

Rhobtb3 Cas9-KO Strategy

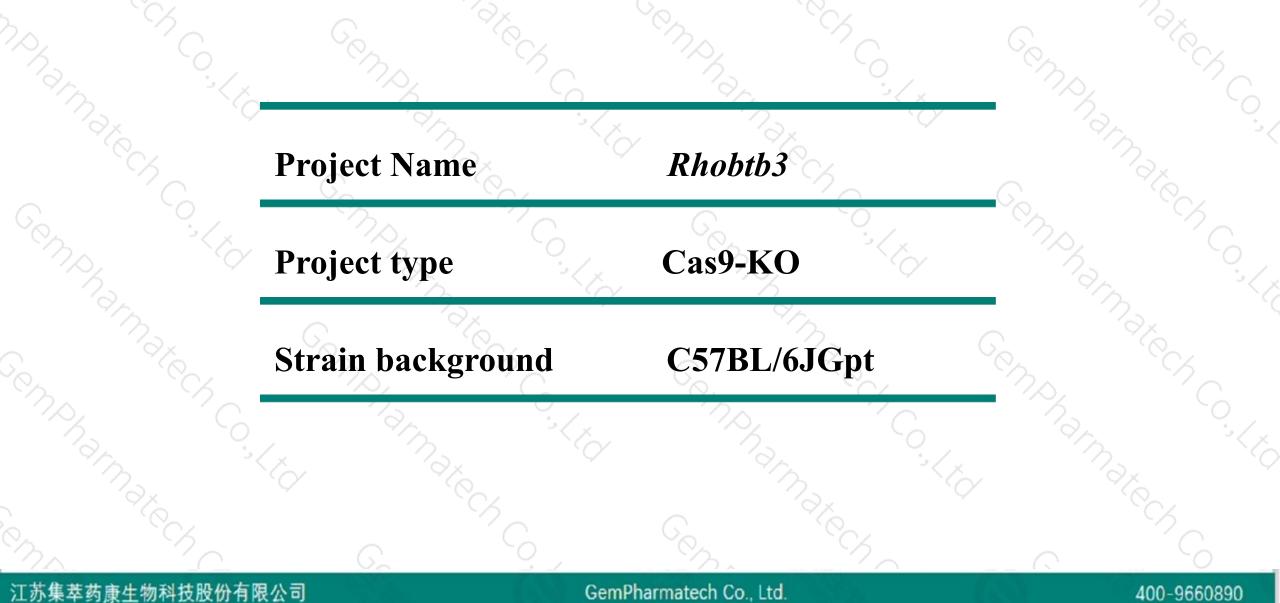
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Reviewer: Xiaojing Li

Design Date: 2020-8-6

Project Overview

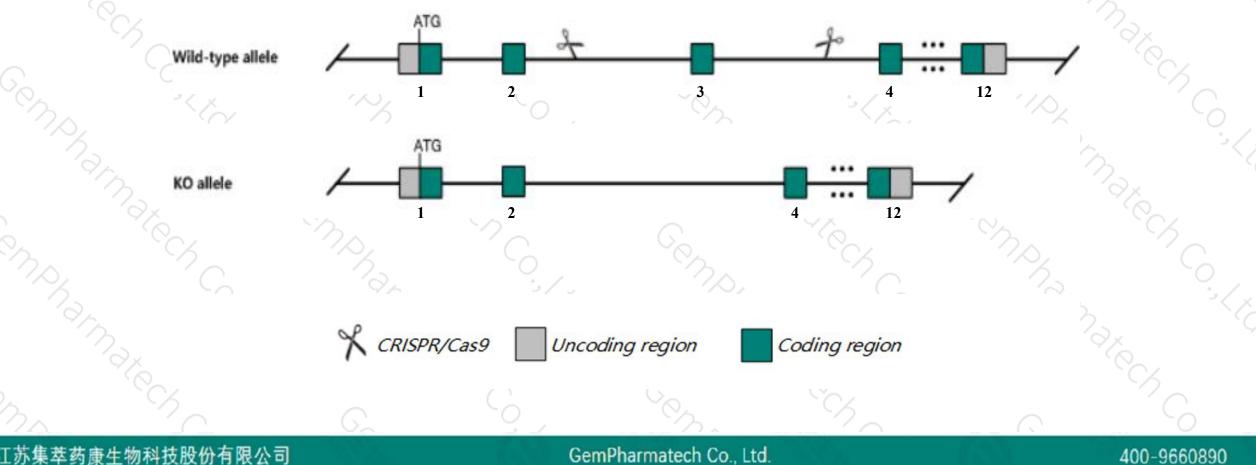




Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Rhobtb3* gene. The schematic diagram is as follows:



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➤ The *Rhobtb3* gene has 5 transcripts. According to the structure of *Rhobtb3* gene, exon3 of *Rhobtb3*-201(ENSMUST00000022078.11) transcript is recommended as the knockout region. The region contains 187bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Rhobtb3* 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 knock-out allele exhibit preweaning lethality, reduced body weight and slightly reduced organ weights that varies by sex.
- The *Rhobtb3* gene is located on the Chr13. 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.

Notice

Gene information (NCBI)



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Rhobtb3 Rho-related BTB domain containing 3 [Mus musculus (house mouse)]

Gene ID: 73296, updated on 13-Mar-2020

Summary

Official Symbol	Rhobtb3 provided by MGI
Official Full Name	Rho-related BTB domain containing 3 provided by MGI
Primary source	MGI:MGI:1920546
See related	Ensembl:ENSMUSG0000021589
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	1700040C17Rik, 2610033K01Rik, 4930503C18Rik, Al317148, AW208826, mKIAA0878
Expression	Broad expression in CNS E11.5 (RPKM 11.7), limb E14.5 (RPKM 9.8) and 23 other tissuesSee more
Orthologs	human all

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Transcript information (Ensembl)



The gene has 5 transcripts, all transcripts are shown below:

Mama	Transprint ID	hn	Protein	Picture	CCDS	UniProt	Flage
Name	Transcript ID	bp	Protein	Biotype	CCDS	UNIProt	Flags
Rhobtb3-201	ENSMUST0000022078.11	4980	<u>611aa</u>	Protein coding	CCDS26652	Q9CTN4	TSL:1 GENCODE basic APPRIS P1
Rhobtb3-202	ENSMUST00000109606.2	1676	<u>260aa</u>	Protein coding		<u>Q8BV11</u>	TSL:1 GENCODE basic
Rhobtb3-205	ENSMUST00000222923.1	303	<u>88aa</u>	Protein coding	2	A0A1Y7VJQ5	CDS 5' incomplete TSL:1
Rhobtb3-204	ENSMUST00000220939.1	712	<u>100aa</u>	Nonsense mediated decay	×	A0A1Y7VMM2	CDS 5' incomplete TSL:3
Rhobtb3-203	ENSMUST00000123353.1	937	No protein	Processed transcript	Ξ.	20	TSL:1

The strategy is based on the design of *Rhobtb3-201* transcript, the transcription is shown below:

< Rhobtb3-201 protein coding

Reverse strand

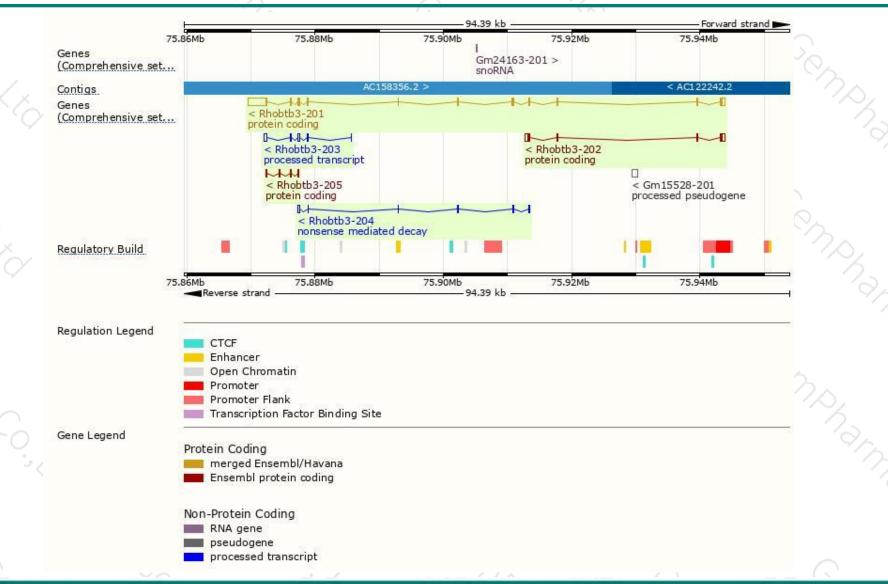
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Genomic location distribution





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Protein domain

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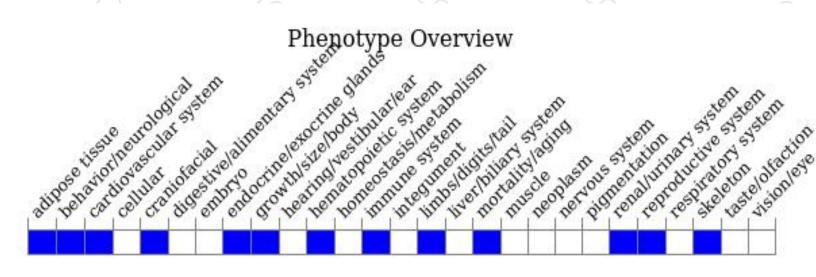
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, ,,	ENSMUSP00000022 Superfamily	No.		SKP1/BTB/POZ domai	n superfamily			5
	SMART	P-loop conta	ining nucleoside triph	osphate hydrolase		BTB/POZ doma	ain	<u>```</u>
	<u>Pfam</u>	Small G	TPase			BTB/POZ domai		
	PROSITE profiles PANTHER	Rho-related BTB domain-c	ontaining protein 3	BTB/POZ domain				
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	CDD			cd18357	-	cd18360	cd18532	-
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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 preweaning lethality, reduced body weight and slightly reduced organ weights that varies by sex.

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



