

# Arhgap44 Cas9-CKO Strategy

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Reviewer: Huimin Su

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# **Project Overview**



**Project Name** 

Arhgap44

**Project type** 

Cas9-CKO

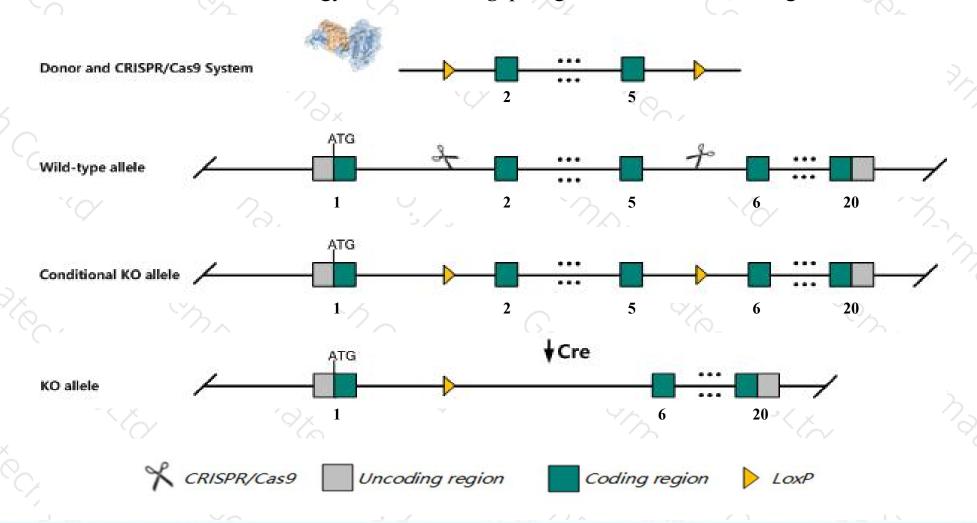
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Arhgap44 gene. The schematic diagram is as follows:



### Technical routes



- The *Arhgap44* gene has 4 transcripts. According to the structure of *Arhgap44* gene, exon2-exon5 of *Arhgap44-201* (ENSMUST00000047463.14) transcript is recommended as the knockout region. The region contains 334bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Arhgap44* 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 knock-out allele exhibit increased brain weight, abnormal dendritic spine morphology, hypoactivity, increased self-grooming behavior, abnormal response to novel objects, and impaired motor learning.
- The KO region contains functional region of the *Gm25991* gene. Knockout the region may affect the function of *Gm25991* gene.
- The *Arhgap44* 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)



#### Arhgap44 Rho GTPase activating protein 44 [Mus musculus (house mouse)]

Gene ID: 216831, updated on 13-Mar-2020

#### Summary

☆ ?

Official Symbol Arhgap44 provided by MGI

Official Full Name Rho GTPase activating protein 44 provided by MGI

Primary source MGI:MGI:2144423

See related Ensembl:ENSMUSG00000033389

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 6330543G20, Al840762, AU040829, AW493732, Rich2

Expression Broad expression in cerebellum adult (RPKM 17.6), frontal lobe adult (RPKM 14.1) and 19 other tissuesSee more

Orthologs <u>human</u> all

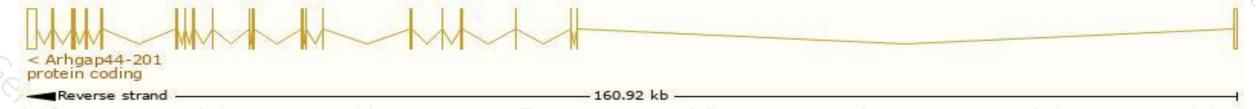
# Transcript information (Ensembl)



The gene has 4 transcripts, all transcripts are shown below:

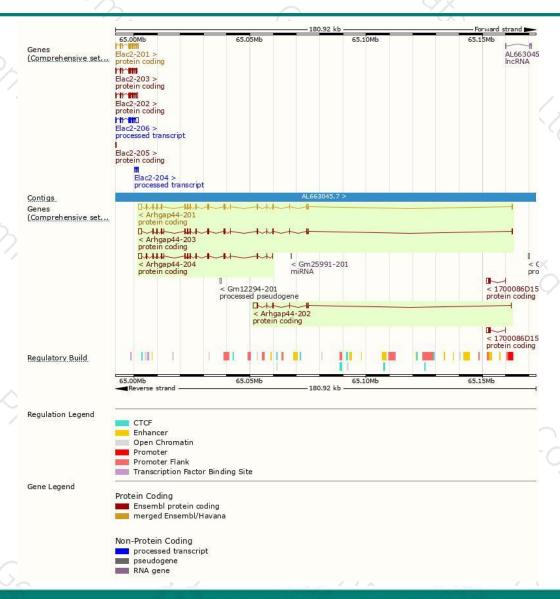
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Arhgap44-201	ENSMUST00000047463.14	4074	764aa	Protein coding	CCDS36180	Q5SSM3	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P2
Arhgap44-203	ENSMUST00000093002.11	3982	<u>814aa</u>	Protein coding	-	Q5SSM3	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Arhgap44-204	ENSMUST00000130420.7	3391	645aa	Protein coding	-	F6T1Y2	CDS 5' incomplete TSL:5
Arhgap44-202	ENSMUST00000093001.4	2256	211aa	Protein coding	- 0	Q5SSM3	TSL:2 GENCODE basic

The strategy is based on the design of Arhgap44-201 transcript, The transcription is shown below



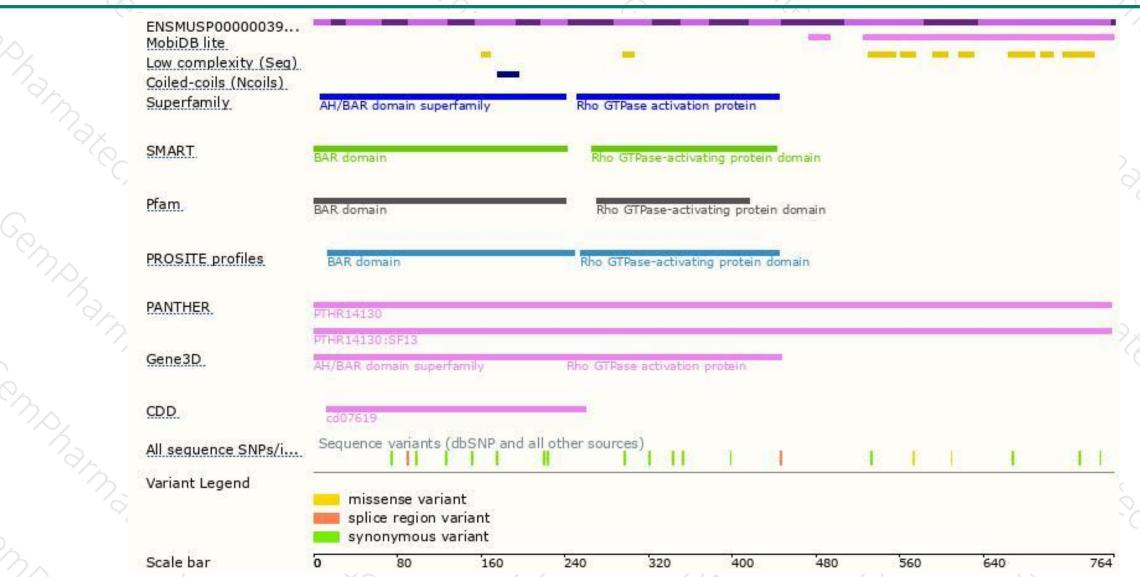
### Genomic location distribution





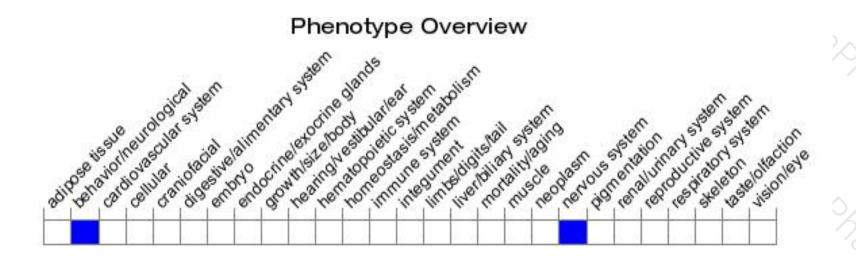
### Protein domain





# 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 brain weight, abnormal dendritic spine morphology, hypoactivity, increased self-grooming behavior, abnormal response to novel objects, and impaired motor learning.



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





