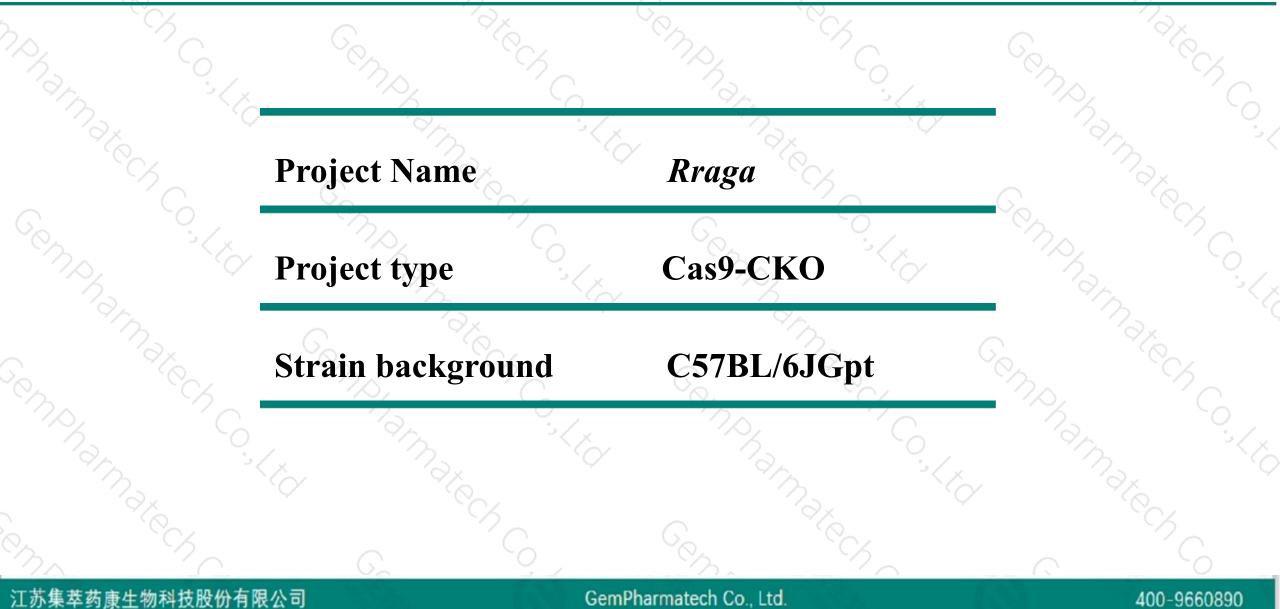


Rraga Cas9-CKO Strategy

Designer: Yanhua Shen Reviewer: Xueting Zhang Design Date: 2020-3-12

Project Overview

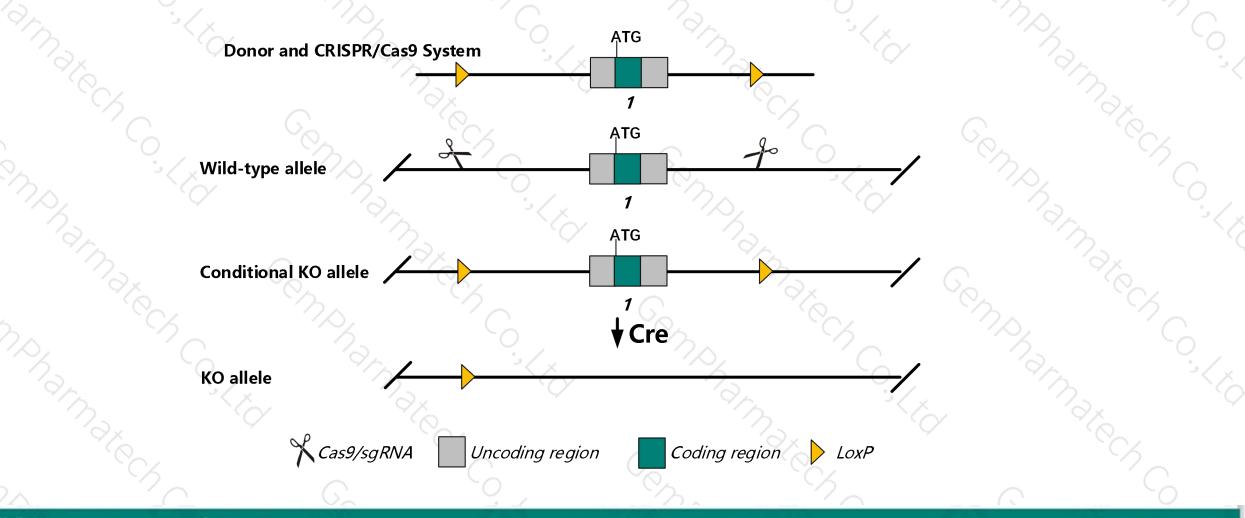




Conditional Knockout strategy



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



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The *Rraga* gene has 1 transcript. According to the structure of *Rraga* gene, exon1 of *Rraga-201* (ENSMUST00000091064.7) transcript is recommended as the knockout region. The region contains all of coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Rraga* 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 null mutations display embryonic lethality during organogenesis, impaired growth and open neural tubes. Mice homozygous for a constitutively active knock-in allele exhibit neonatal lethality associated with impaired hepatic gluconeogenesis and impaired autophagy.
- The *Rraga* gene is located on the Chr4. 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)



Rraga Ras-related GTP binding A [Mus musculus (house mouse)]

Gene ID: 68441, updated on 27-Feb-2020

Summary

< ?

Official SymbolRraga provided by MGIOfficial Full NameRas-related GTP binding A provided by MGIPrimary sourceMGI:MGI:1915691See relatedEnsembl:ENSMUSG0000070934Gene typeprotein codingRefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
Myomorpha; Muroidea; Muridae; Musimae; Mus; MusAlso known asRAGA; FIP-1; Al255374; 1300010C19Rik
human all

Genomic context

Location: 4; 4 C4

Exon count:

☆ ?

400-9660890

See Rraga in Genome Data Viewer

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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	4
Rraga-201	ENSMUST0000091064.7	1618	<u>313aa</u>	Protein coding	<u>CCDS18306</u> @	<u>Q80X95</u> @	TSL:NA	GENCODE basic	APPRIS P1

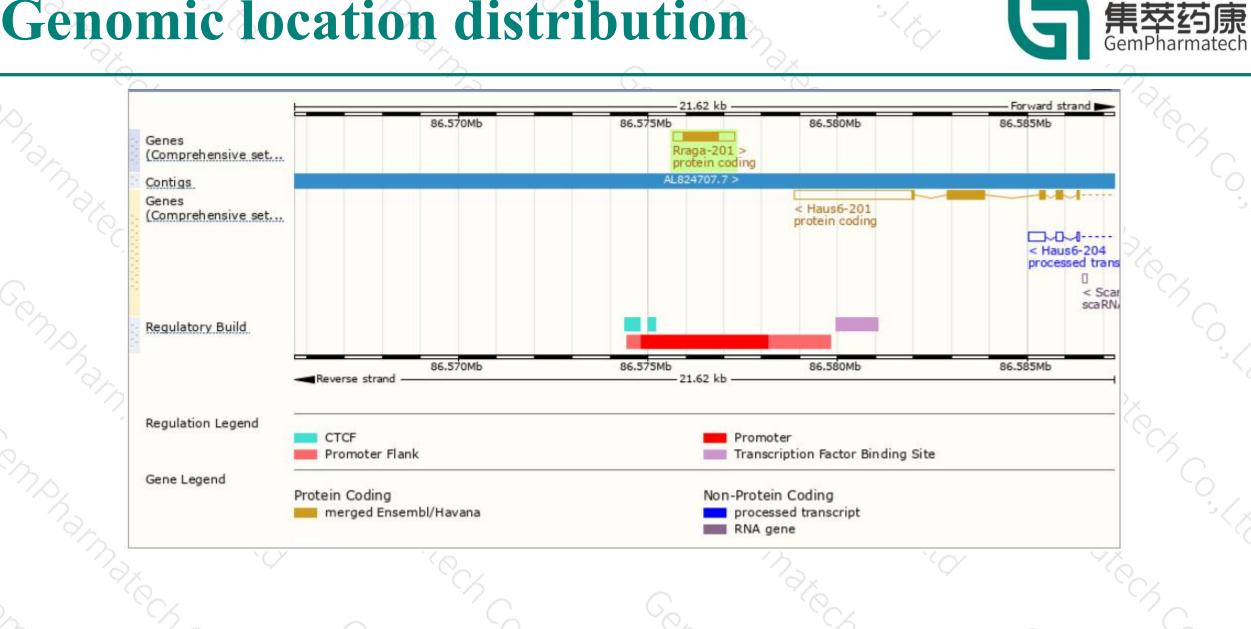
The strategy is based on the design of *Rraga-201* transcript, The transcription is shown below

Rraga-201 > protein coding		1.62 kb	 Forward strand 🗩
traga-201 > protein coding			
raga-201 > rotein coding			
protein coding	raga-201 >		
	otein coding		

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Genomic location distribution



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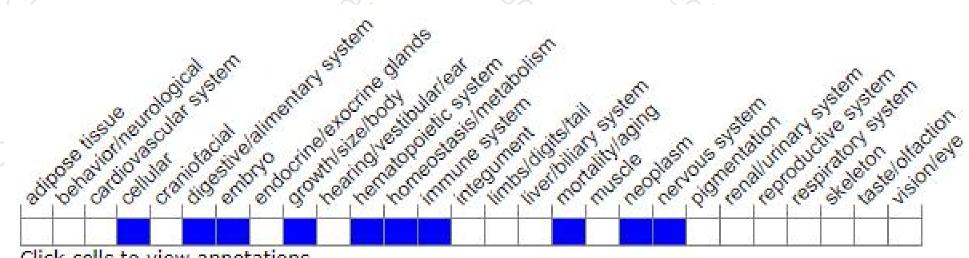
Protein domain



0	Co.				\sim	``CA
ENSMUSP0000088 Superfamily	P-loop containing nu	ucleoside triphosphate hydrolase	*			
Pfam.	Gtr1/RagA G proteir					
PANTHER	PTHR11259:SF7					
18 (A)	Gtr1/RagA G protein					
Gene3D	3.40.50.300			3.30.450.190		
CDD.	Alexandra and a second s					1 20
	RagA/B	bSNP and all other sources)				6
All sequence SNPs/i	Sequence variants (o	DSNP and all other sources)		1.		
Variant Legend	synonymous va	riant				
Scale bar	o 40	80	120 160	200	240	313
TRA C	Support	SG.	Cons,	ALC C	Choho	e contra
~~~~		í G		°°C/		6

## Mouse phenotype description(MGI)





Click cells to view annotations.

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 null mutations display embryonic lethality during organogenesis, impaired growth and open neural tubes. Mice homozygous for a constitutively active knock-in allele exhibit neonatal lethality associated with impaired hepatic gluconeogenesis and impaired autophagy.

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



