

# Arhgap24 Cas9-CKO Strategy

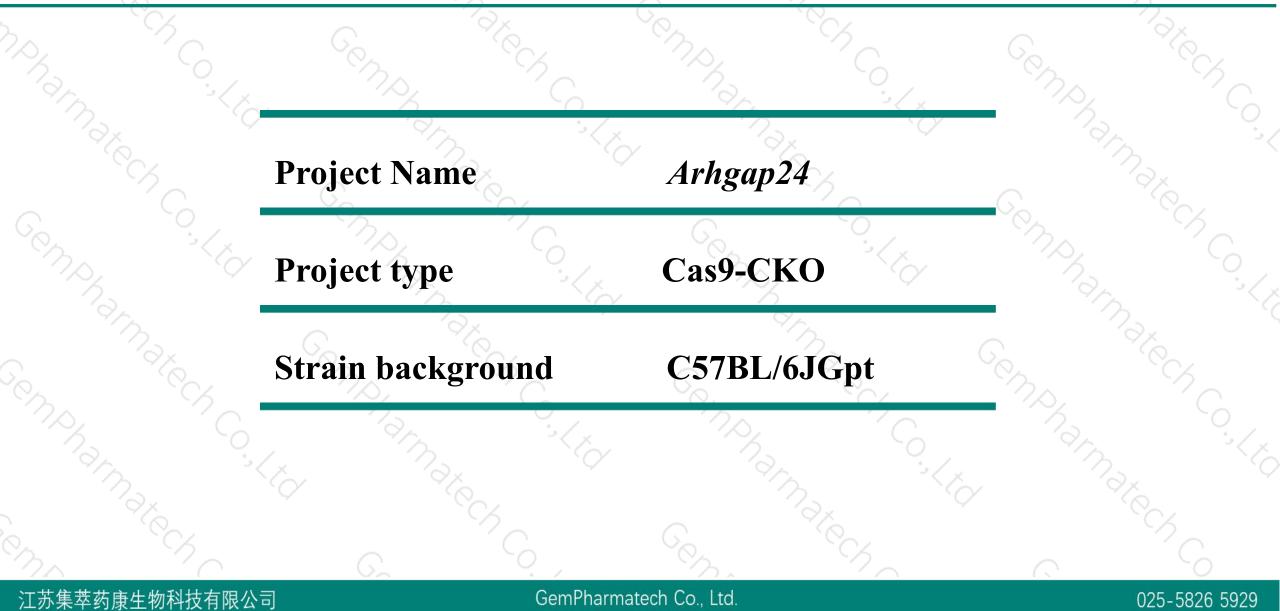
**Designer: Xueting Zhang** 

**Reviewer: Daohua Xu** 

Design Date: 2020-8-13

# **Project Overview**



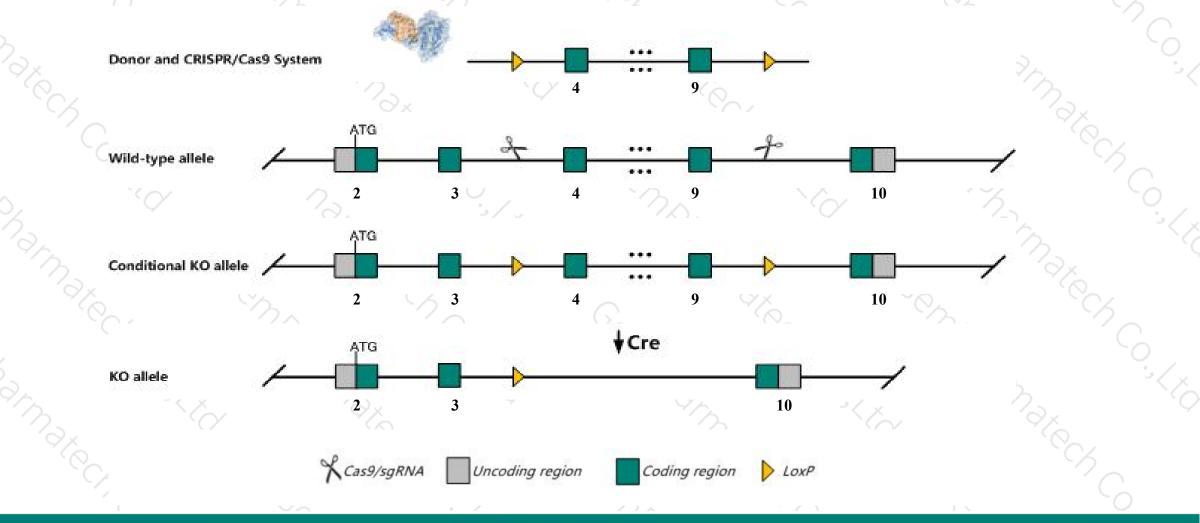


### **Conditional Knockout strategy**



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This model will use CRISPR/Cas9 technology to edit the Arhgap24 gene. The schematic diagram is as follows:



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The Arhgap24 gene has 8 transcripts. According to the structure of Arhgap24 gene, exon4-exon9 of Arhgap24-203(ENSMUST00000094559.8) transcript is recommended as the knockout region. The region contains 1738bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Arhgap24* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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.



- > The *Arhgap24* gene is located on the Chr5. 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.
- Transcript *Arhgap24*-207 may not be affected.
- > 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)**



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#### Arhgap24 Rho GTPase activating protein 24 [Mus musculus (house mouse)]

Gene ID: 231532, updated on 13-Mar-2020

#### Summary

Official Symbol	Arhgap24 provided by MGI
Official Full Name	Rho GTPase activating protein 24 provided by MGI
Primary source	MGI:MGI:1922647
See related	Ensembl:ENSMUSG0000057315
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	0610025G21Rik
Expression	Biased expression in kidney adult (RPKM 13.2), bladder adult (RPKM 1.9) and 11 other tissues See more
Orthologs	human all

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# **Transcript information (Ensembl)**



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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Arhgap24-201	ENSMUST0000070000.5	3632	<u>657aa</u>	Protein coding	CCD584922	D3Z5T4	TSL:5 GENCODE basic	
Arhgap24-203	ENSMUST0000094559.8	3346	<u>747aa</u>	Protein coding	CCDS19474	<u>G3X9N1</u>	TSL:1 GENCODE basic APPRIS P	
Arhgap24-202	ENSMUST0000073302.11	3151	<u>654aa</u>	Protein coding	CCDS19475	<u>Q8C4V1</u>	TSL:1 GENCODE basic	
Arhgap24-206	ENSMUST00000112854.7	2991	<u>654aa</u>	Protein coding	CCDS19475	<u>Q8C4V1</u>	TSL:5 GENCODE basic	
Arhgap24-205	ENSMUST00000112853.7	2853	<u>654aa</u>	Protein coding	CCDS19475	<u>Q8C4V1</u>	TSL:5 GENCODE basic	
Arhgap24-204	ENSMUST00000112852.7	2783	<u>654aa</u>	Protein coding	CCDS19475	<u>Q8C4V1</u>	TSL:1 GENCODE basic	
Arhgap24-208	ENSMUST00000130222.1	632	No protein	Processed transcript			TSL:3	
Arhgap24-207	ENSMUST00000126125.1	4277	No protein	Retained intron	- 27	120	TSL:1	

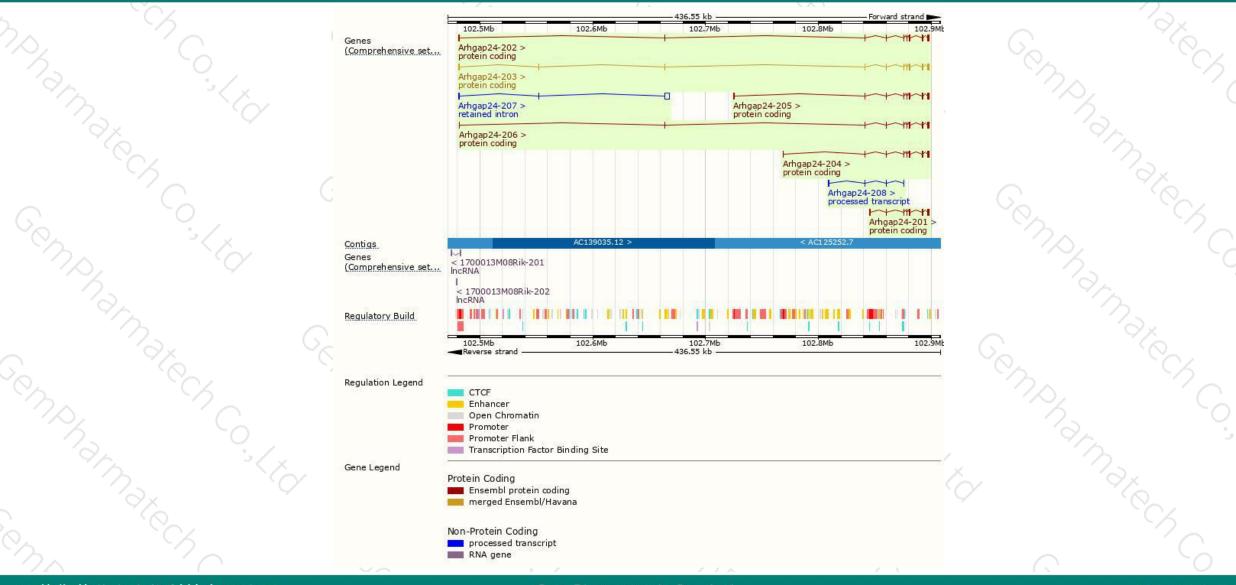
The strategy is based on the design of *Arhgap24-203* transcript, the transcription is shown below:



### **Genomic location distribution**



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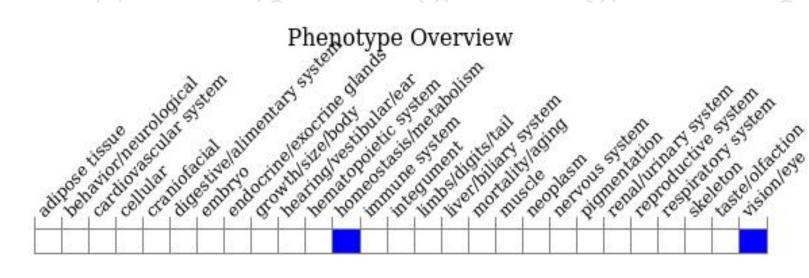
# **Protein domain**



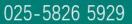
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## Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).





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



