

Arhgap32 Cas9-CKO Strategy

Designer: Xiaojing Li

Design Date: 2020-1-22

Reviewer: JiaYu

Project Overview



Project Name

Arhgap32

Project type

Cas9-CKO

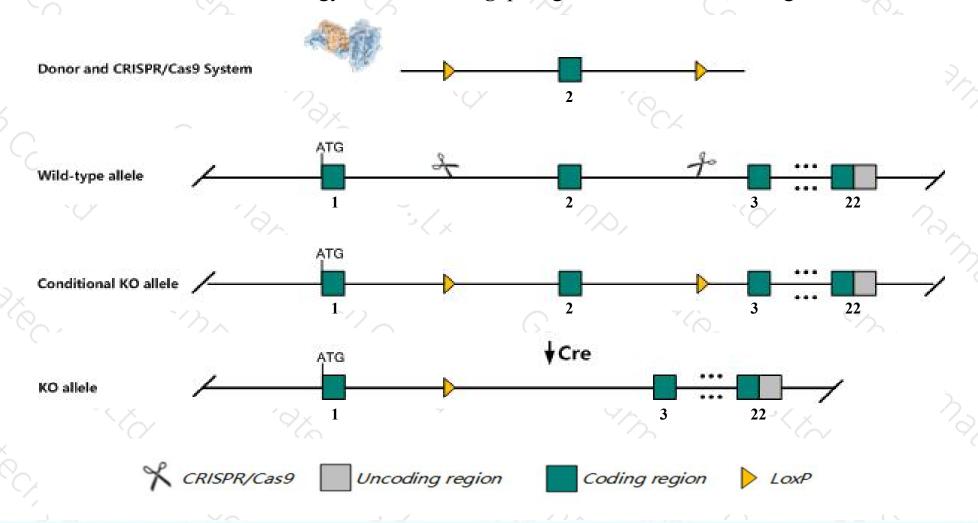
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Arhgap32* gene. The schematic diagram is as follows:



Technical routes



- The *Arhgap32* gene has 7 transcripts. According to the structure of *Arhgap32* gene, exon2 of *Arhgap32-203* (ENSMUST00000174641.7) transcript is recommended as the knockout region. The region contains 109bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Arhgap32* 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.

Notice



- > According to the existing MGI data, Mice homozygous for a null mutation are fertile but display abnormal neurite growth.
- ➤ This strategy has no effect on *Arhgap32*-201, *Arhgap32*-206, *Arhgap32*-207.
- The *Arhgap32* gene is located on the Chr9. 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)



Arhgap32 Rho GTPase activating protein 32 [Mus musculus (house mouse)]

Gene ID: 330914, updated on 12-Aug-2019

Summary

Official Symbol Arhgap32 provided by MGI

Official Full Name Rho GTPase activating protein 32 provided by MGI

Primary source MGI:MGI:2450166

See related Ensembl: ENSMUSG00000041444

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 Grit; Rics; Gc-gap; Px-rics; p250Gap; mKIAA0712; p200Rhogap; 3426406O18Rik

Annotation information Note: There are conflicting opinions about the protein-coding potential of this locus. [17 Jun 2011]

Expression Broad expression in cortex adult (RPKM 9.5), frontal lobe adult (RPKM 9.3) and 22 other tissues See more

Orthologs human all

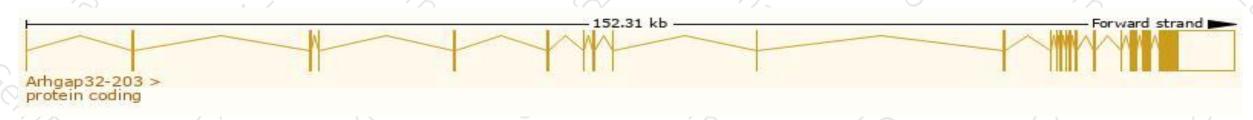
Transcript information (Ensembl)



The gene has 7 transcripts, all transcripts are shown below:

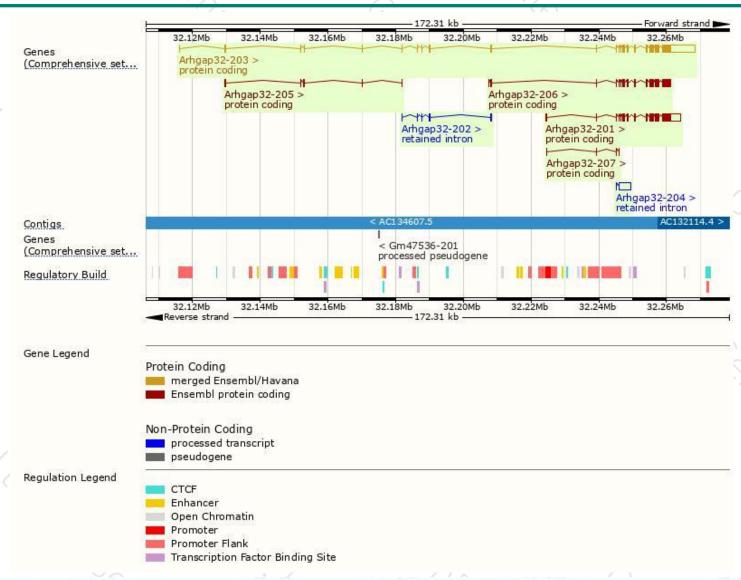
Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
ENSMUST00000174641.7	13568	2089aa	Protein coding	CCDS57666	Q811P8	TSL:5 GENCODE basic APPRIS P1
ENSMUST00000168954.8	8703	<u>1740aa</u>	Protein coding	CCDS22951	Q811P8	TSL:5 GENCODE basic
ENSMUST00000182802.7	5621	<u>1740aa</u>	Protein coding	CCDS22951	Q811P8	TSL:1 GENCODE basic
ENSMUST00000182310.1	540	<u>180aa</u>	Protein coding	358	S4R2G6	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:
ENSMUST00000183121.1	380	<u>91aa</u>	Protein coding	1271	S4R2C0	CDS 3' incomplete TSL:5
ENSMUST00000174730.1	3638	No protein	Retained intron	9 4 9		TSL:2
ENSMUST00000174314.1	543	No protein	Retained intron	020	-	TSL:3
	ENSMUST00000174641.7 ENSMUST00000168954.8 ENSMUST00000182802.7 ENSMUST00000182310.1 ENSMUST00000183121.1 ENSMUST00000174730.1	ENSMUST00000174641.7 13568 ENSMUST00000168954.8 8703 ENSMUST00000182802.7 5621 ENSMUST00000182310.1 540 ENSMUST00000183121.1 380 ENSMUST00000174730.1 3638	ENSMUST00000174641.7 13568 2089aa ENSMUST00000168954.8 8703 1740aa ENSMUST00000182802.7 5621 1740aa ENSMUST00000182310.1 540 180aa ENSMUST00000183121.1 380 91aa ENSMUST00000174730.1 3638 No protein	ENSMUST00000174641.7 13568 2089aa Protein coding ENSMUST00000168954.8 8703 1740aa Protein coding ENSMUST00000182802.7 5621 1740aa Protein coding ENSMUST00000182310.1 540 180aa Protein coding ENSMUST00000183121.1 380 91aa Protein coding ENSMUST00000174730.1 3638 No protein Retained intron	ENSMUST00000174641.7 13568 2089aa Protein coding CCDS57666 ENSMUST00000168954.8 8703 1740aa Protein coding CCDS22951 ENSMUST00000182802.7 5621 1740aa Protein coding CCDS22951 ENSMUST00000182310.1 540 180aa Protein coding - ENSMUST00000183121.1 380 91aa Protein coding - ENSMUST00000174730.1 3638 No protein Retained intron -	ENSMUST00000174641.7 13568 2089aa Protein coding CCDS57666 Q811P8 ENSMUST00000168954.8 8703 1740aa Protein coding CCDS22951 Q811P8 ENSMUST00000182802.7 5621 1740aa Protein coding CCDS22951 Q811P8 ENSMUST00000182310.1 540 180aa Protein coding - S4R2G6 ENSMUST00000183121.1 380 91aa Protein coding - S4R2C0 ENSMUST00000174730.1 3638 No protein Retained intron - -

The strategy is based on the design of Arhgap32-203 transcript, The transcription is shown below



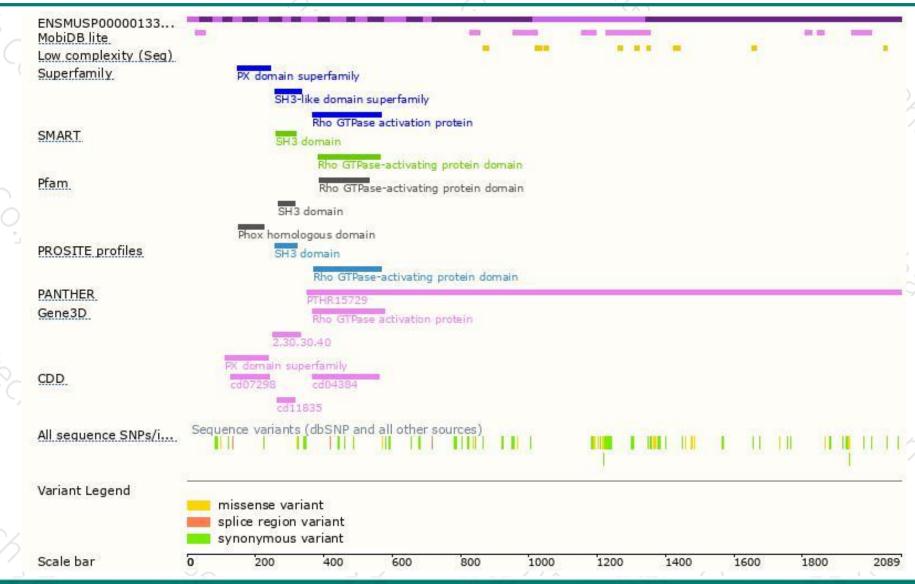
Genomic location distribution





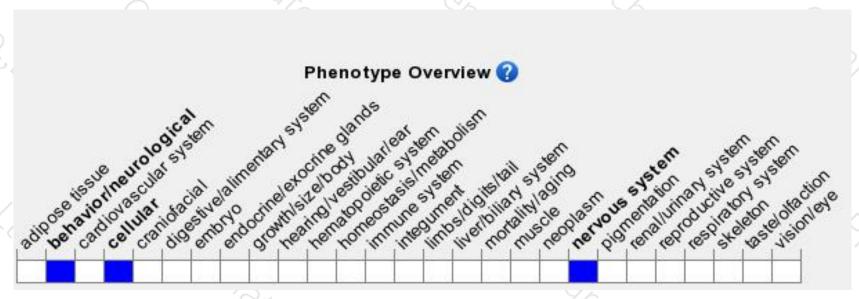
Protein domain





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 null mutation are fertile but display abnormal neurite growth.



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





