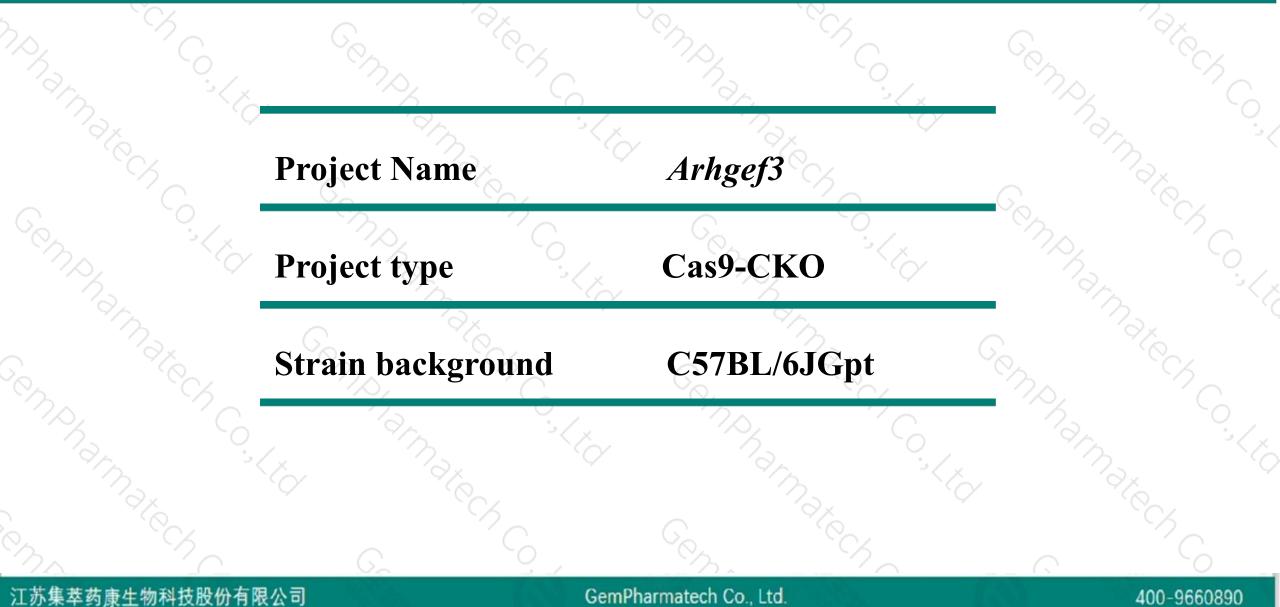


Arhgef3 Cas9-CKO Strategy

Designer: Reviewer: Design Date: JiaYu Xiaojing Li 2020-3-24

Project Overview

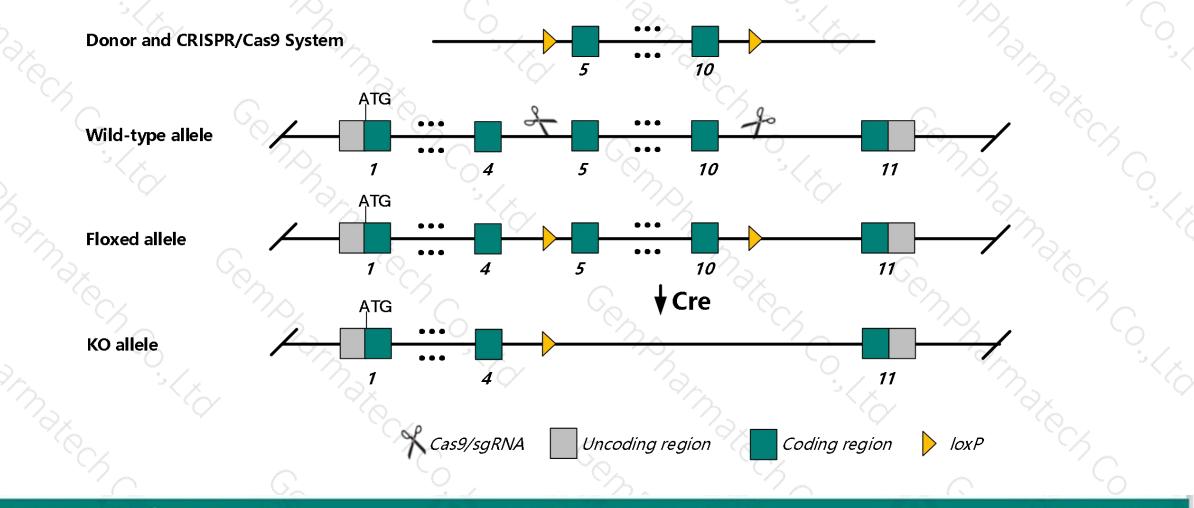




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Arhgef3* gene. The schematic diagram is as follows:



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The Arhgef3 gene has 4 transcripts. According to the structure of Arhgef3 gene, exon5-exon10 of Arhgef3-201 (ENSMUST00000049206.5) transcript is recommended as the knockout region. The region contains 853bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Arhgef3* 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.



> According to the existing MGI data, Mice homozygous for a knock-out allele exhibit increased mean platelet volume and a mild delay in platelet recovery in response to thrombocytopenia.

Some amino acids will remain at the N-terminus and some functions may be retained.

- The Arhgef3 gene is located on the Chr14. 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.

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Gene information (NCBI)



\$?

Arhgef3 Rho guanine nucleotide exchange factor (GEF) 3 [Mus musculus (house mouse)]

Gene ID: 71704, u	updated on	13-Mar-2020
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Summary

Official SymbolArhgef3 provided by MGIOfficial Full NameRho guanine nucleotide exchange factor (GEF) 3 provided by MGIPrimary sourceMGI:MGI:1918954See relatedEnsembl:ENSMUSG0000021895Gene typeprotein codingRefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
Myomorpha; Muroidea; Murinae; Mus; MusAlso known asXpln; C76747; 1200004124Rik; 9830169H03RikExpressionUbiquitous expression in lung adult (RPKM 12.9), bladder adult (RPKM 8.6) and 28 other tissues See more
human all

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Transcript information (Ensembl)



The gene has 4 transcripts, all transcripts are shown below:

Name 🍦	Transcript ID 🖕	bp 🖕	Protein 🖕	Biotype 👙	CCDS 🖕	UniProt 🖕	Flags 🖕	
Arhgef3-201	ENSMUST0000049206.5	3585	<u>531aa</u>	Protein coding	CCDS26886 伊	Q91X46@	TSL:1 GENCODE basic APPRIS P2	
Arhgef3-202	ENSMUST00000224981.1	3 <mark>618</mark>	<u>524aa</u>	Protein coding	100	A0A2X0SFN9@Q91X46@	GENCODE basic APPRIS ALT1	
Arhgef3-204	ENSMUST00000225949.1	3198	<u>551aa</u>	Protein coding	100	A0A286YDE6@	GENCODE basic	
Arhgef3-203	ENSMUST00000225494.1	572	No protein	Processed transcript	100	1.54		

The strategy is based on the design of Arhgef3-201 transcript, The transcription is shown below

165.87 kb

Arhgef3-201 > protein coding

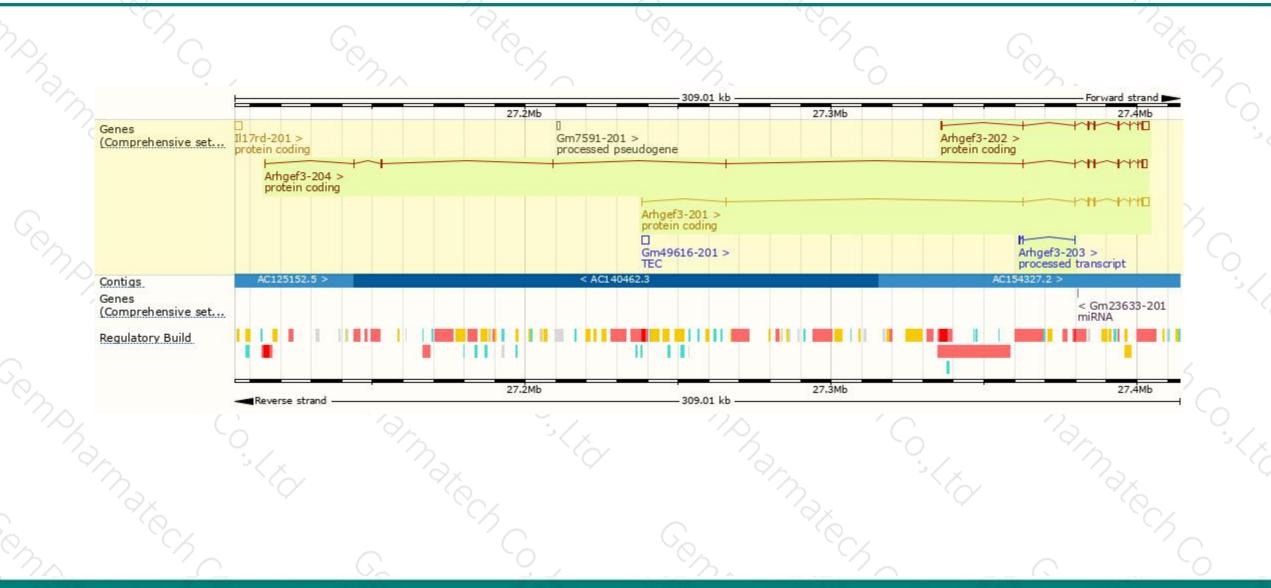
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Forward strand

Genomic location distribution



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Protein domain



3~	5	6			<u>`</u> ?	5	\sim	<u> </u>
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	Pfam PROSITE profiles		Dbl homology (DH) dom	ain	Pleckstrin homolo	ogy domain	_	
Con	PROSITE patterns PANTHER	PTHR46006	Dbl homology (DH) domai		ne-nucleotide dissociation	n stimulator, CDC24, conserved si	te	×S
	Gene3D	PTHR46006:SF2	Dbl homology (DH) domain supe	erfamily	PH-lik)	e domain superfamily		
	CDD		Dbl homology (DH) domai	in	cd1057.	2	-	
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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 knock-out allele exhibit increased mean platelet volume and a mild delay in platelet recovery in response to thrombocytopenia.

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



