

Arf5 Cas9-KO Strategy

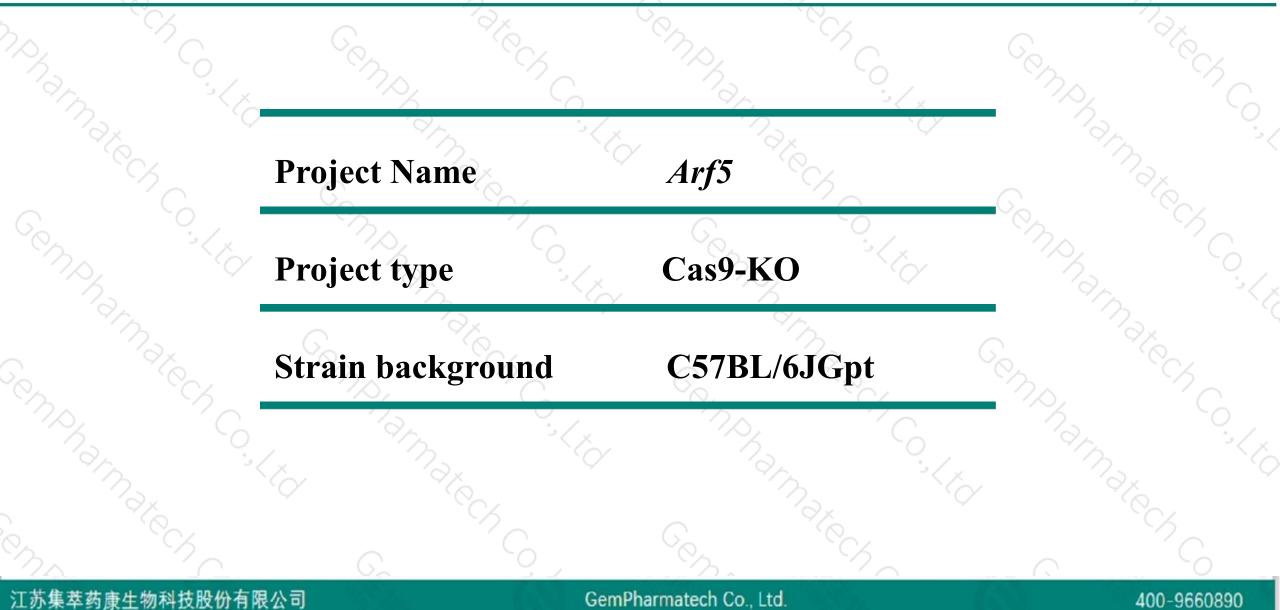
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Reviewer: Jiayuan Yao

Design Date: 2020/11/24

Project Overview

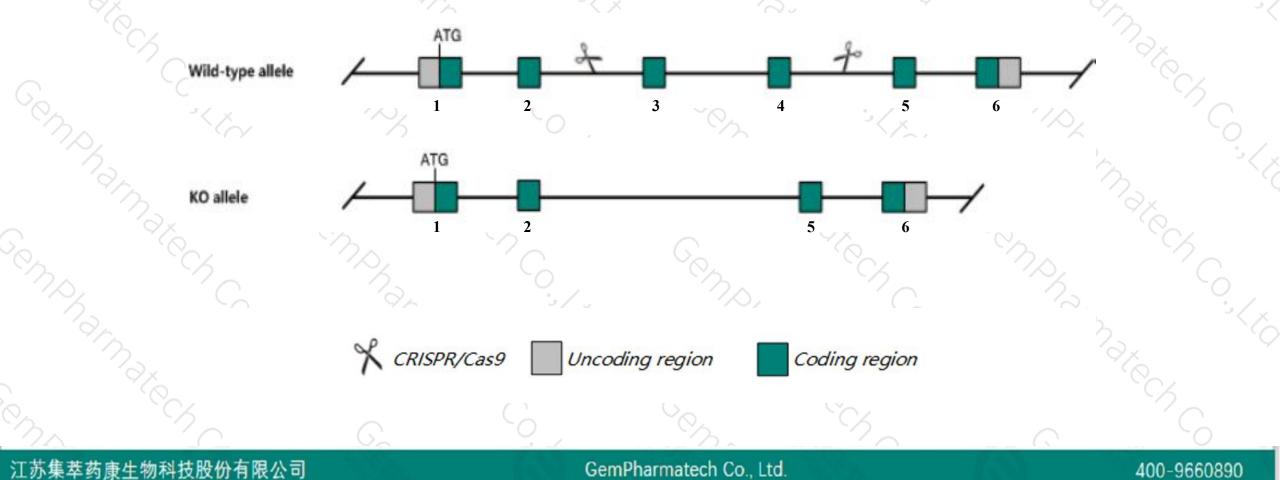




Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Arf5* gene. The schematic diagram is as follows:





> The *Arf5* gene has 3 transcripts. According to the structure of *Arf5* gene, exon3-exon4 of *Arf5*-201(ENSMUST0000020717.11) transcript is recommended as the knockout region. The region contains 182bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Arf5* gene. The brief process is as follows: CRISPR/Cas9 system 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.



According to the existing MGI data,mice homozygous for a null allele exhibit no abnormalities. Mice also heterozygous for an arf4 null allele exhibit impaired coordination, tremors and altered brain wave pattern and Purkinje cell electrophysiology.
The KO region contains functional region of the *Gcc1* gene. Knockout of this region may affect the function of *Gcc1* gene. The KO region is about 2.5kb from the N-terminal of *Fscn3* gene. Knockout of this region may affect the function of *Fscn3* gene.
The *Arf5* 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)



Arf5 ADP-ribosylation factor 5 [Mus musculus (house mouse)]

Gene ID: 11844, updated on 26-Mar-2020

Summary

Official SymbolArf5 provided by MGIOfficial Full NameADP-ribosylation factor 5 provided byMGIPrimary sourceMGI:MGI:99434See relatedEnsembl:ENSMUSG0000020440Gene typeprotein codingRefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
Muroidea; Muridae; Murinae; Mus; MusExpressionUbiquitous expression in duodenum adult (RPKM 584.2), small intestine adult (RPKM 299.8) and 28 other tissues
See more
human all

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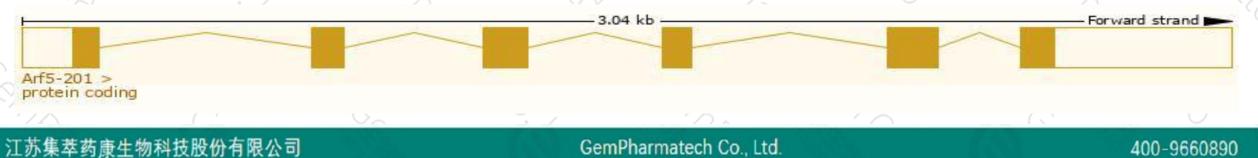
Transcript information (Ensembl)



The gene has 3 transcripts, all transcripts are shown below:

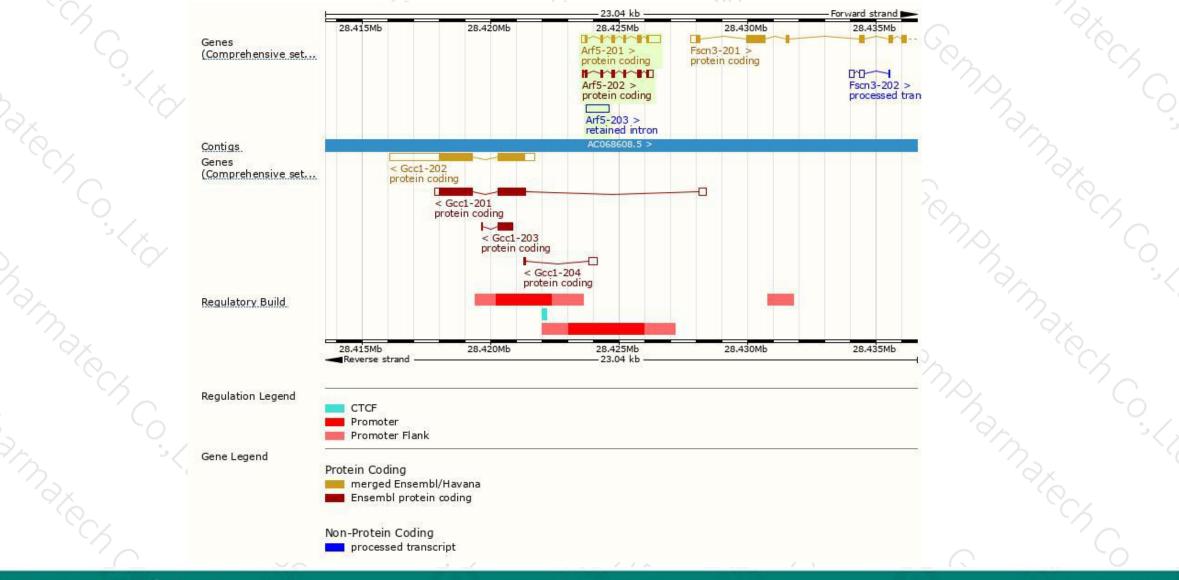
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Arf5-201	ENSMUST0000020717.11	1116	<u>180aa</u>	Protein coding	CCDS19952	P84084	TSL:1 GENCODE basic APPRIS P1
Arf5-202	ENSMUST00000169841.1	768	<u>180aa</u>	Protein coding	CCDS19952	P84084	TSL:5 GENCODE basic APPRIS P1
Arf5-203	ENSMUST00000202028.1	919	No protein	Retained intron	2	-	TSL:NA

The strategy is based on the design of *Arf5-201* transcript, the transcription is shown below:



Genomic location distribution





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Protein domain



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	ENSMUSP00000127 Coiled-coils (Ncoils) TIGRFAM		Small GTP-b	inding protein	domain		2		-		
	Superfamily	1	-loop contain	ing nucleoside	triphosphate	e hydrolase					
	<u>SMART</u>	SM00177	SM00175								C
	Prints	SM00178	Small (TD	ase superfamil							-0
	Pfam.	Small		rfamily, ARF/S		ype					
	PROSITE profiles	PS	51417	(+21), (A)	25672						
	PANTHER	PTHR1171	1							-	
		PTHR1171	1;SF314								2
	Gene3D		3,40,50,30	0							· · ./ .
	CDD		cd04150							-	
	Scale bar	0	20	40	60	80	100	120	140	160 180	5
				S.		Good a		7		° CA	

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



