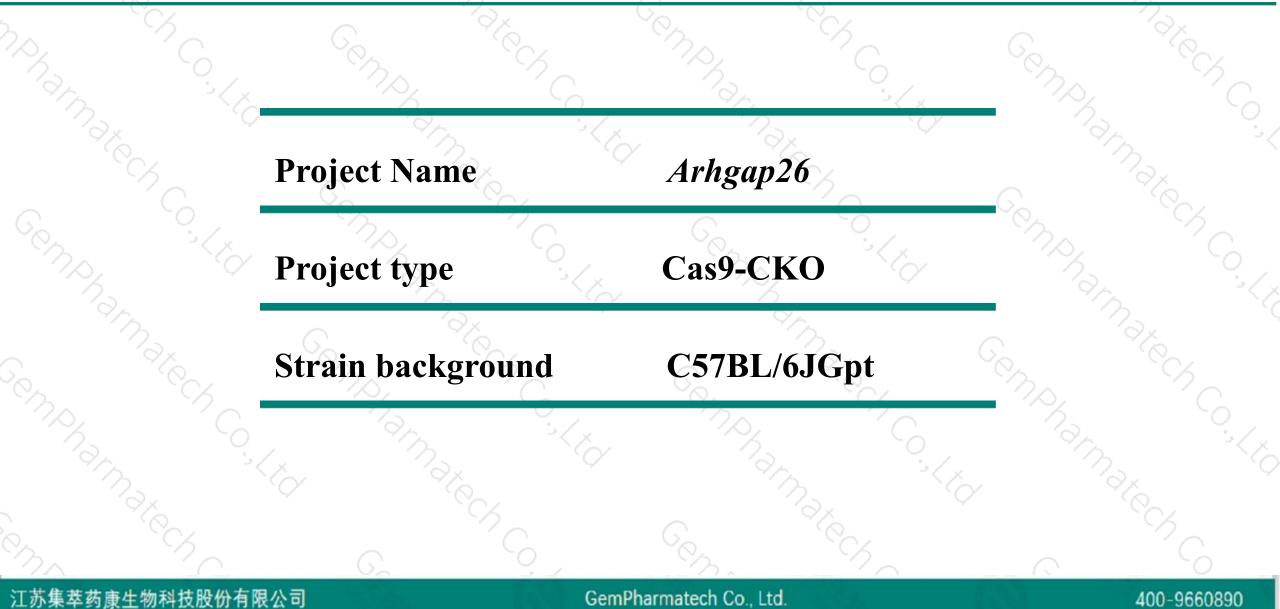


# Arhgap26 Cas9-CKO Strategy

Designer: Reviewer: Design Date: JiaYu Xiaojing Li 2020-3-26

## **Project Overview**

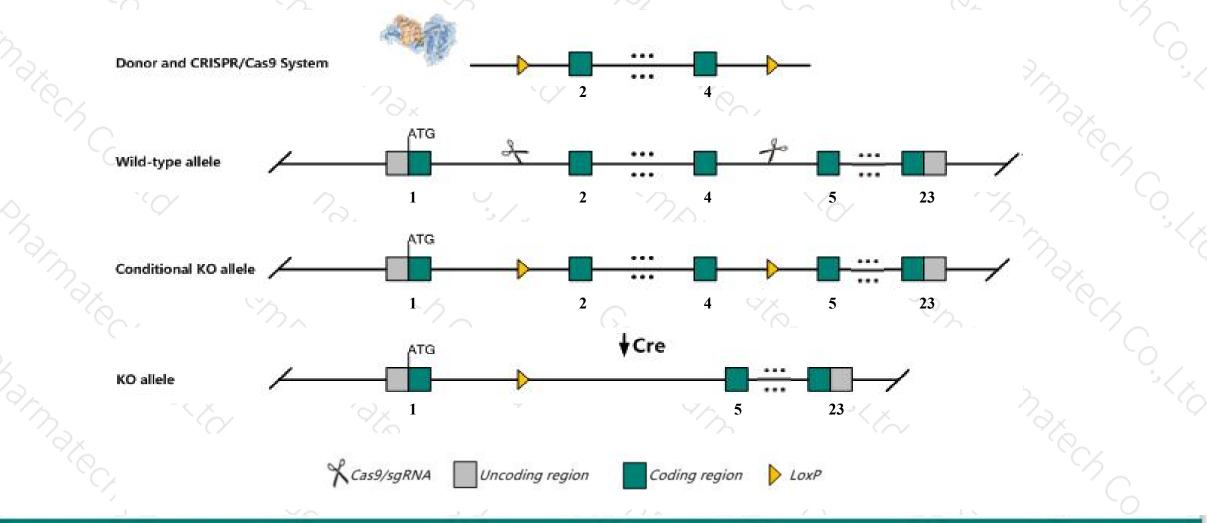




## **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the Arhgap26 gene. The schematic diagram is as follows:



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The Arhgap26 gene has 12 transcripts. According to the structure of Arhgap26 gene, exon2-exon4 of Arhgap26-201 (ENSMUST00000097593.8) transcript is recommended as the knockout region. The region contains 230bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Arhgap26* 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 hypomorphic allele display reduced myofiber size, impaired myoblast fusion and abnormal muscle regeneration.

≻Transcript 206,209,211 CDS 5' incomplete the influences is unknown.

The Arhgap26 gene is located on the Chr18. 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)**



\$ ?

Arhgap26 Rho GTPase activating protein 26 [ Mus musculus (house mouse) ]

Gene ID: 71302, updated on 13-Mar-2020

#### Summary

Official SymbolArhgap26 provided by MGIOfficial Full NameRho GTPase activating protein 26 provided by MGIPrimary sourceMGI:MGI:1918552See relatedEnsembl:ENSMUSG00000036452Gene typeprotein codingRefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;<br/>Myomorpha; Muroidea; Muridae; Musika; 2610010G17Rik; 4933432P15RikAlso known asAl853435; mKIAA0621; 1810044B20Rik; 2610010G17Rik; 4933432P15RikExpressionBroad expression in cerebellum adult (RPKM 9.3), cortex adult (RPKM 5.9) and 23 other tissues See more<br/>human all

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## **Transcript information (Ensembl)**



### The gene has 12 transcripts, all transcripts are shown below:

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Name 🖕	Transcript ID	bp 🖕	Protein 🖕	Biotype	CCDS 🖕	UniProt 🝦	Flags	
Arhgap26-201	ENSMUST0000097593.8	7898	<u>814aa</u>	Protein coding	<u>CCDS29205</u> &	<u>Q6ZQ82</u> &	TSL:5 GENCODE basic APPRIS P2	
Arhgap26-205	ENSMUST00000137497.8	6415	<u>722aa</u>	Protein coding	5	F6T836 &	TSL:1 GENCODE basic APPRIS ALT1	
Arhgap26-209	ENSMUST00000154551.7	2515	<u>388aa</u>	Protein coding	5	F6XTB7团	CDS 5' incomplete TSL:1	
Arhgap26-210	ENSMUST00000155576.7	2280	<u>759aa</u>	Protein coding	5	E9QAQ3립	TSL:5 GENCODE basic APPRIS ALT1	
Arhgap26-206	ENSMUST00000141058.7	607	<u>144aa</u>	Protein coding	5	<u>F6Q7M1</u> 团	CDS 5' incomplete TSL:2	
Arhgap26-211	ENSMUST00000235660.1	524	<u>132aa</u>	Protein coding		<u>A0A494B9M9</u> 교	CDS 5' incomplete	
Arhgap26-208	ENSMUST00000151757.7	529	<u>115aa</u>	Nonsense mediated decay		<u>F7D661</u> &	CDS 5' incomplete TSL:5	
Arhgap26-207	ENSMUST00000148399.1	867	No protein	Processed transcript		17	TSL:1	
Arhgap26-203	ENSMUST00000123820.1	775	No protein	Processed transcript	5	ka.	TSL:5	
Arhgap26-212	ENSMUST00000235817.1	577	No protein	Processed transcript	5	ka.	-	
Arhgap26-204	ENSMUST00000133247.1	556	No protein	Processed transcript		ka.	TSL:1	
Arhgap26-202	ENSMUST00000115574.1	1159	No protein	Retained intron		la <del>x</del>	TSL:1	
				[			15	

The strategy is based on the design of Arhgap26-201 transcript, The transcription is shown below

383.14 kb

Arhgap26-201 > protein coding

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Forward stran

### **Genomic location distribution**

39.0Mb

protein coding

Arhgap26-213 >

protein coding

protein coding

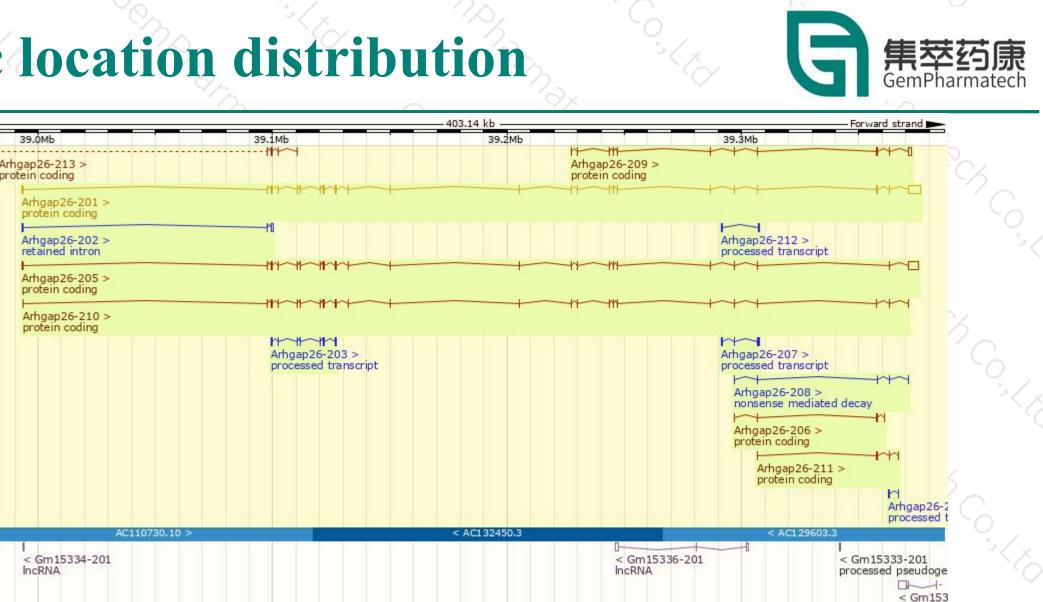
protein coding

IncRNA

39.0Mb

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Reverse strand



Regulatory Build

(Comprehensive set...

Contigs Genes

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Genes

(Comprehensive set...

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39.2Mb

403.14 kb -

39.1Mb

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IncRNA

39.3Mb

## **Protein domain**



										· · · · /	
	ENSMUSP00000095 MobiDB lite Low complexity (Seg) Coiled-coils (Ncoils) Superfamily	AH/BAR domain superfamily	SSF50	729	Rho GTPase a	tivation protein	_		SH3-like dor	nain superfamily	- <sup>2</sup> ~ ~ ~
<	SMART			trin homology doma	in Rho GTPase-ac	ivating protein do	nain			SH3 dom	ain
	Pfam.	PF16746		strin homology dom						SH3 don	
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	PANTHER	Rho GTPase-activating protein 26									- `0
	Gene3D	PTHR12552 AH/BAR domain superfamily	PH-like	domain superfamily	Rho GTPase acti	vation protein				2,30,30,40	
	CDD	GRAF, BAR domain	cd012	140						GRAF, SH	
		GRAF, BAR DOMAIN	20012		04374		_			URAR SH	13 00
	All sequence SNPs/i	Sequence variants (dbSNP and all oth	ner sources)	110		m	$\mathbf{r} = \mathbf{s}$	ш п	a	L II	<u>"</u> C
5	Variant Legend	missense variant synonymous variant		splice region variant							
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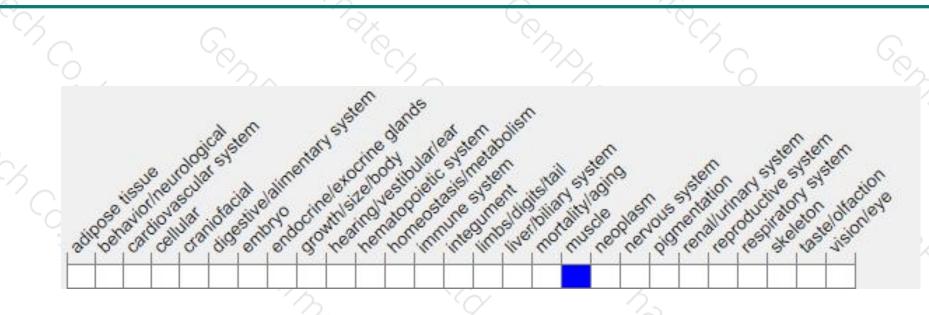
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### Mouse phenotype description(MGI)





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

According to the existing MGI data, Mice homozygous for a hypomorphic allele display reduced myofiber size, impaired myoblast fusion and abnormal muscle regeneration.



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



