Arap3 Cas9-KO Strategy

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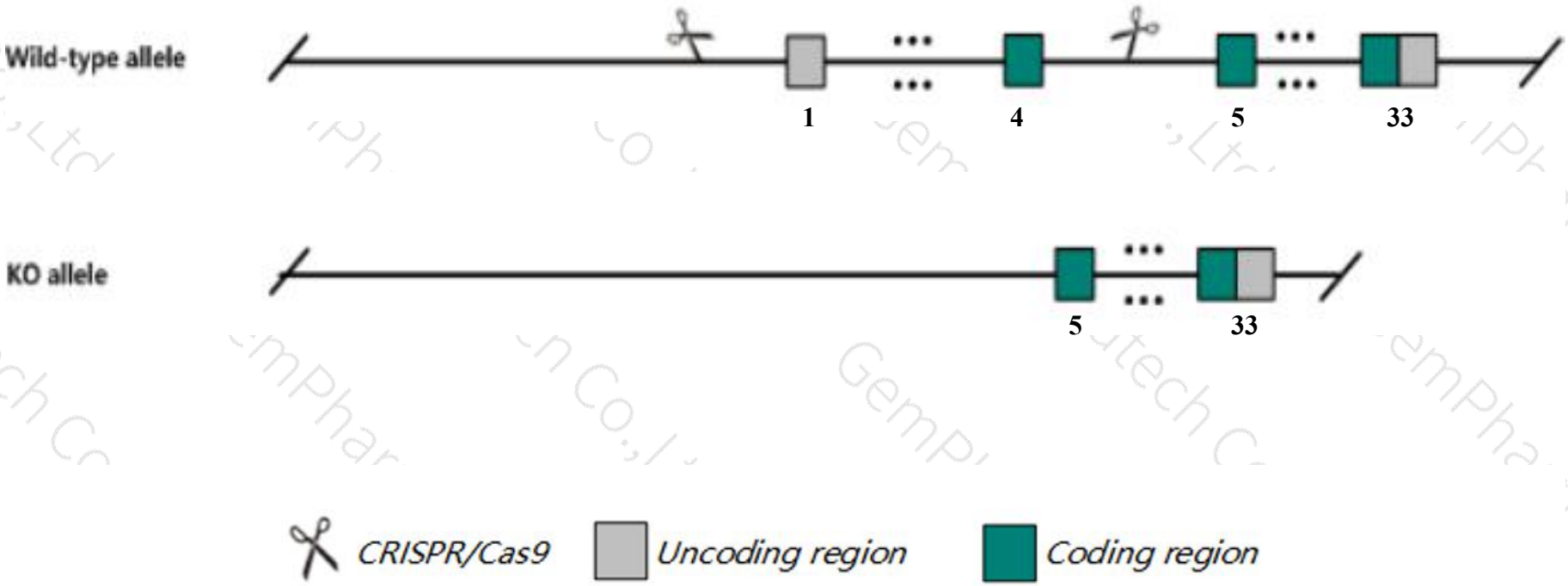
Design Date: 2020-7-23

Project Overview

Project Name	***Arap3***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Arap3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Arap3* gene has 9 transcripts. According to the structure of *Arap3* gene, exon1-exon4 of *Arap3-201*(ENSMUST00000042944.8) transcript is recommended as the knockout region.The region contains 695bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Arap3* gene. The brief process is as follows: CRISPR/Cas9 system 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.

Notice

➢ According to the existing MGI data,mice homozygous for a knock-out allele die around E11 exhibiting pallor, embryonic growth arrest, yolk sac and placental abnormalities, and an endothelial cell-autonomous defect in sprouting angiogenesis. Knock-in mice homozygous for a point mutation display similar angiogenesis defects.

➢ The *Arap3* gene is located on the Chr18. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

Arap3 ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 3 [Mus musculus (house mouse)]

Gene ID: 106952, updated on 13-Mar-2020

Summary

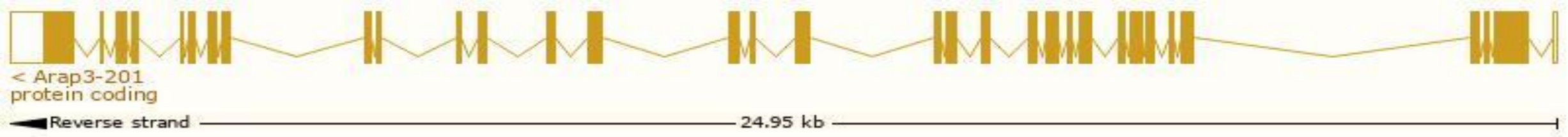
Official Symbol	Arap3 provided by MGI
Official Full Name	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 3 provided byMGI
Primary source	MGI:MGI:2147274
See related	Ensembl:ENSMUSG00000024451
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Centd3, Drag1, E030006K04Rik, cnt-d3
Summary	This gene encodes a phosphoinositide binding protein containing ARF-GAP, RHO-GAP, RAS-associating, and pleckstrin homology domains. The ARF-GAP and RHO-GAP domains cooperate in mediating rearrangements in the cell cytoskeleton and cell shape. It is a specific PtdIns(3,4,5)P3/PtdIns(3,4)P2-stimulated Arf6-GAP protein. Two transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Sep 2015]
Expression	Biased expression in lung adult (RPKM 62.4), subcutaneous fat pad adult (RPKM 16.7) and 13 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

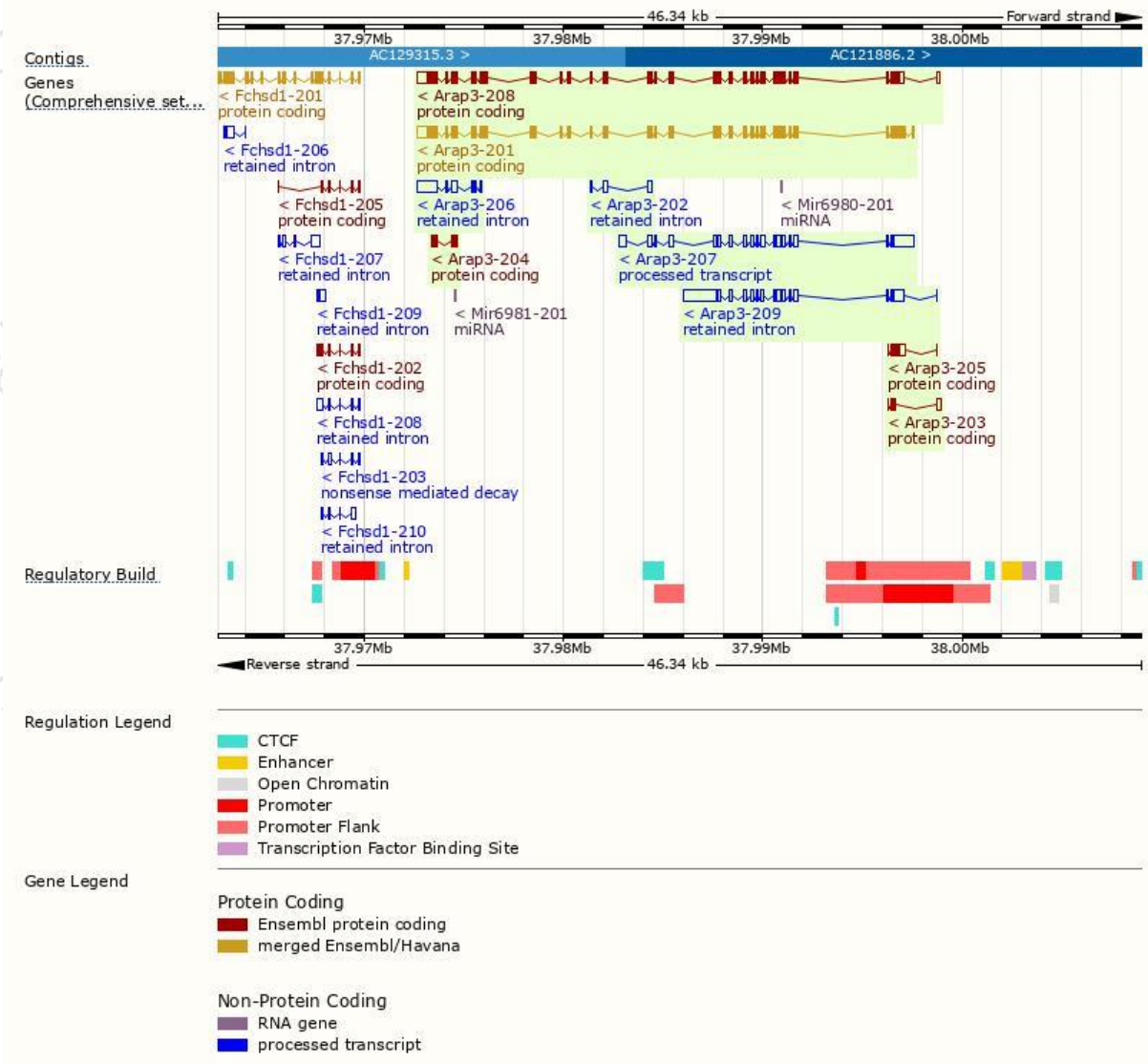
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Arap3-201	ENSMUST00000042944.8	5219	1538aa	Protein coding	CCDS29196	Q8R5G7	TSL:1 GENCODE basic APPRIS P1
Arap3-208	ENSMUST00000237272.1	5359	1460aa	Protein coding	-	Q8R5G7	GENCODE basic
Arap3-205	ENSMUST00000236588.1	683	124aa	Protein coding	-	A0A494BA67	CDS 3' incomplete
Arap3-204	ENSMUST00000236534.1	464	154aa	Protein coding	-	A0A494BAF2	CDS 5' and 3' incomplete
Arap3-203	ENSMUST00000236134.1	434	47aa	Protein coding	-	A0A494BA60	CDS 3' incomplete
Arap3-207	ENSMUST00000237188.1	3601	No protein	Processed transcript	-	-	
Arap3-209	ENSMUST00000237677.1	3880	No protein	Retained intron	-	-	
Arap3-206	ENSMUST00000236772.1	1556	No protein	Retained intron	-	-	
Arap3-202	ENSMUST00000235391.1	499	No protein	Retained intron	-	-	

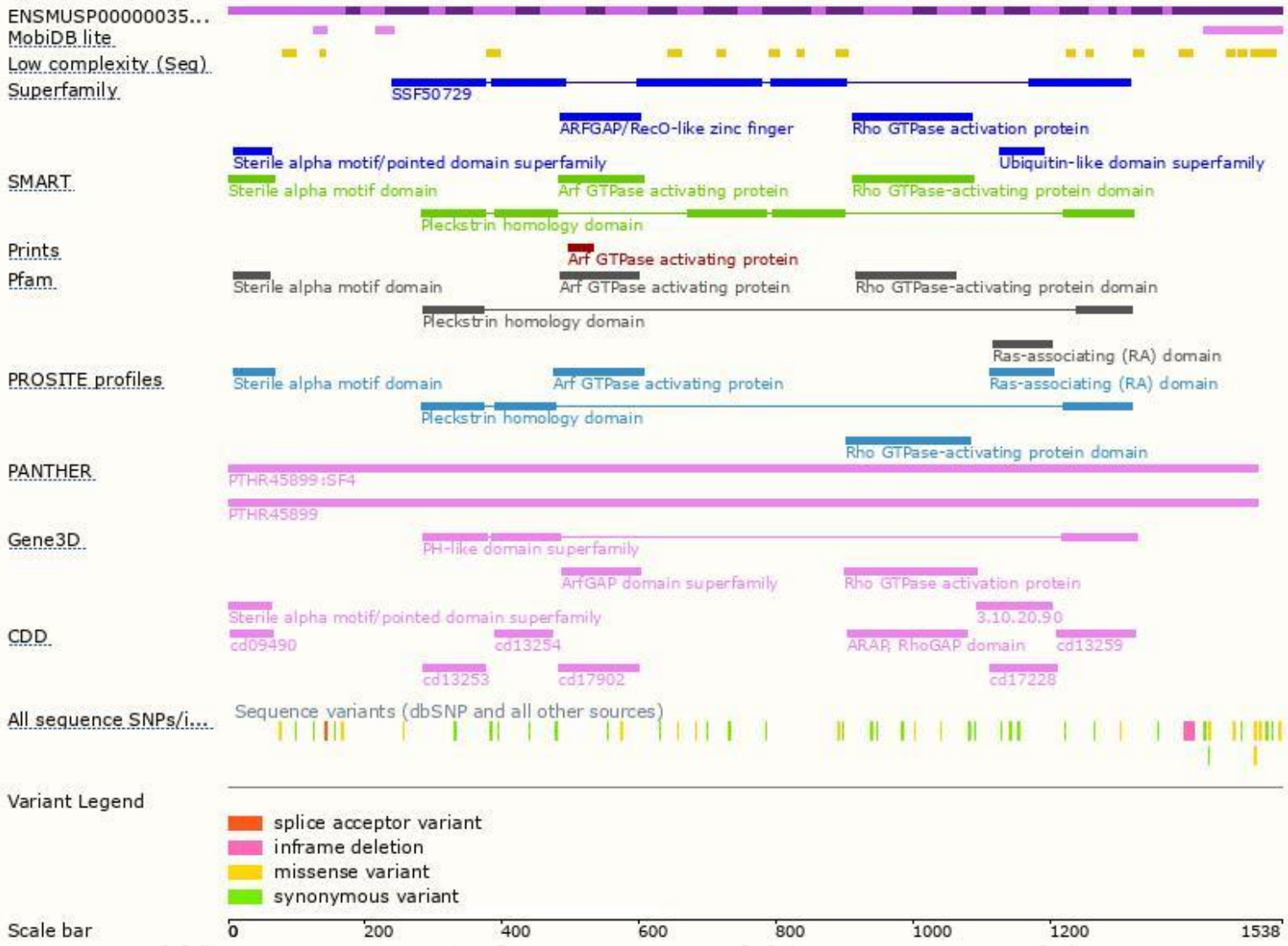
The strategy is based on the design of *Arap3-201* transcript,the transcription is shown below:



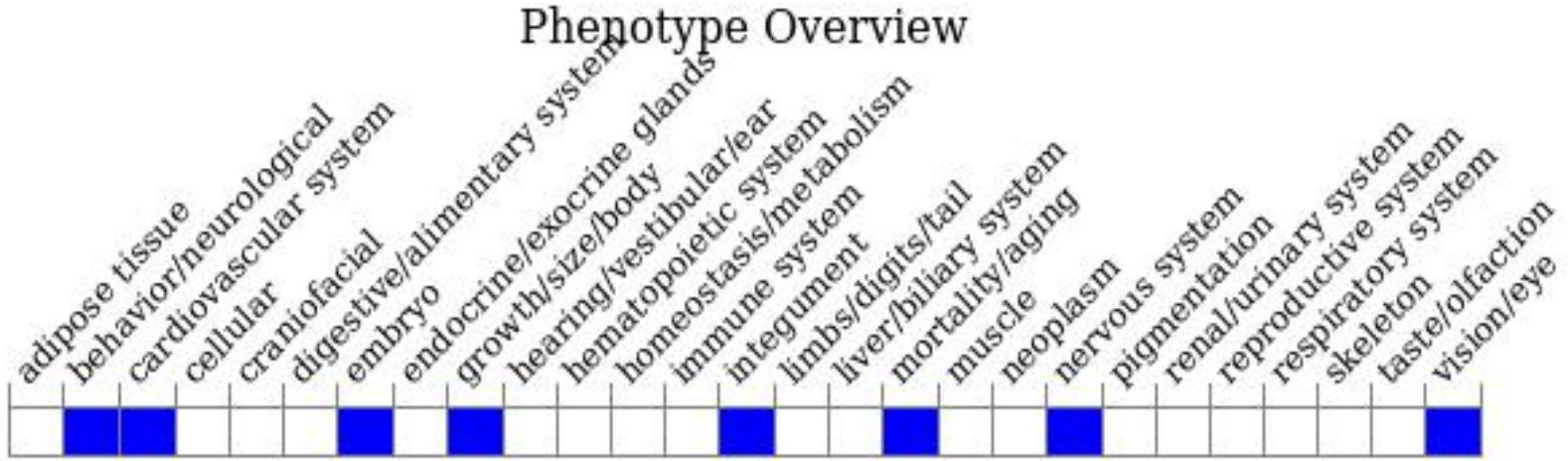
Genomic location distribution



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 knock-out allele die around E11 exhibiting pallor, embryonic growth arrest, yolk sac and placental abnormalities, and an endothelial cell-autonomous defect in sprouting angiogenesis. Knock-in mice homozygous for a point mutation display similar angiogenesis defects.

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

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