

Rc3h2 Cas9-KO Strategy

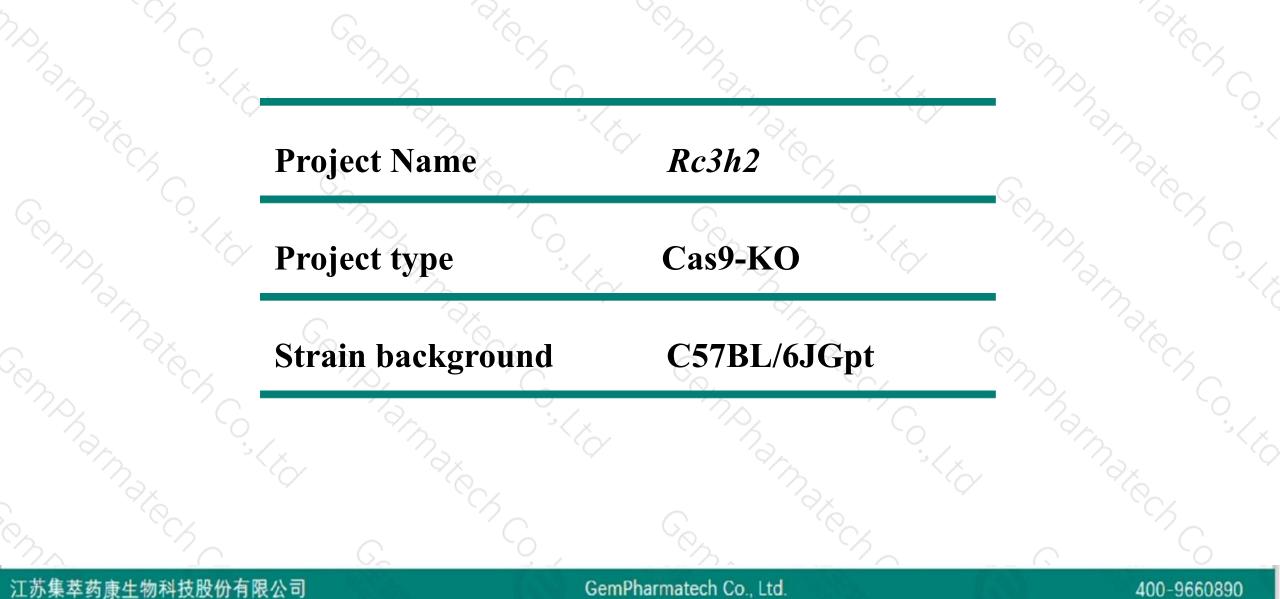
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Reviewer: Xiaojing Li

Design Date: 2020-7-29

Project Overview

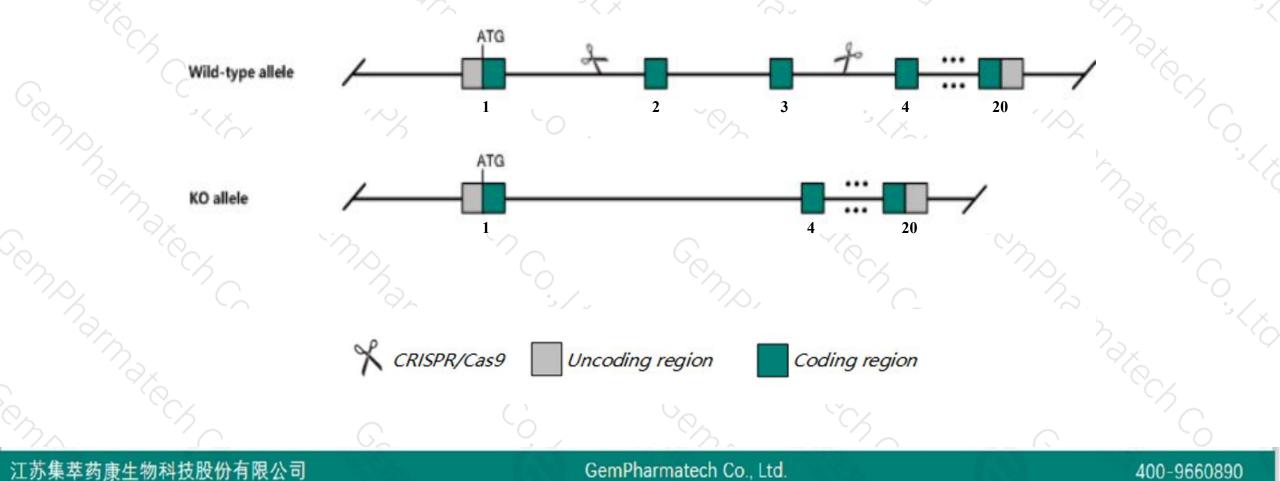




Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Rc3h2* gene. The schematic diagram is as follows:





> The *Rc3h2* gene has 10 transcripts. According to the structure of *Rc3h2* gene, exon2-exon3 of *Rc3h2-201*(ENSMUST00000100143.9) transcript is recommended as the knockout region. The region contains 352bp coding sequence. Knock out the region will result in disruption of protein function.

> In this project we use CRISPR/Cas9 technology to modify Rc3h2 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,homozygotes for a knock-out allele are viable and healthy but show increased TNF production by macrophages in response to LPS. Homozygotes for a different knock-out allele show postnatal lethality, decreased body size and weight, and an immature lung phenotype with decreased alveolar expansion.
The *Rc3h2* gene is located on the Chr2. 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.

Gene information (NCBI)



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Rc3h2 ring finger and CCCH-type zinc finger domains 2 [Mus musculus (house mouse)]

Gene ID: 319817, updated on 13-Mar-2020

Summary

Official Symbol	Rc3h2 provided by MGI
Official Full Name	ring finger and CCCH-type zinc finger domains 2 provided by <u>MGI</u>
Primary source	MGI:MGI:2442789
See related	Ensembl:ENSMUSG0000075376
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
	Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2900024N03Rik, 9430019J22Rik, D930043C02Rik, Mnab, Rnf164
Expression	Ubiquitous expression in whole brain E14.5 (RPKM 8.3), CNS E18 (RPKM 7.9) and 28 other tissuesSee more
Orthologs	human all

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Transcript information (Ensembl)



The gene has 10 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rc3h2-202	ENSMUST00000112934.7	8948	<u>1125aa</u>	Protein coding	CCDS71046	A2AVP4	TSL:1 GENCODE basic APPRIS ALT1
Rc3h2-201	ENSMUST00000100143.9	8799	<u>1187aa</u>	Protein coding	CCDS38116	P0C090	TSL:5 GENCODE basic APPRIS P3
Rc3h2-203	ENSMUST00000112936.3	5828	<u>1187aa</u>	Protein coding	CCD538116	P0C090	TSL:5 GENCODE basic APPRIS P3
Rc3h2-205	ENSMUST00000125619.6	5754	<u>478aa</u>	Nonsense mediated decay		A0A0N4SVF7	TSL:5
Rc3h2-204	ENSMUST00000124218.1	513	No protein	Processed transcript	2		TSL:3
Rc3h2-207	ENSMUST00000204690.1	3479	No protein	Retained intron	-	673	TSL:NA
Rc3h2-206	ENSMUST00000143826.1	3083	No protein	Retained intron	-	-	TSL:2
Rc3h2-209	ENSMUST00000204962.1	2698	No protein	Retained intron	-	020	TSL:NA
Rc3h2-210	ENSMUST00000205124.1	2236	No protein	Retained intron	π.	(7 5)	TSL:NA
Rc3h2-208	ENSMUST00000204959.1	575	No protein	Retained intron	÷.	8 - 81	TSL:2
						-	

The strategy is based on the design of *Rc3h2-201* transcript, the transcription is shown below:

< Rc3h2-201 protein coding

Reverse strand -

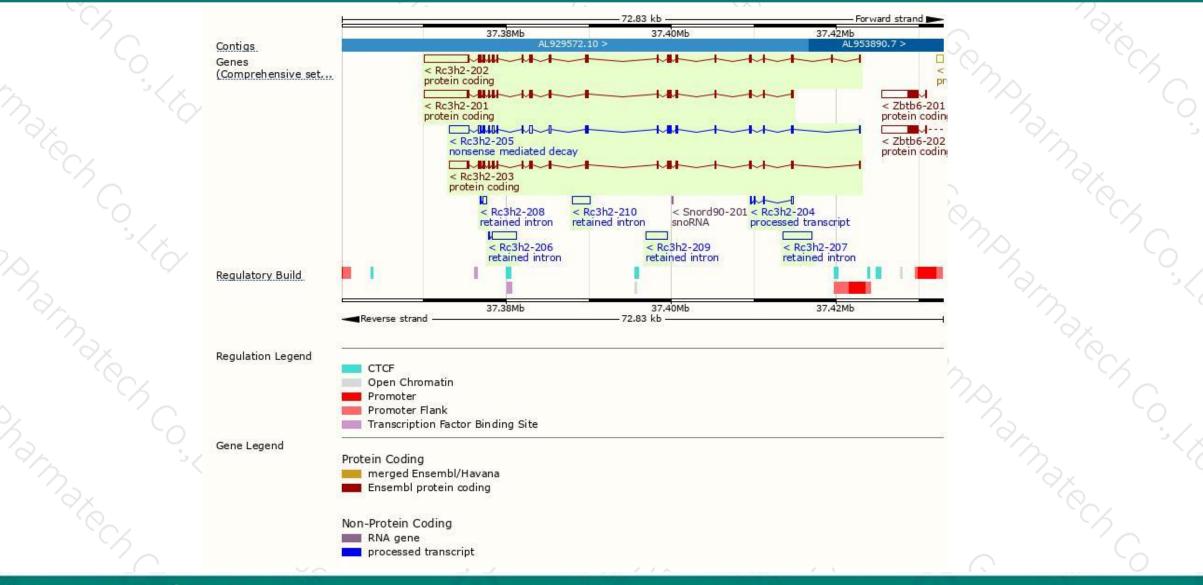
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Genomic location distribution





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Protein domain



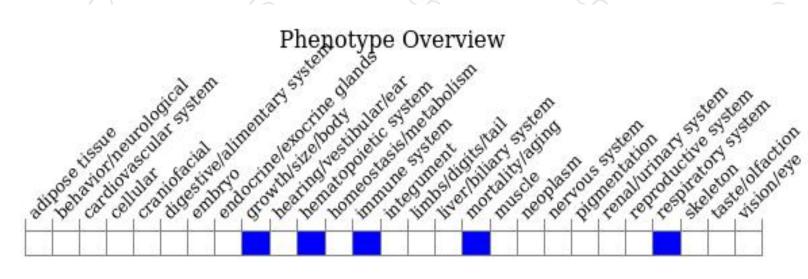


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Mouse phenotype description(MGI)





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

According to the existing MGI data, homozygotes for a knock-out allele are viable and healthy but show increased TNF production by macrophages in response to LPS. Homozygotes for a different knock-out allele show postnatal lethality, decreased body size and weight, and an immature lung phenotype with decreased alveolar expansion.

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



