

Rab5a Cas9-CKO Strategy

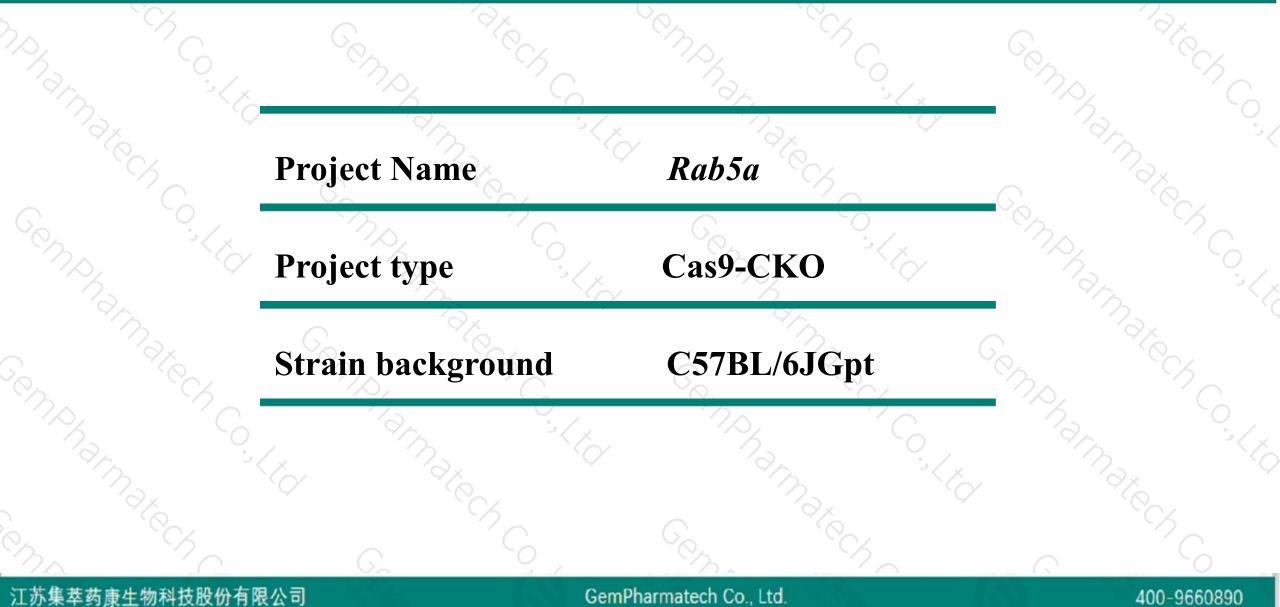
Designer: Reviewer:

Design Date:

Daohua Xu Huimin Su 2019-9-28

Project Overview

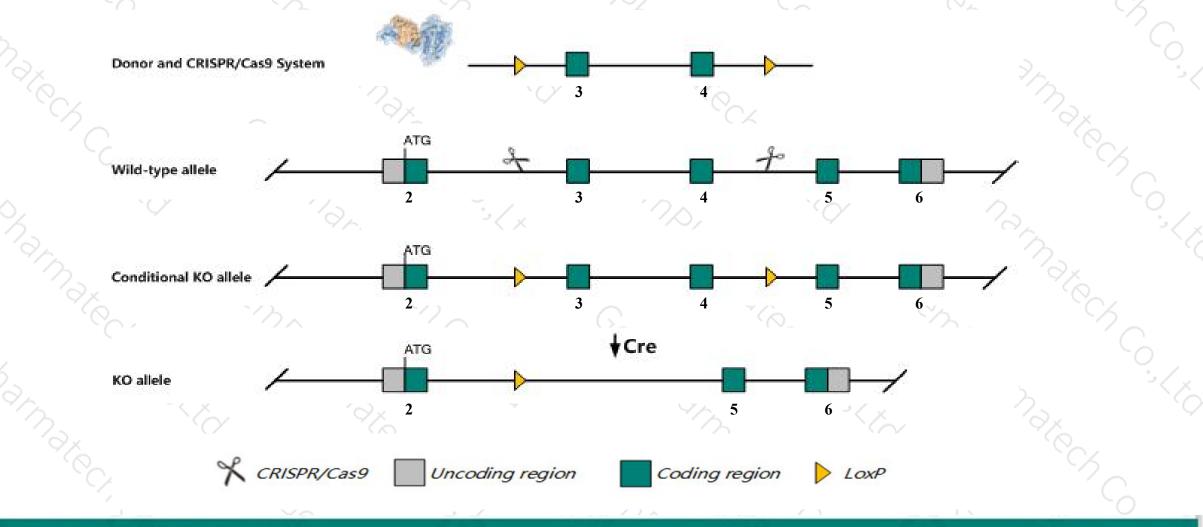




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Rab5a* gene. The schematic diagram is as follows:



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The Rab5a gene has 2 transcripts. According to the structure of Rab5a gene, exon3-exon4 of Rab5a-201 (ENSMUST00000017975.6) transcript is recommended as the knockout region. The region contains 275bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Rab5a* 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 Rab5a gene is located on the Chr17. 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)



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Rab5a RAB5A, member RAS oncogene family [Mus musculus (house mouse)]

Gene ID: 271457, updated on 7-Apr-2019

Summary

Official Symbol	Rab5a provided by MGI
Official Full Name	RAB5A, member RAS oncogene family provided by MGI
Primary source	MGI:MGI:105926
See related	Ensembl:ENSMUSG00000017831
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	2410015H04Rik, Al663973, AU021172, nnyRab5a
Expression	Broad expression in CNS E11.5 (RPKM 27.6), CNS E18 (RPKM 24.0) and 25 other tissues See more
Orthologs	human all

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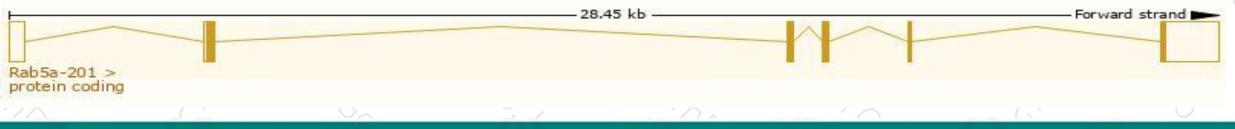
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The gene has 2 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rab5a-201	ENSMUST00000017975.6	2366	<u>215aa</u>	Protein coding	CCDS28879	Q9CQD1	TSL:1 GENCODE basic APPRIS P1
Rab5a-202	ENSMUST00000233347.1	3226	No protein	Retained intron	-8		

The strategy is based on the design of Rab5a-201 transcript, The transcription is shown below

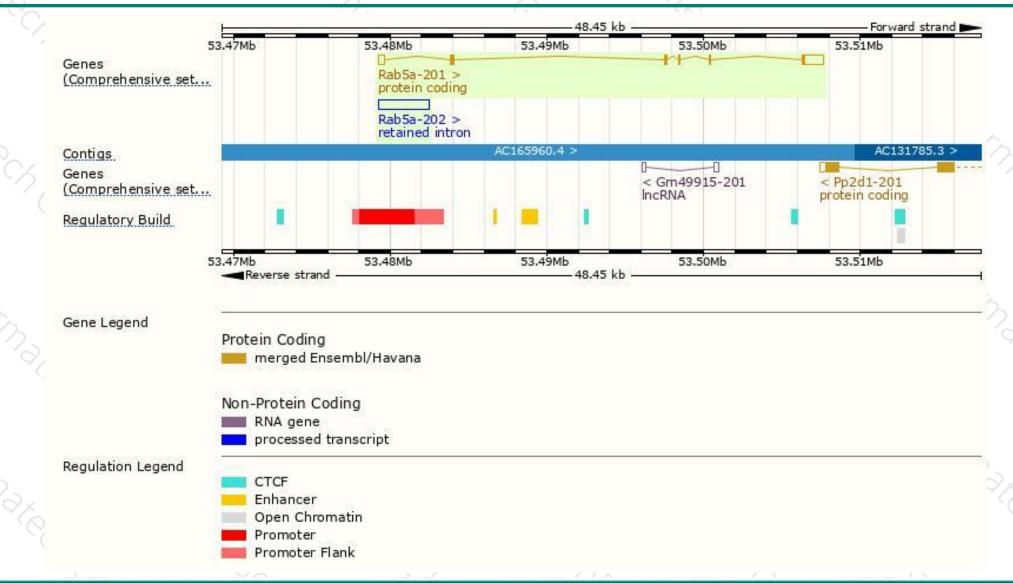


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Genomic location distribution





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Protein domain

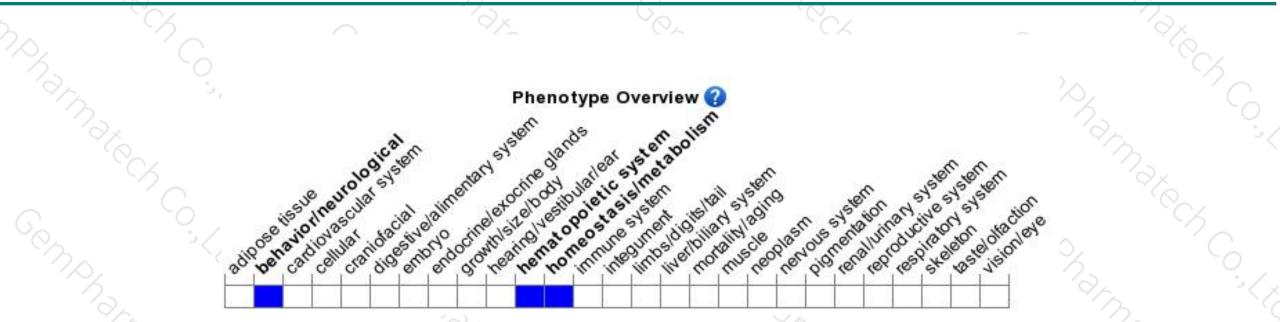
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Mouse phenotype description(MGI)





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



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



