

Rnf148 Cas9-CKO Strategy

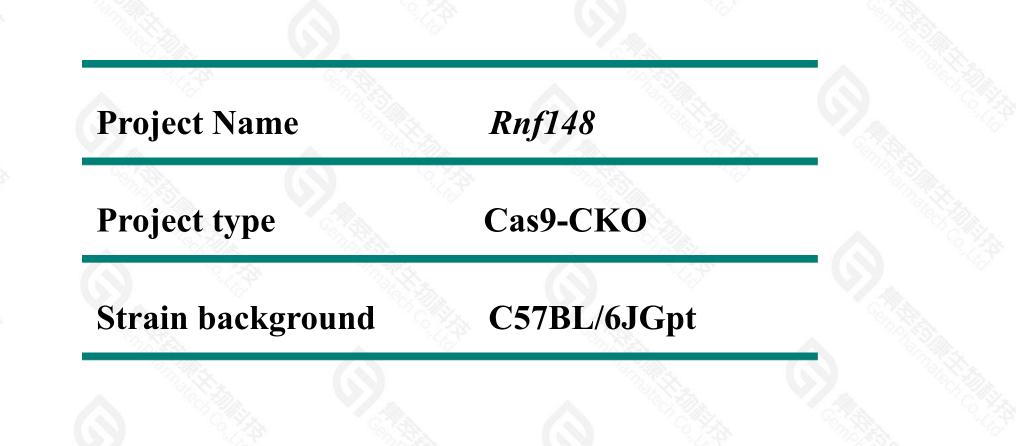
Designer: Daohua Xu

Reviewer: Xueting Zhang

Design Date: 2021-2-9

Project Overview

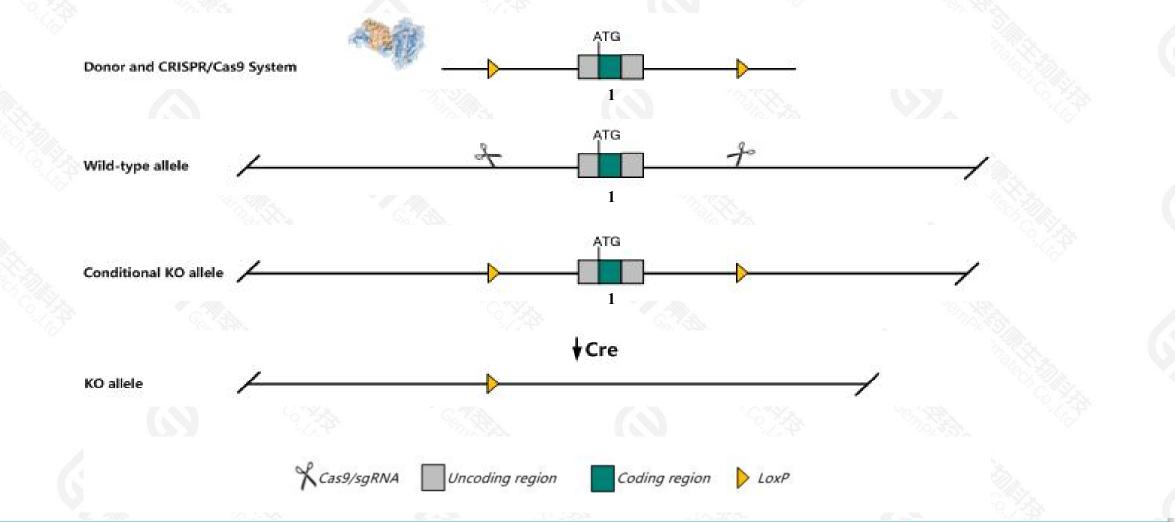




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Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the Rnf148 gene. The schematic diagram is as follows:



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Technical routes



> The *Rnf148* gene has 1 transcript. According to the structure of *Rnf148* gene, exon1 of *Rnf148*-201(ENSMUST00000104979.1) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Rnf148* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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 KO region contains partial intron of the Rnf148 gene.Knockout the region may affect the function of *Cadps2* gene.
 The *Rnf148* gene is located on the Chr6. 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)



☆ ?

Rnf148 ring finger protein 148 [Mus musculus (house mouse)]

Gene ID: 71300, updated on 13-Mar-2020

Summary

Official Symbol	Rnf148 provided by MGI
Official Full Name	ring finger protein 148 provided by <u>MGI</u>
Primary source	MGI:MGI:1918550
See related	Ensembl:ENSMUSG0000078179
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	4933432M07Rik, Greul3
Orthologs	human all

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400-9660890

Transcript information (Ensembl)

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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rnf148-201	ENSMUST00000104979.1	1239	<u>316aa</u>	Protein coding	CCDS51726	G3X9R7	TSL:NA GENCODE basic APPRIS P1

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The strategy is based on the design of *Rnf148-201* transcript, the transcription is shown below:

< Rnf148-201 protein coding	9					
Reverse st	rand —		 kb ———			1
S. T.D.S.	1 C - Mar	23.25	0.255	to the second	1. X 1. 4 S	954
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Genomic location distribution



		NO. 100 100 100 100 100 100 100 100 100 10	21.24 kb			
	23.645Mb	23.650Mb	23.655Mb	23.660Mb	23.66	
Contigs			< AC1 55850.6			
Genes (Comprehensive set	< Cadps2-212 protein coding					
	< Cadps2-213 protein coding					
	< Cadps2-205 protein coding					
	< Cadps2-201 protein coding					
	< Cadps2-209 protein coding					
	< Cadps2-206 protein coding					
	< Cadps2-202 protein coding					
	< Cadps2-204 protein coding					
	< Cadps2-208 retained intron	<pre>< Rnf133-201 protein coding </pre>	<pre>< Rnf148-201 protein coding</pre>			
Regulatory Build						
	23.645Mb Reverse strand	23.650Mb	23.655Mb 21.24 kb	23.660Mb	23.66	
Regulation Legend	Enhancer					
Gene Legend	Protein Coding				9	

Ensembl protein coding merged Ensembl/Havana

Non-Protein Coding processed transcript

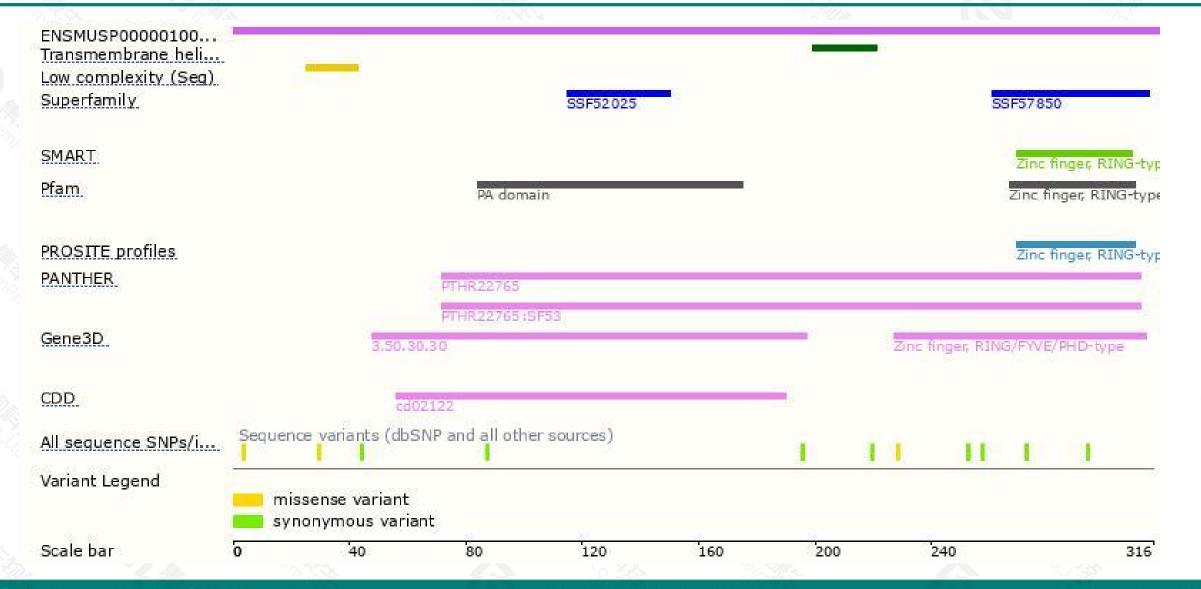
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Protein domain





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If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



