

Arhgef15 Cas9-CKO Strategy

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Design Date:2019-8-12

Project Overview



Project Name

Arhgef15

Project type

Cas9-CKO

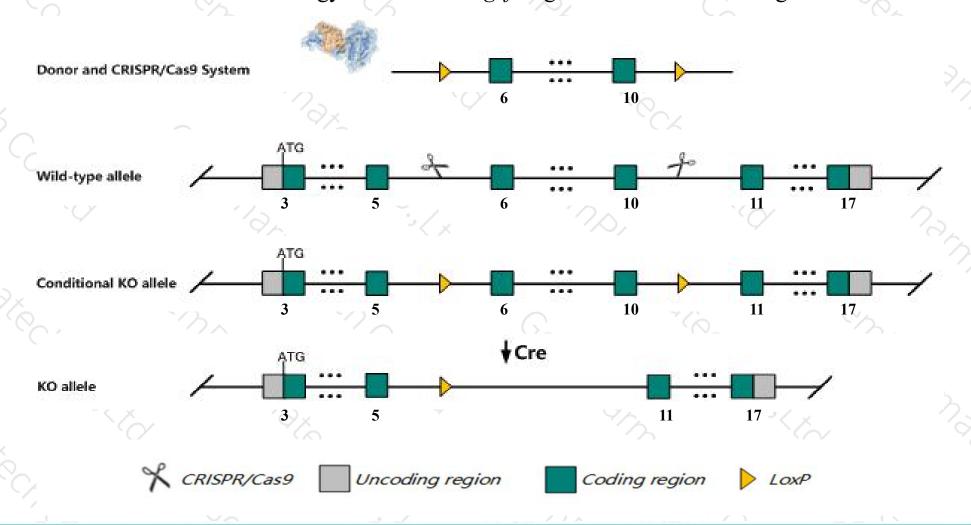
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Arhgef15 gene. The schematic diagram is as follows:



Technical routes



- The *Arhgef15* gene has 6 transcripts. According to the structure of *Arhgef15* gene, exon4-exon8 of *Arhgef15-201* (ENSMUST0000065040.12) transcript is recommended as the knockout region. The region contains 715bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Arhgef15* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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.

Notice



- ➤ According to the existing MGI data, Mice homozygous for a knock out allele exhibit increased excitatory synapse formation. Mice homozygous for a knock-out allele exhibit delayed radial growth, sparse vasculature and empty baselment membrane sleeves in the retina.
- ➤ Transcript *Arhgef15*-204&205&206 may not be affected.
- > The *Arhgef15* 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)



Arhgef15 Rho guanine nucleotide exchange factor (GEF) 15 [Mus musculus (house mouse)]

Gene ID: 442801, updated on 31-Jan-2019

Summary

☆ ?

Official Symbol Arhgef15 provided by MGI

Official Full Name Rho guanine nucleotide exchange factor (GEF) 15 provided by MGI

Primary source MGI:MGI:3045246

See related Ensembl: ENSMUSG00000052921

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 D130071N09, D530030K12Rik, E5

Expression Broad expression in lung adult (RPKM 39.8), adrenal adult (RPKM 12.1) and 15 other tissuesSee more

Orthologs <u>human</u> all

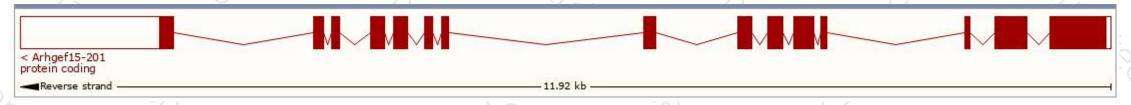
Transcript information (Ensembl)



The gene has 6 transcripts, all transcripts are shown below:

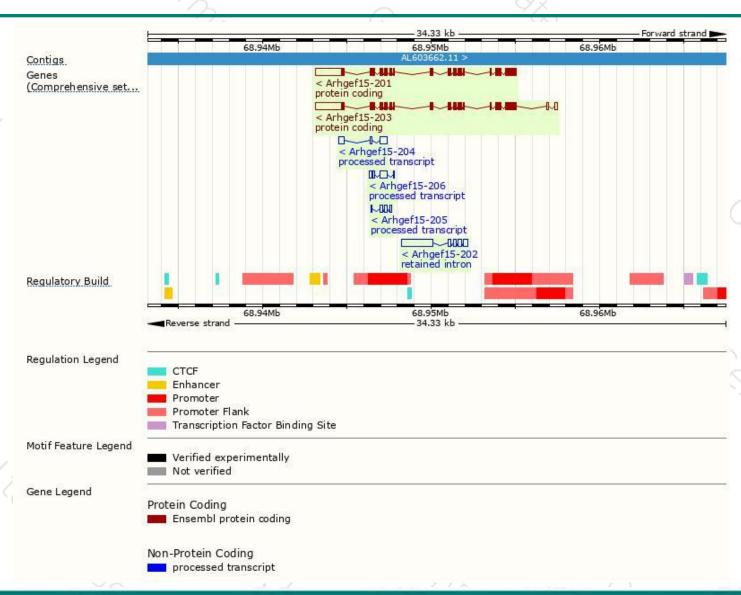
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Arhgef15-203	ENSMUST00000108671.1	4372	849aa	Protein coding	CCDS24873	Q5FWH6	TSL:5 GENCODE basic APPRIS P1
Arhgef15-201	ENSMUST00000065040.12	4123	849aa	Protein coding	CCDS24873	Q5FWH6	TSL:5 GENCODE basic APPRIS P1
Arhgef15-204	ENSMUST00000131686.1	805	No protein	Processed transcript	5	-	TSL:2
Arhgef15-206	ENSMUST00000151520.1	726	No protein	Processed transcript	<u> </u>	10	TSL:3
Arhgef15-205	ENSMUST00000141747.1	428	No protein	Processed transcript	ā	-	TSL:5
Arhgef15-202	ENSMUST00000108670.1	2659	No protein	Retained intron		-	TSL:1

The strategy is based on the design of Arhgef15-201 transcript, The transcription is shown below



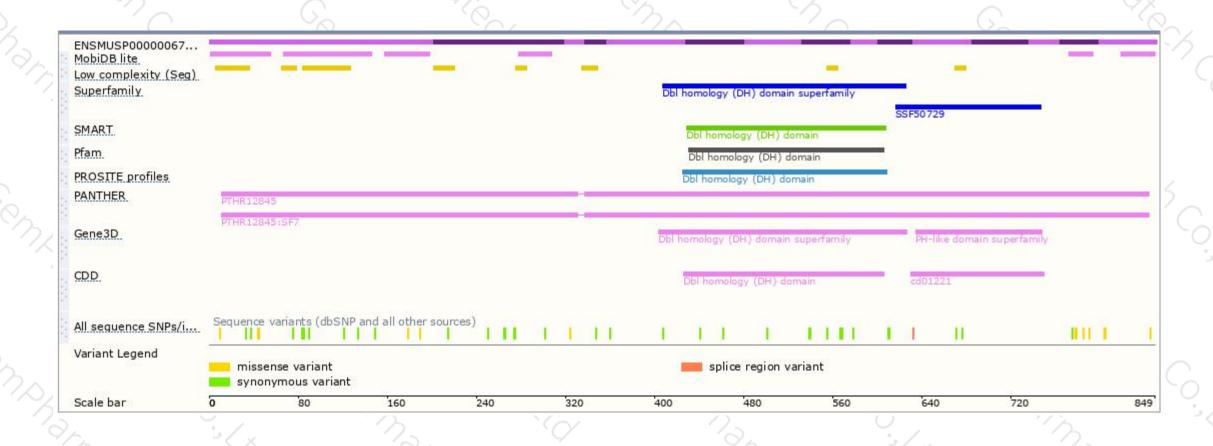
Genomic location distribution





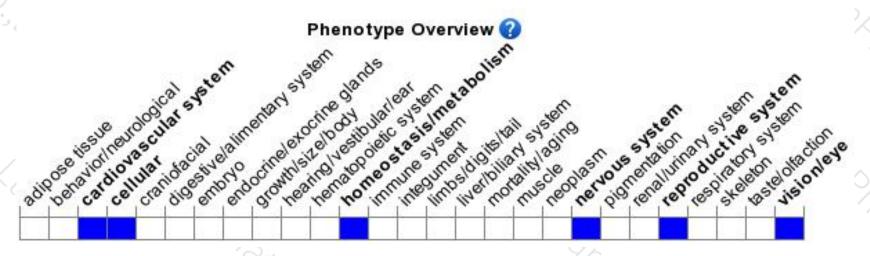
Protein domain





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 exhibit increased excitatory synapse formation. Mice homozygous for a knock-out allele exhibit delayed radial growth, sparse vasculature and empty baselment membrane sleeves in the retina.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





