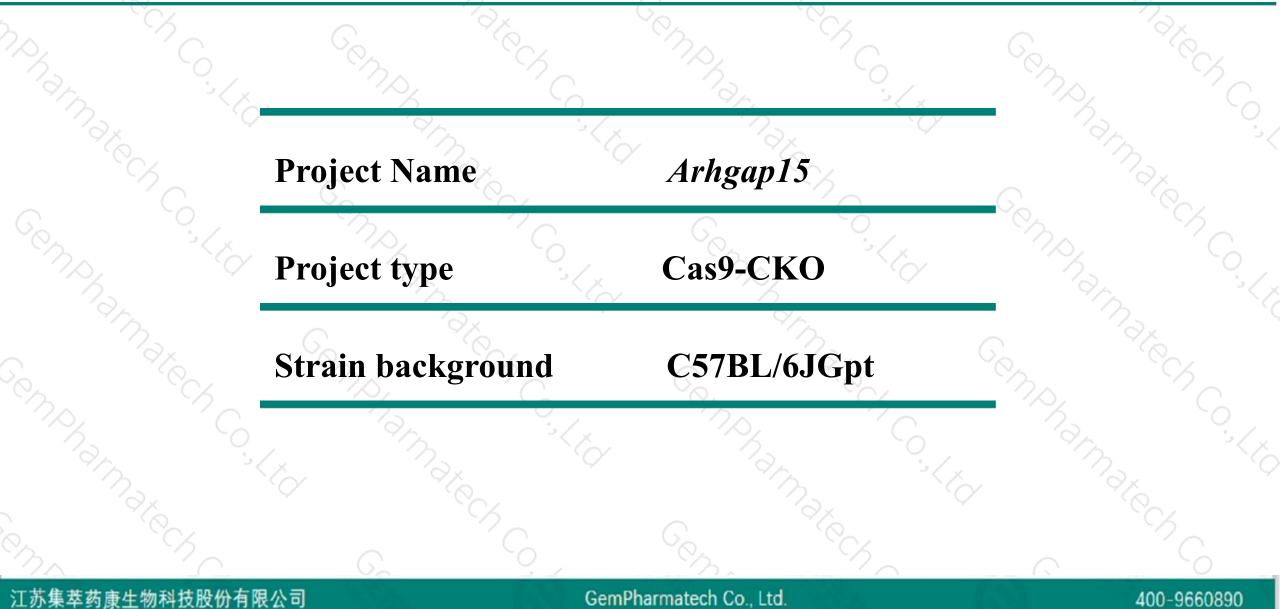


Arhgap15 Cas9-CKO Strategy

Designer: Reviewer: Design Date: Daohua Xu Huimin Su 2020-3-30

Project Overview



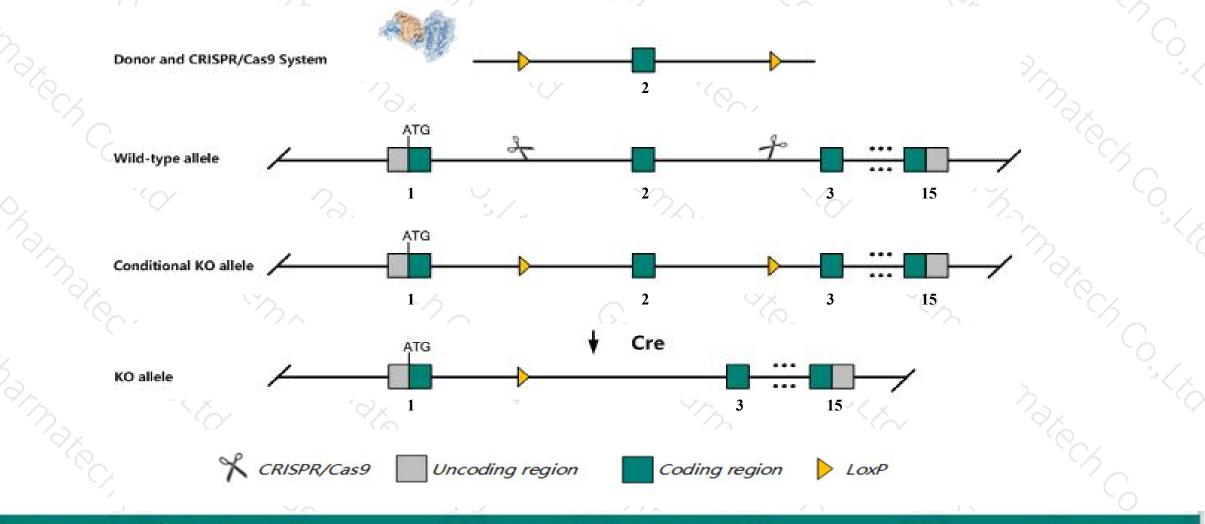


Conditional Knockout strategy



400-9660890

This model will use CRISPR/Cas9 technology to edit the Arhgap15 gene. The schematic diagram is as follows:



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The Arhgap15 gene has 5 transcripts. According to the structure of Arhgap15 gene, exon2 of Arhgap15-203 (ENSMUST00000112824.7) transcript is recommended as the knockout region. The region contains 178bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Arhgap15* 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 disruption of this gene display reduced leukocyte numbers and abnormally shaped macrophage. Chemotactic responses of macrophage are normal while neutrophile chemoattraction and bacterial pagocytosis are increased.
- ➤The Arhgap15 gene is located on the Chr2. 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)



Arhgap15 Rho GTPase activating protein 15 [Mus musculus (house mouse)]

Gene ID: 76117, updated on 13-Mar-2020

Summary

☆ ?

Official Full Name Rho GTPase activating protein 15 provided by MGI Primary source MGI:MGI:1923367 See related Ensembl:ENSMUSG00000049744 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 Also known as 5830480G12Rik Summary The protein encoded by this gene is a RAC GTPase-activating protein that is regulated through its PH domain and by recruitment to the membrane. The protein accelerates hydrolysis of guanosine triphosphate to guanosine diphosphate to repress Rac activity. Knock-out of Arhgap15 function demonstrates that this gene is required to regulate multiple functions in macrophages and neutrophils. Alternative splicing
See related Ensembl:ENSMUSG0000049744 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; Muridae; Mus Also known as 5830480G12Rik Summary The protein encoded by this gene is a RAC GTPase-activating protein that is regulated through its PH domain and by recruitment to the membrane. The protein accelerates hydrolysis of guanosine triphosphate to guanosine diphosphate to repress Rac activity. Knock-out of
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results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Sep 2014]
Expression Broad expression in thymus adult (RPKM 1.0), cortex adult (RPKM 0.9) and 21 other tissues See more
Orthologs human all

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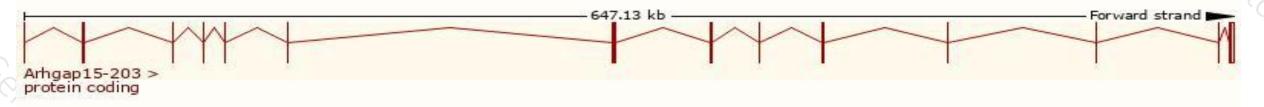
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Transcript information (Ensembl)



Name 🖕	Transcript ID	bp 🖕	Protein 🖕	Biotype .	CCDS 🖕	UniProt B1AW40 ₢	Flags			
Arhgap15-203	ENSMUST00000112824.7	3118	<u>477aa</u>	Protein coding	CCDS79793 ₽		TSL:1 GENCODE basic			
Arhgap15-201	ENSMUST0000055776.7	2604	<u>481aa</u>	Protein coding	<u>CCDS16019</u> &	<u>Q811M1</u> &	TSL:1 GENCODE basic APPRIS P			
Arhgap15-202	ENSMUST00000112822.7	2714	<u>108aa</u>	Nonsense mediated decay	-	<u>Q811M1</u> &	TSL:1			
Arhgap15-205	ENSMUST00000140528.1	4709	No protein	Processed transcript	-		TSL:2			
Arhgap15-204	ENSMUST00000128630.1	397	No protein	Processed transcript		-	TSL:3			

The strategy is based on the design of Arhgap15-203 transcript, The transcription is shown below

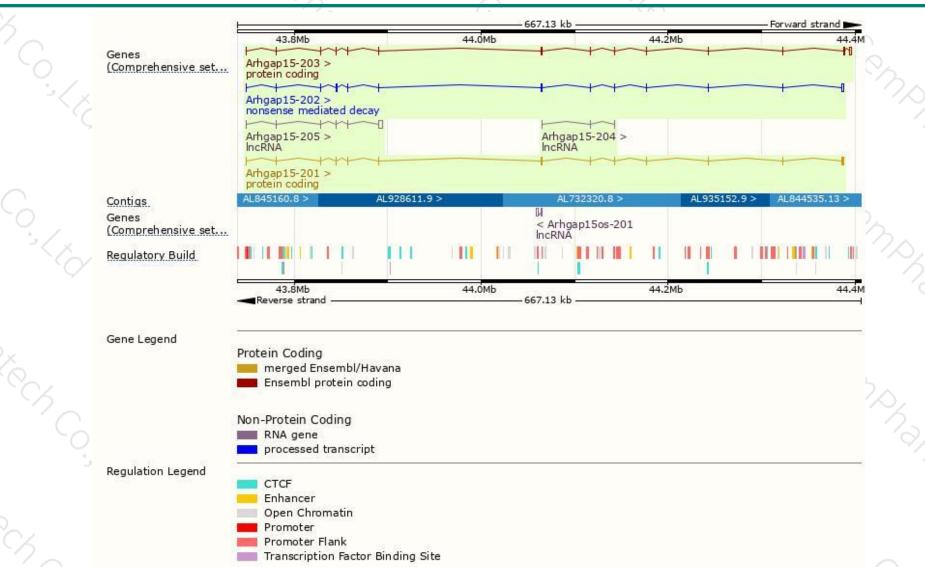


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Genomic location distribution





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Protein domain

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	ENSMUSP00000108	12				12	1000		20					

Mouse phenotype description(MGI)



Phenotype Overview 🕜 cardiovascular system nematopoletics homeostasisim mmune system inerbilary syrenaliumary notaitylagit nenous syste pignentation nearingivest imbeldigitest integument reproductive endocrimelt respiratory neoplasm skeleton ombryo Click cells to view annotations.

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 disruption of this gene display reduced leukocyte numbers and abnormally shaped macrophage. Chemotactic responses of macrophage are normal while neutrophile chemoattraction and bacterial pagocytosis are increased.

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



