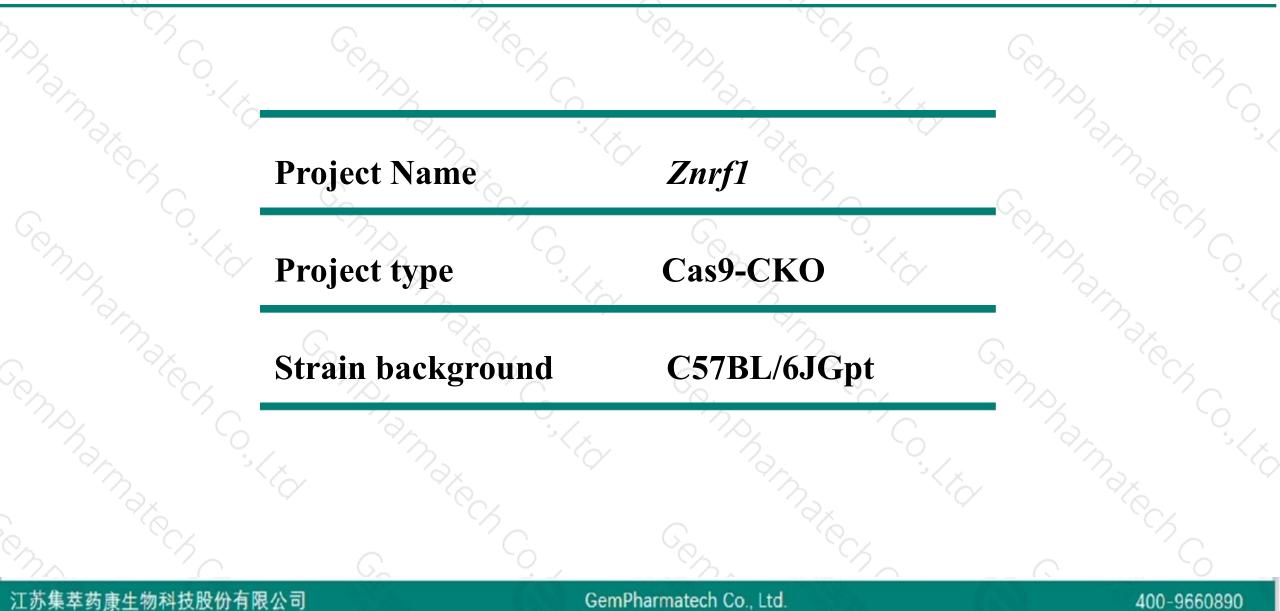


# Znrfl Cas9-CKO Strategy

Designer: Reviewer: Design Date: JiaYu Xiaojing Li 2020-2-26

# **Project Overview**



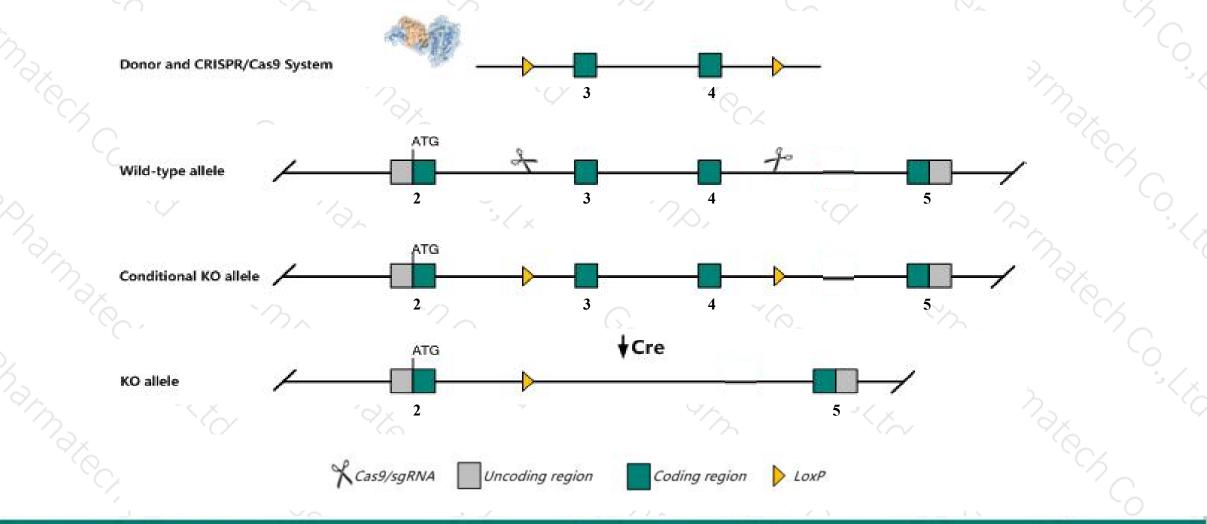


## **Conditional Knockout strategy**



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



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The Znrfl gene has 15 transcripts. According to the structure of Znrfl gene, exon3-exon4 of Znrfl-205 (ENSMUST00000172856.7) transcript is recommended as the knockout region. The region contains 202bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Znrf1* 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 Znrfl gene is located on the Chr8. 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.
- Some amino acids will remain at the N-terminus and some functions may be retained.
- 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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### Znrf1 zinc and ring finger 1 [Mus musculus (house mouse)]

Gene ID: 170737, updated on 3-Feb-2019

#### Summary

Official Symbol	Znrf1 provided by MGI
Official Full Name	zinc and ring finger 1 provided by MGI
Primary source	MGI:MGI:2177308
See related	Ensembl:ENSMUSG00000033545
Gene type	protein coding
<b>RefSeq status</b>	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	B830022L21Rik, Rnf42, Zrfp1, nin283
Expression	Broad expression in thymus adult (RPKM 159.2), whole brain E14.5 (RPKM 40.2) and 20 other tissues See more
Orthologs	human all

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# **Transcript information (Ensembl)**



### The gene has 15 transcripts, all transcripts are shown below:

		1						
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Znrf1-204	ENSMUST00000171182.7	5466	<u>195aa</u>	Protein coding CCDS52673 E9PZB7 TSL:3 GENCOD		TSL:3 GENCODE basic		
Znrf1-205	ENSMUST00000172856.7	4600	<u>227aa</u>	Protein coding	CCDS22676	<u>Q91V17</u>	TSL:5 GENCODE basic APPRIS P1	
Znrf1-203	ENSMUST00000168428.7	4451	<u>227aa</u>	Protein coding CCDS22676 Q91V17 TSL:1 GENCODE basic A		TSL:1 GENCODE basic APPRIS P1		
Znrf1-202	ENSMUST00000166859.7	3650	<u>127aa</u>	Protein coding	CCDS52674	E9PWL2	TSL:5 GENCODE basic	
Znrf1-201	ENSMUST00000095176.11	3108	<u>227aa</u>	Protein coding	CCDS22676	Q91V17	TSL:1 GENCODE basic APPRIS P1	
Znrf1-207	ENSMUST00000173506.7	1238	<u>227aa</u>	Protein coding	CCDS22676	<u>Q91V17</u>	TSL:2 GENCODE basic APPRIS P1	
Znrf1-213	ENSMUST00000174333.7	515	<u>127aa</u>	Protein coding	CCDS52674	E9PWL2	TSL:3 GENCODE basic	
Znrf1-209	ENSMUST00000173726.7	549	<u>88aa</u>	Protein coding	20	G3UWY5	CDS 5' incomplete TSL:3	
Znrf1-215	ENSMUST00000174454.7	494	<u>65aa</u>	Protein coding	-	<u>G3UYU9</u>	TSL:3 GENCODE basic	
Znrf1-210	ENSMUST00000173781.1	337	<u>65aa</u>	Protein coding	-8	<u>G3UYU9</u>	TSL:3 GENCODE basic	
Znrf1-211	ENSMUST00000173819.1	297	<u>41aa</u>	Protein coding	<b>1</b> 2	G3UWN6	CDS 5' incomplete TSL:3	
Znrf1-212	ENSMUST00000173922.1	559	<u>28aa</u>	Nonsense mediated decay	-	<u>G3UZ70</u>	CDS 5' incomplete TSL:2	
Znrf1-214	ENSMUST00000174376.7	501	<u>54aa</u>	Nonsense mediated decay	-	G3UWW9	CDS 5' incomplete TSL:5	
Znrf1-208	ENSMUST00000173692.1	446	No protein	IncRNA	-8	-5	TSL:3	
Znrf1-206	ENSMUST00000173481.1	413	No protein	IncRNA	10	-	TSL:3	

The strategy is based on the design of Znrf1-205 transcript, The transcription is shown below

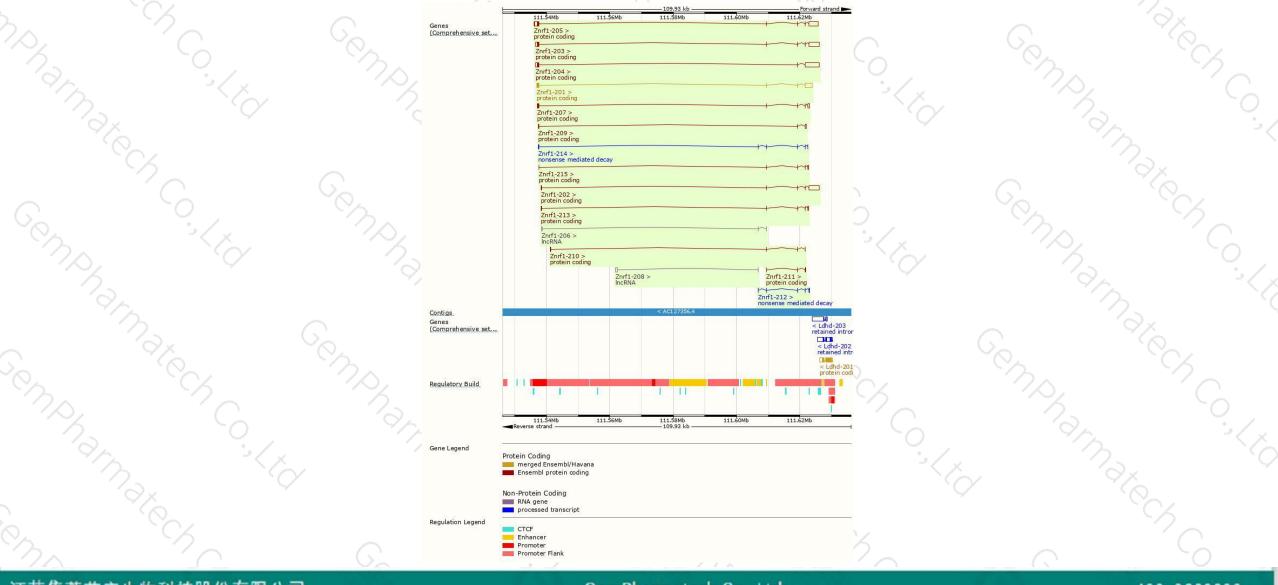


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### **Genomic location distribution**





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## **Protein domain**



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Superfamily					SSF57850	_
SMART					Zinc finger, RING-type	_
<u>Pfam</u>					Zinc finger, RING-type	
PROSITE profiles					Zinc finger, RING-type	
	7HR46661					
Cana3D	THR46661:SF2					
Gene3D CDD				Zinc finge	er, RING/FYVE/PHD-type	
		J.			cd16694	
All sequence SNPs/i	Sequence variants (dbSNP and all o	ther sources)				1.1
Variant Legend	synonymous variant					
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If you have any questions, you are welcome to inquire. Tel: 400-9660890



