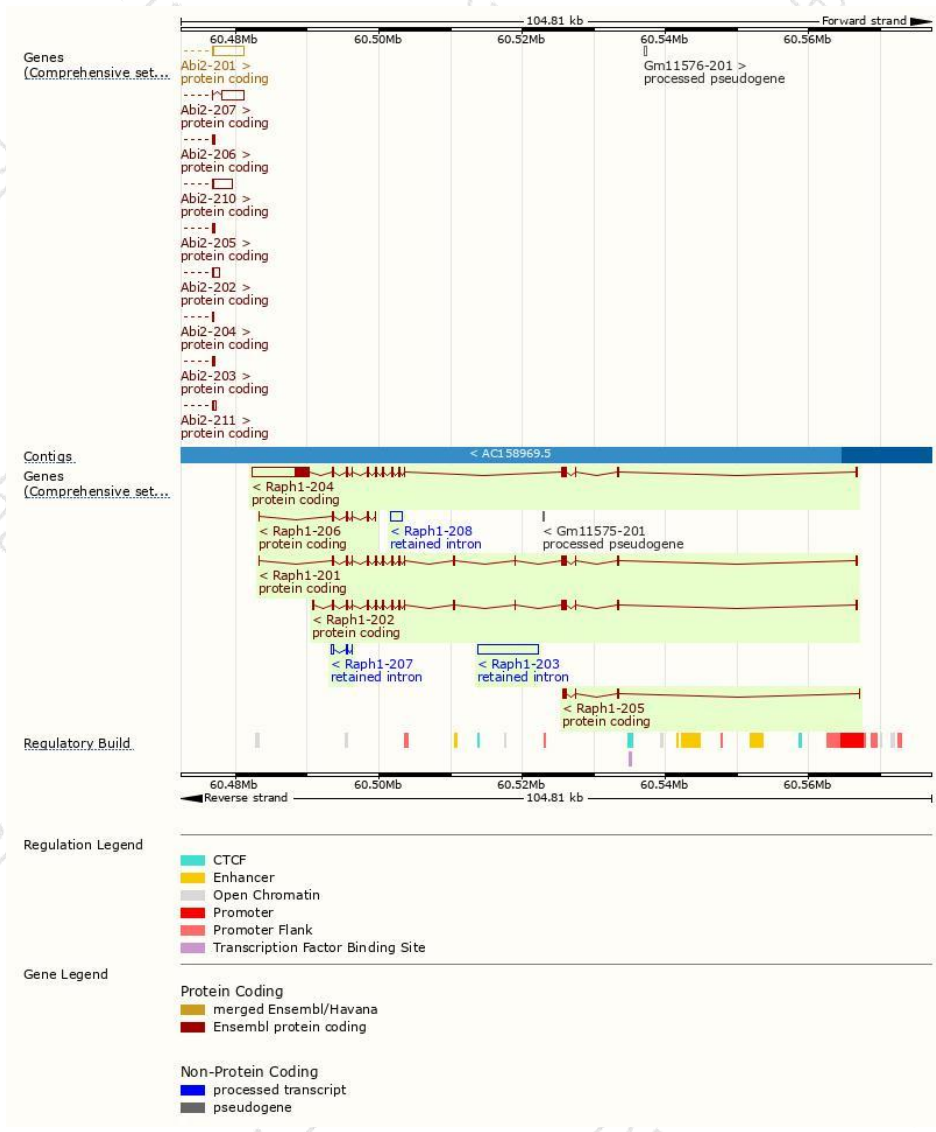
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Raph1-204	ENSMUST00000140485.7	10024	1266aa	Protein coding	CCDS48275	F2Z3U3	TSL:5 GENCODE basic APPRIS P2
Raph1-202	ENSMUST00000090293.10	2409	645aa	Protein coding	-	F2Z4B7	TSL:5 GENCODE basic APPRIS ALT2
Raph1-201	ENSMUST00000027168.11	2241	679aa	Protein coding	-	G5E867	TSL:5 GENCODE basic APPRIS ALT2
Raph1-206	ENSMUST00000182085.7	701	216aa	Protein coding	-	S4R2K5	CDS 5' incomplete TSL:3
Raph1-205	ENSMUST00000142258.2	690	213aa	Protein coding	-	F2Z3X7	CDS 3' incomplete TSL:3
Raph1-203	ENSMUST00000127573.2	8483	No protein	Retained intron	-	-	TSL:NA
Raph1-208	ENSMUST00000189383.1	1663	No protein	Retained intron	-	-	TSL:NA
Raph1-207	ENSMUST00000188511.1	536	No protein	Retained intron	-	-	TSL:3

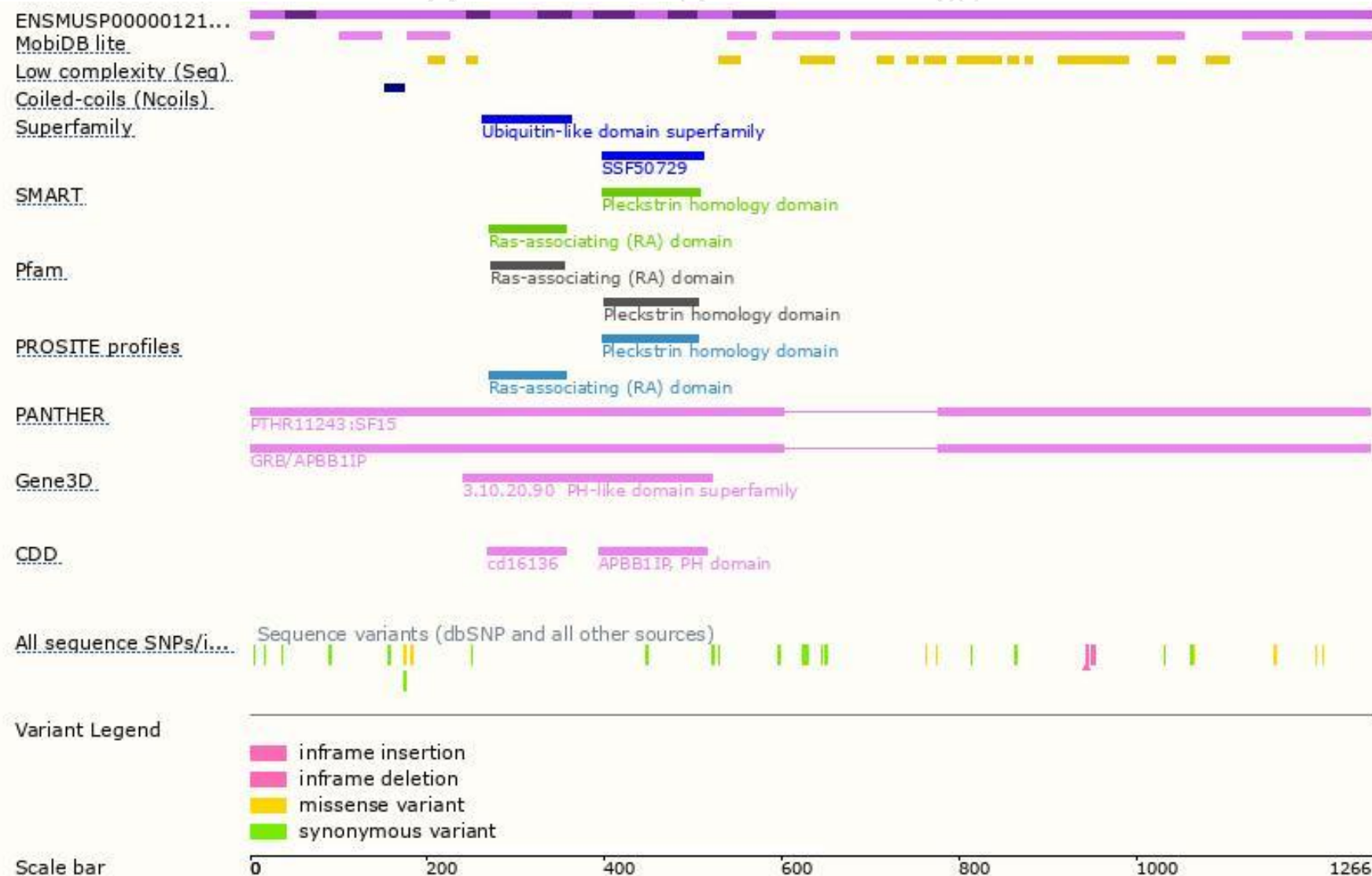
The strategy is based on the design of *Raph1-204* transcript,the transcription is shown below:



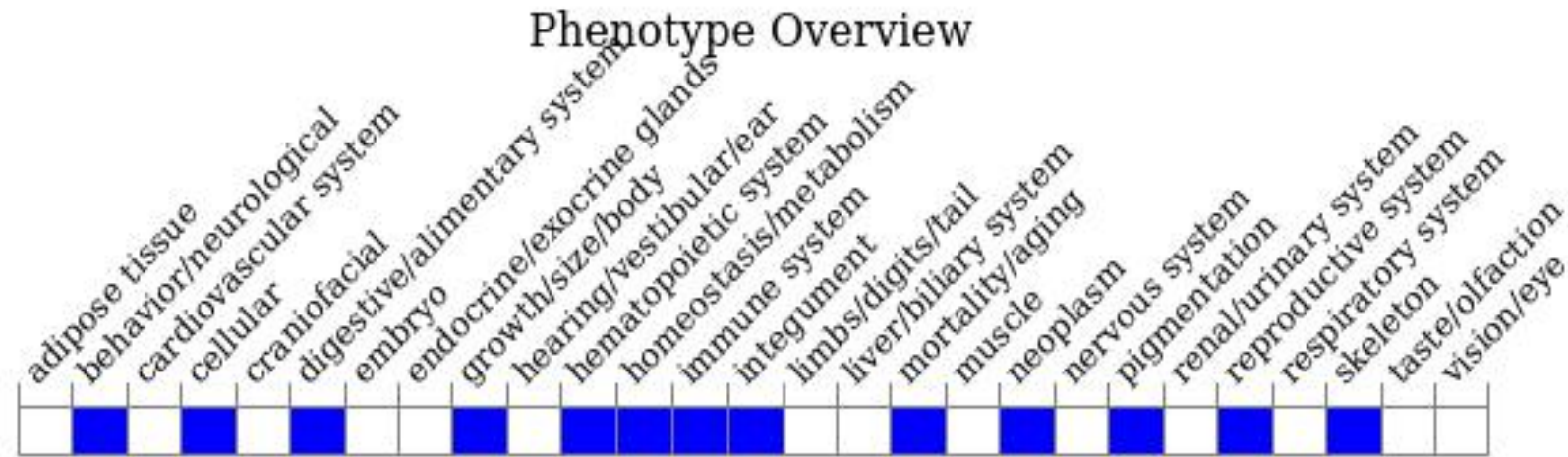
Genomic location distribution



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 conditional allele activated in all cells exhibit background sensitive neonatal or postnatal lethality, decreased body size, belly spotting and decreased melanocyte numbers in the trunk.

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

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