# Arhgef5 Cas9-KO Strategy

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**Reviewer:** Huimin Su

**Design Date:** 2020-3-30

### **Project Overview**



**Project Name** 

Arhgef5

**Project type** 

Cas9-KO

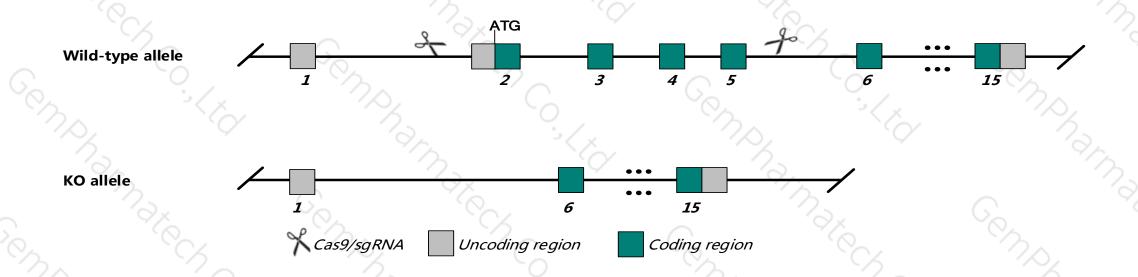
Strain background

C57BL/6JGpt

### **Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the Arhgef5 gene. The schematic diagram is as follows:



### **Technical routes**



- The *Arhgef5* gene has 8 transcripts. According to the structure of *Arhgef5* gene, exon2~exon5 of *Arhgef5*-201 (ENSMUST00000031750.13) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Arhgef5* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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**



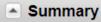
- According to the existing MGI data, mice homozygous for a knock-out allele exhibit decreased Th2 response in an ovalbumin-induced asthma model.
- ➤ The KO region contains functional region of the *Gm44253* gene. Knockout the region may affect the function of *Gm44253* gene.
- The *Arhgef5* gene is located on the Chr6. 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)



#### Arhgef5 Rho guanine nucleotide exchange factor (GEF) 5 [ Mus musculus (house mouse) ]

Gene ID: 54324, updated on 13-Mar-2020





Official Symbol Arhgef5 provided by MGI

Official Full Name Rho guanine nucleotide exchange factor (GEF) 5 provided by MGI

Primary source MGI:MGI:1858952

See related Ensembl: ENSMUSG00000033542

Gene type protein coding
RefSeq status PROVISIONAL
Organism <u>Mus musculus</u>

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae;

Murinae; Mus; Mus

**Also known as** Tim1; AA717842; AW495314; 2210412D05Rik

Expression Broad expression in large intestine adult (RPKM 16.0), placenta adult (RPKM 11.8) and 23 other tissues See more

Orthologs human all

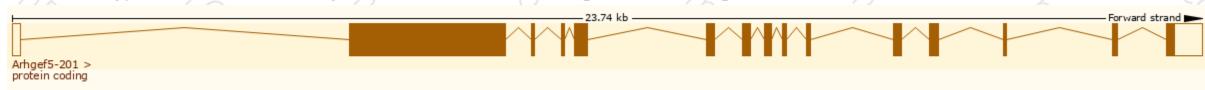
# Transcript information (Ensembl)



The gene has 8 transcripts, and all transcripts are shown below:

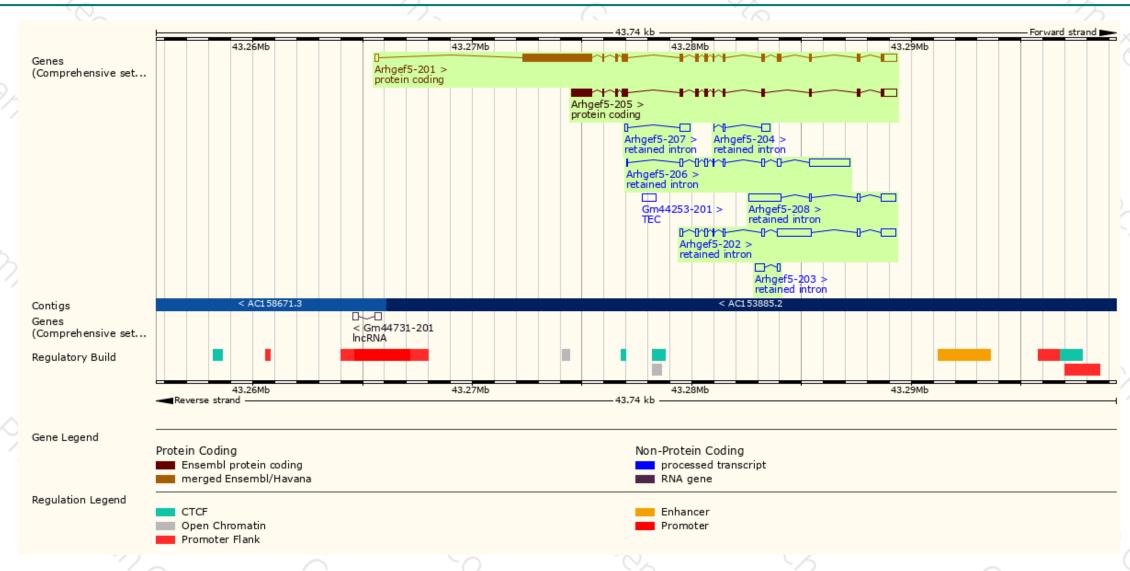
Name 🝦	Transcript ID 🗼	bp 🍦	Protein 🍦	Biotype 🍦	CCDS	UniProt 🍦	Flags -
Arhgef5-201	ENSMUST00000031750.13	5469	<u>1581aa</u>	Protein coding	CCDS51759 ₽	E9Q7D5 ₽	TSL:5 GENCODE basic APPRIS P1
Arhgef5-205	ENSMUST00000182924.1	2938	<u>796aa</u>	Protein coding	-	<u>S4R1F1</u> ₽	CDS 5' incomplete TSL:5
Arhgef5-202	ENSMUST00000182190.7	3098	No protein	Retained intron	-	-	TSL:1
Arhgef5-206	ENSMUST00000183094.7	2777	No protein	Retained intron	-	-	TSL:5
Arhgef5-208	ENSMUST00000183313.1	2319	No protein	Retained intron	-	-	TSL:1
Arhgef5-207	ENSMUST00000183227.1	598	No protein	Retained intron	-	-	TSL:1
Arhgef5-203	ENSMUST00000182647.1	573	No protein	Retained intron	-	-	TSL:2
Arhgef5-204	ENSMUST00000182752.1	519	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Arhgef5*-201 transcript, the transcription is shown below:



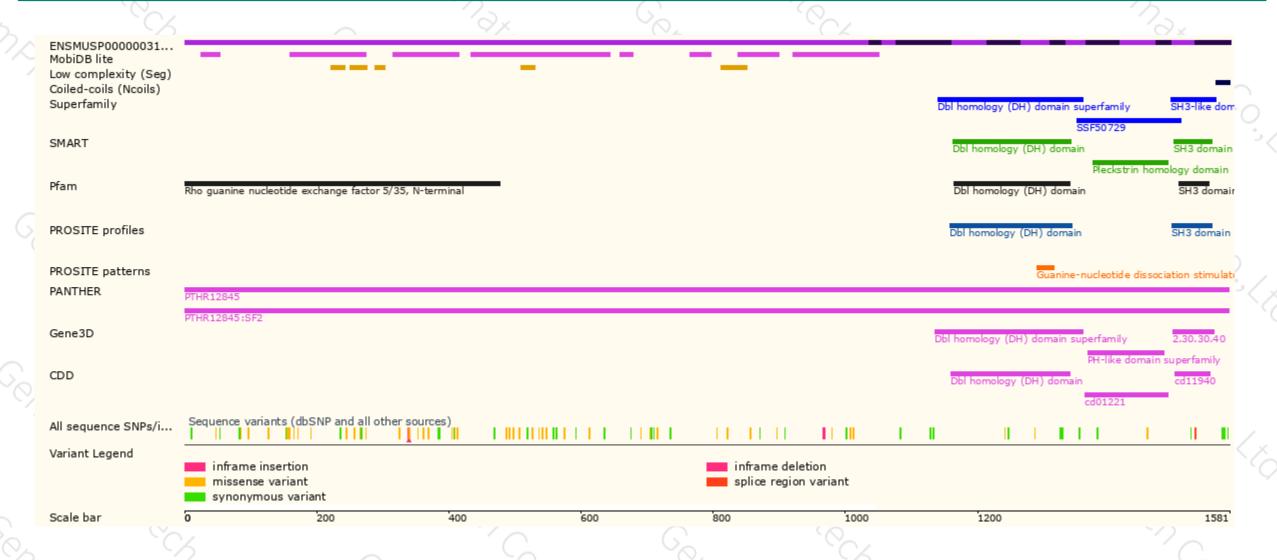
## Genomic location (Ensembl)





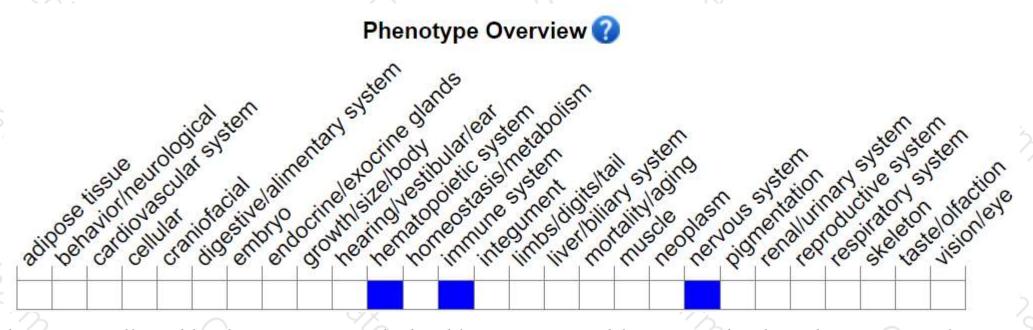
## Protein domain (Ensembl)





### 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 decreased Th2 response in an ovalbumin-induced asthma model.

If you have any questions, you are welcome to inquire. Tel: 025-5864 1534





