

Arhgap4 Cas9-CKO Strategy

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Project Overview

Project Name

Arhgap4

Project type

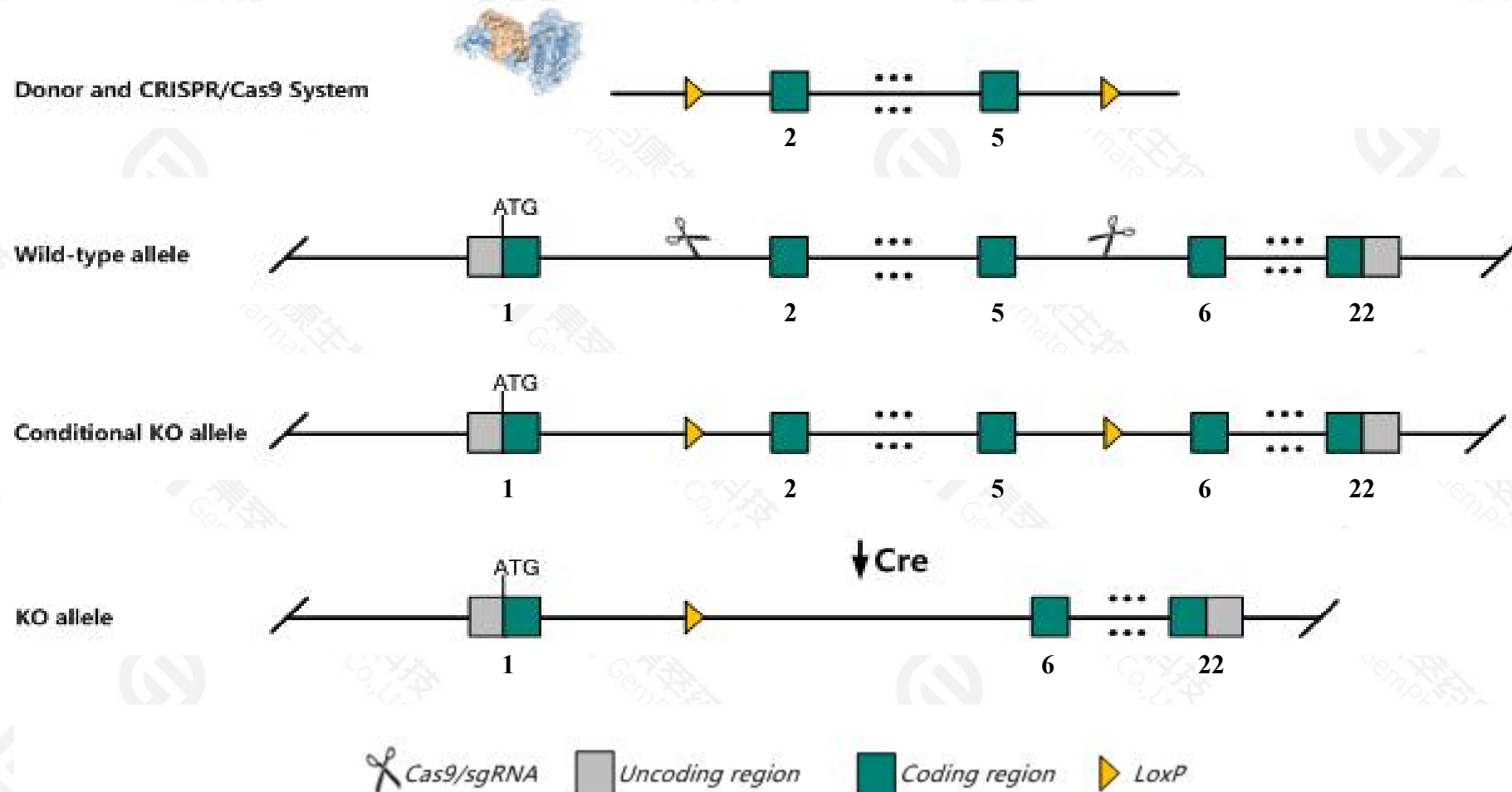
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Arhgap4* gene. The schematic diagram is as follows:



- The *Arhgap4* gene has 20 transcripts. According to the structure of *Arhgap4* gene, exon2-exon5 of *Arhgap4-201*(ENSMUST00000064376.13) transcript is recommended as the knockout region. The region contains 647bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Arhgap4* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor 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.

- The *Arhgap4* gene is located on the ChrX. 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)

Arhgap4 Rho GTPase activating protein 4 [Mus musculus (house mouse)]

Gene ID: 171207, updated on 13-Dec-2020

Summary



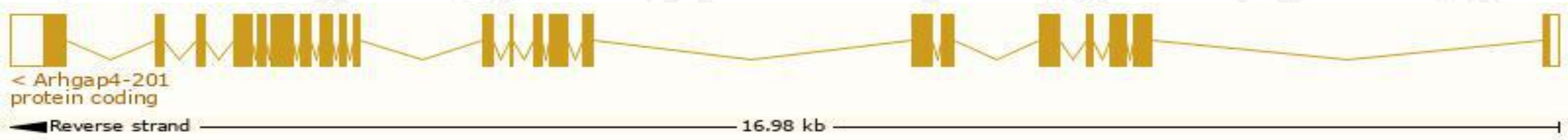
Official Symbol	Arhgap4 provided by MGI
Official Full Name	Rho GTPase activating protein 4 provided by MGI
Primary source	MGI:MGI:2159577
See related	Ensembl:ENSMUSG00000031389
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	A130009C12Rik, A530015A20Rik, Rgc, Rgc1, c1, mKIAA0131
Expression	Biased expression in thymus adult (RPKM 79.1), spleen adult (RPKM 57.3) and 5 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

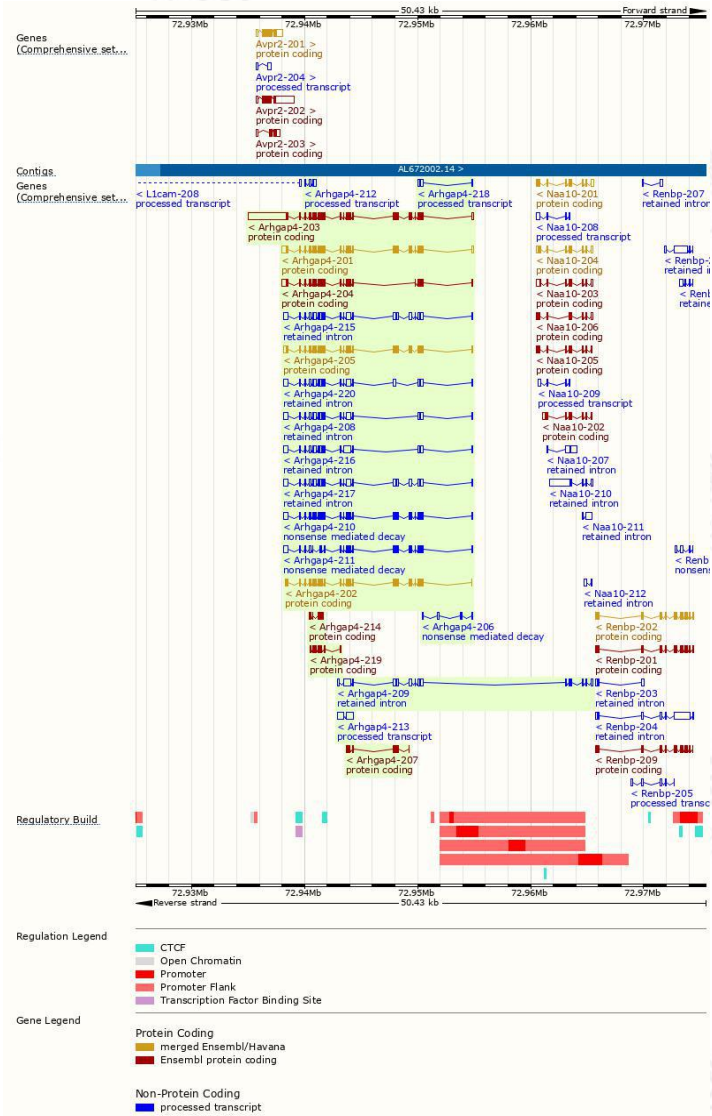
The gene has 20 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Arhgap4-201	ENSMUST00000064376.13	3353	955aa	Protein coding	CCDS41014		TSL:1, GENCODE basic, APPRIS P2,
Arhgap4-205	ENSMUST00000114407.9	3051	934aa	Protein coding	CCDS53100		TSL:1, GENCODE basic,
Arhgap4-202	ENSMUST00000114404.8	2895	944aa	Protein coding	CCDS53101		TSL:1, GENCODE basic,
Arhgap4-203	ENSMUST00000114405.8	6299	965aa	Protein coding	-		TSL:5, GENCODE basic, APPRIS ALT2,
Arhgap4-204	ENSMUST00000114406.9	2749	767aa	Protein coding	-		TSL:1, GENCODE basic,
Arhgap4-219	ENSMUST00000149774.2	823	274aa	Protein coding	-		CDS 5' and 3' incomplete, TSL:5,
Arhgap4-207	ENSMUST00000124798.2	642	214aa	Protein coding	-		CDS 5' and 3' incomplete, TSL:5,
Arhgap4-214	ENSMUST00000143696.8	597	199aa	Protein coding	-		CDS 5' and 3' incomplete, TSL:5,
Arhgap4-210	ENSMUST00000130976.8	3123	847aa	Nonsense mediated decay	-		TSL:1,
Arhgap4-211	ENSMUST00000140393.8	2690	605aa	Nonsense mediated decay	-		TSL:5,
Arhgap4-206	ENSMUST00000123283.2	555	44aa	Nonsense mediated decay	-		TSL:3,
Arhgap4-213	ENSMUST00000143536.2	1196	No protein	Processed transcript	-		TSL:2,
Arhgap4-218	ENSMUST00000149135.2	563	No protein	Processed transcript	-		TSL:1,
Arhgap4-212	ENSMUST00000142925.2	527	No protein	Processed transcript	-		TSL:5,
Arhgap4-215	ENSMUST00000145159.8	3208	No protein	Retained intron	-		TSL:2,
Arhgap4-217	ENSMUST00000148319.8	3145	No protein	Retained intron	-		TSL:2,
Arhgap4-216	ENSMUST00000147510.8	2806	No protein	Retained intron	-		TSL:2,
Arhgap4-220	ENSMUST00000151600.8	2803	No protein	Retained intron	-		TSL:2,
Arhgap4-208	ENSMUST00000127163.8	2580	No protein	Retained intron	-		TSL:2,
Arhgap4-209	ENSMUST00000128329.2	2367	No protein	Retained intron	-		TSL:2,

The strategy is based on the design of *Arhgap4-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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