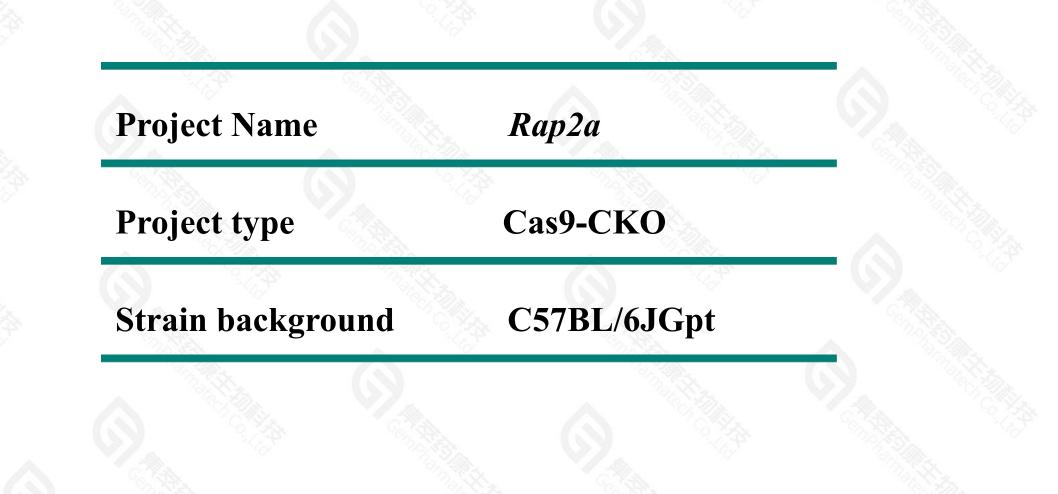


Rap2a Cas9-CKO Strategy

Designer: Reviewer: Design Date: Daohua Xu Huimin Su 2019-11-14

Project Overview





江苏集萃药康生物科技股份有限公司。

GemPharmatech Co., Ltd.

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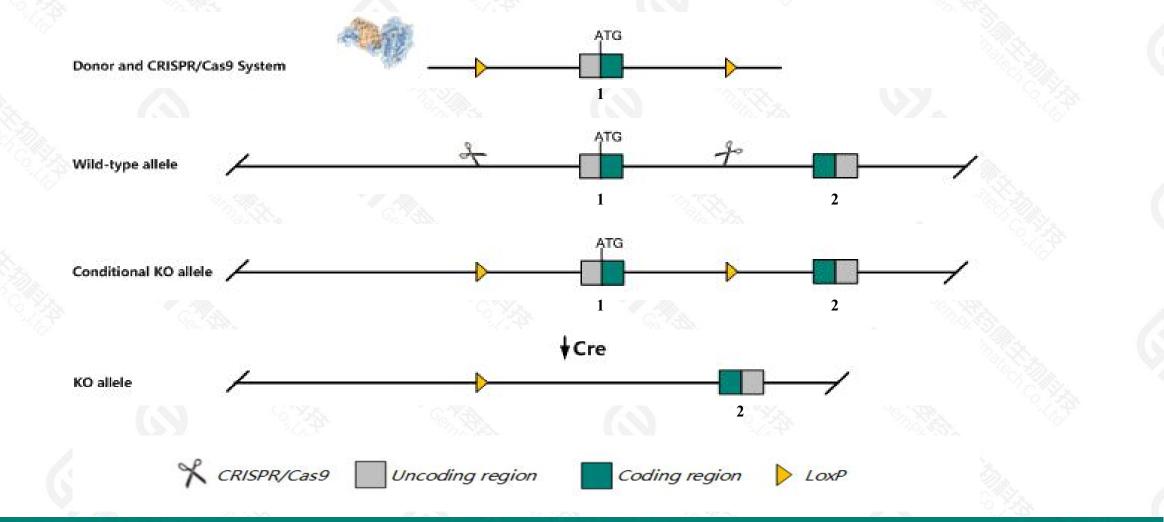
Conditional Knockout strategy

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This model will use CRISPR/Cas9 technology to edit the Rap2a gene. The schematic diagram is as follows:



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Technical routes



> The *Rap2a* gene has 1 transcript. According to the structure of *Rap2a* gene, exon1 of *Rap2a*-201(ENSMUST00000062117.14) transcript is recommended as the knockout region. The region contains start codon ATG.Knock out the region will result in disruption of protein function.

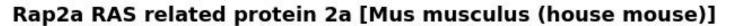
> In this project we use CRISPR/Cas9 technology to modify *Rap2a* gene. The brief process is as follows: CRISPR/Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. 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.



- > The Rap2a 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.

Gene information (NCBI)



Gene ID: 76108, updated on 3-Jan-2021

Summary

Official Symbol	Rap2a provided by MGI
Official Full Name	RAS related protein 2a provided by MGI
Primary source	MGI:MGI:97855
See related	Ensembl:ENSMUSG00000051615
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
	Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	5830461H18Rik
Expression	Ubiquitous expression in lung adult (RPKM 22.3), frontal lobe adult (RPKM 18.8) and 28 other tissues See more
Orthologs	human all

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Transcript information (Ensembl)

The gene has 1 transcript, and the transcript is shown below:

Name		Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
	Rap2a-201	ENSMUST0000062117.14	4191	<u>183aa</u>	Protein coding	CCDS27341		TSL:1, GENCODE basic, APPRIS P1,	

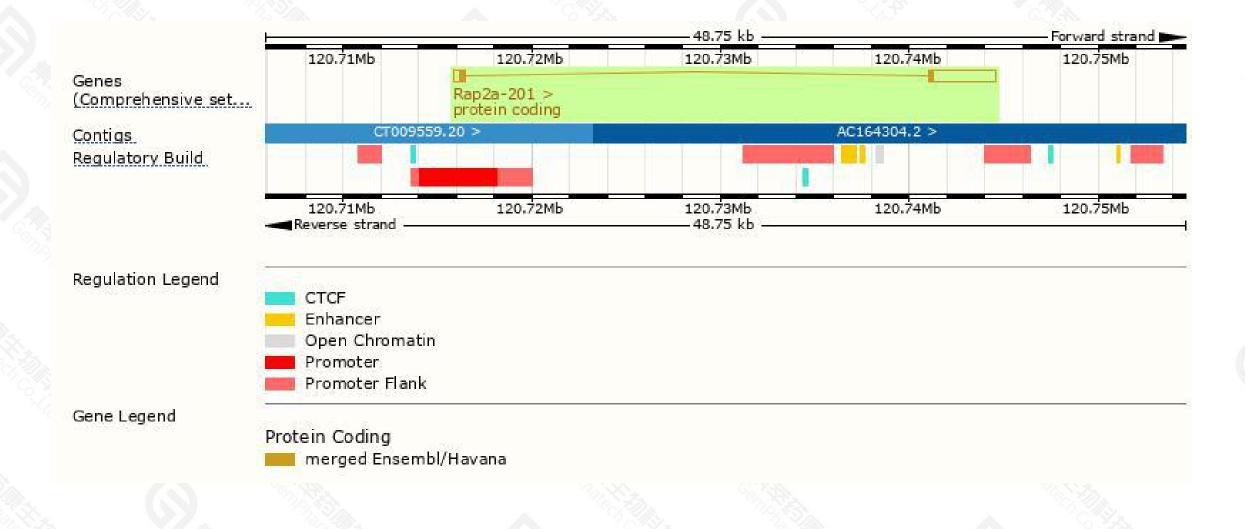
The strategy is based on the design of *Rap2a-201* transcript, the transcription is shown below:

	 Forward strand				
Rap2a-201 2					
Rap2a-201 > protein codir	ng				
Sec. The sec.	100	10000	50 M.S.	Star Star	1. Z 1. 1 5

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Genomic location distribution





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Protein domain



	(5) NAX		1 A 10	10.5255	10,385	C		57.5		
0	20	40	60	80	100	120	140	160	183	
syr	ionymous va	riant								
Sequen	ce variants (d	IbSNP and a	ll other source	s)				140		
	Ras-related	protein Rap2								
3,40,50,5	300							#		
Small GTPase superfamily, Ras-type										
and the second s	and an and a strategy									
Small GT	Pase superfam	nily, Ras-type								
Contraction of the	No. of Concession, Name				-					
COLUMN STATE							-	-		
1										
SM00173	3									
SM	00176								-	
P-loop c	ontaining nucle	eoside triphos	phate hydrolase	9				-		
Small GT	P-binding prot	ein domain						-		
-			WARNER.		_				-C.X. X-	
	Small GT P-loop c SM SM00173 SM001 SM001 SM001 PR004 Small GT Small GT 3,40,50,3 Sequent Sequent	Small GTP-binding prot P-loop containing nucl SM00176 SM00173 SM00174 SM00175 PR00449 Small GTPase Small GTPase superfam PTHR24070;SF221 Small GTPase superfam 3,40,50,300 Ras-related Sequence variants (or	Small GTP-binding protein domain P-loop containing nucleoside triphos SM00176 SM00173 SM00174 SM00175 PR00449 Small GTPase Small GTPase superfamily, Ras-type PTHR24070;SF221 Small GTPase superfamily, Ras-type 3,40,50,300 Ras-related protein Rap2 Sequence variants (dbSNP and a	Small GTP-binding protein domain P-loop containing nucleoside triphosphate hydrolase SM00176 SM00173 SM00174 SM00175 PR00449 Small GTPase Small GTPase superfamily, Ras-type PTHR24070;SF221 Small GTPase superfamily, Ras-type 3.40.50.300 Ras-related protein Rap2 Sequence variants (dbSNP and all other source	Small GTP-binding protein domain P-loop containing nucleoside triphosphate hydrolase SM00176 SM00173 SM00174 SM00175 PR00449 Small GTPase Small GTPase superfamily, Ras-type PTHR24070;SF221 Small GTPase superfamily, Ras-type 3,40,50,300 Ras-related protein Rap2 Sequence variants (dbSNP and all other sources)	Small GTP-binding protein domain P-loop containing nucleoside triphosphate hydrolase SM00176 SM00173 SM00174 SM00175 PR00449 Small GTPase Small GTPase Small GTPase superfamily, Ras-type PTHR24070;SF221 Small GTPase superfamily, Ras-type 3,40,50,300 Ras-related protein Rap2 Sequence variants (dbSNP and all other sources)	Small GTP-binding protein domain P-bop containing nucleoside triphosphate hydrolase SM00176 SM00173 SM00174 SM00174 SM00175 PR00449 Small GTPase Small GTPase Small GTPase superfamily, Ras-type PTHR24070:SF221 Small GTPase superfamily, Ras-type 3.40.50.300 Ras-related protein Rap2 Sequence variants (dbSNP and all other sources)	Small GTP-binding protein domain P-loop containing nucleoside triphosphate hydrolase SM00176 SM00173 SM00174 SM00175 PR00449 Small GTPase Small GTPase Small GTPase superfamily, Ras-type PTHR240701SF221 Small GTPase superfamily, Ras-type 3.40, S0, 300 Ras-related protein Rap2 Sequence variants (dbSNP and all other sources)	Small GTP-binding protein domain P-loop containing nucleoside triphosphate hydrolase SMD0176 SM00173 SM00174 SM00175 PR00449 Small GTPase Small GTPase Small GTPase superfamily, Ras-type PTHR24070:SF221 Small GTPase superfamily, Ras-type 3:40.50.300 Ras-related protein Rap2 Sequence variants (dbSNP and all other sources)	Small GTP-binding protein domain P-loop containing nucleoside triphosphate hydrolase SM00176 SM00173 SM00174 SM00175 PR00449 Small GTPase superfamily, Ras-type PTHR24070:SF221 Small GTPase superfamily, Ras-type 3.40.50.300 Ras-related protein Rap2 Sequence variants (dbSNP and all other sources)



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



