

# Ing4 Cas9-KO Strategy

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Reviewer: Xingkai Xiao

Design Date: 2024-4-16

#### Overview

#### Target Gene Name

• Ing4

Project Type

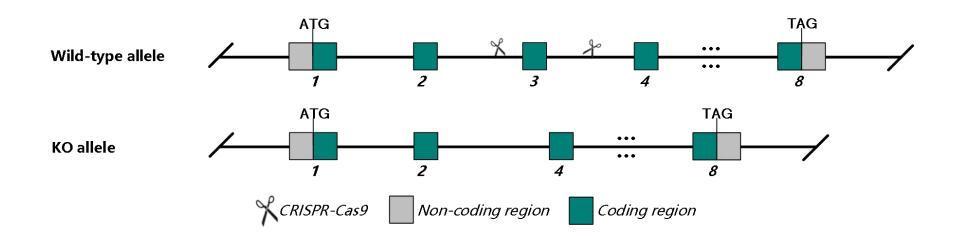
• Cas9-KO

Genetic Background

• C57BL/6JGpt



## Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the Ing4 gene.

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## **Technical Information**

- The *Ing4* gene has 9 transcripts. According to the structure of *Ing4* gene, exon 3 of *Ing4*-201 (ENSMUST00000032480.14) is recommended as the knockout region. The region contains 167 bp of coding sequence. Knocking out the region will result in disruption of gene function.
- In this project we use CRISPR-Cas9 technology to modify *Ing4* gene. The brief process is as follows: CRISPR-Cas9 system and gRNA 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.



#### Gene Information

Ing4 inhibitor of growth family, member 4 [ Mus musculus (house mouse) ]

Gene ID: 28019, updated on 10-Mar-2024

Summary

Official Symbol Ing4 provided by MGI Official Full Name inhibitor of growth family, member 4 provided by MGI Primary source MGI:MGI:107307 See related Ensembl:ENSMUSG00000030330 AllianceGenome:MGI:107307 Gene type protein coding RefSeg 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 D6Xrf92; p29ING4; D6Wsu147e Summary Predicted to enable methylated histone binding activity and transcription coactivator activity. Involved in positive regulation of apoptotic process. Located in nucleus. Is expressed in several structures, including cerebral cortex; early conceptus; genitourinary system; sensory organ; and skeleton. Orthologous to human ING4 (inhibitor of growth family member 4). [provided by Alliance of Genome Resources, Apr 2022] Expression Ubiquitous expression in limb E14.5 (RPKM 43.3), CNS E14 (RPKM 42.0) and 28 other tissues See more Orthologs human all Try the new Gene table NEW Try the new Transcript table Genomic context \$ ? Location: 6 F2: 6 59.17 cM See Ing4 in Genome Data Viewer Exon count: 8

#### https://www.ncbi.nlm.nih.gov/gene/28019



**±** Download Datasets

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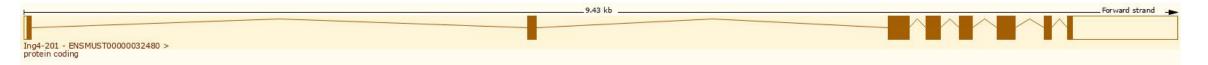
#### **Transcript Information**

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#### The gene has 9 transcripts, all transcripts are shown below:

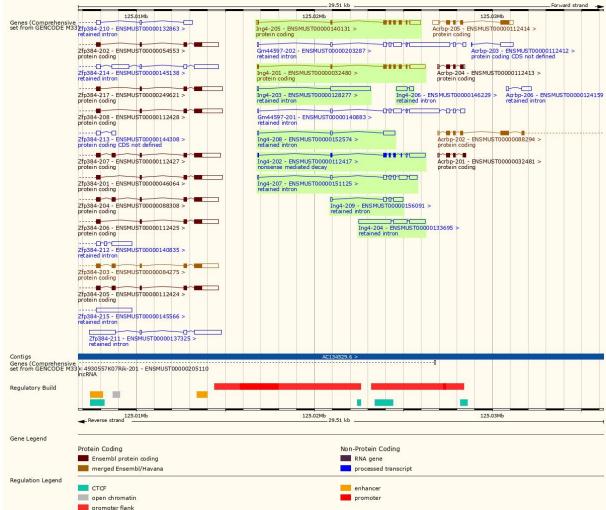
Transcript ID	Name 🍦	bp 🍦	Protein v	Biotype 🍦	CCDS	UniProt Match	Flags						
ENSMUST00000140131.8	Ing4-205	1446	<u>249aa</u>	Protein coding	4	Q8C0D7 🗗	Ensembl Canonical GENCODE basic APPRIS ALT1 TSL:1						
ENSMUST0000032480.14	Ing4-201	1629	<u>248aa</u>	Protein coding	<u>CCDS39632</u> &	<u>Q8C0D7-2</u> &	GENCODE basic APPRIS P4 TSL:1						
ENSMUST00000112417.9	Ing4-202	1487	<u>168aa</u>	Nonsense mediated decay	· · · · · · · · · · · · · · · · · · ·	<u>Q8C0D7-5</u> &	TSL:1						
ENSMUST00000133695.2	Ing4-204	3458	No protein	Retained intron		-	TSL:1						
ENSMUST00000128277.2	Ing4-203	2294	No protein	Retained intron		-	TSL:1						
ENSMUST00000151125.8	Ing4-207	1383	No protein	Retained intron		-	TSL:1						
ENSMUST00000156091.2	Ing4-209	879	No protein	Retained intron		-	TSL:5						
ENSMUST00000146229.2	Ing4-206	849	No protein	Retained intron			TSL:1						
ENSMUST00000152574.8	Ing4-208	791	No protein	Retained intron	9	-	TSL:2						

#### The strategy is based on the design of *Ing4*-201 transcript, the transcription is shown below:



Source: http://asia.ensembl.org/

#### Genomic Information



Source: http://asia.ensembl.org/

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#### Protein Information

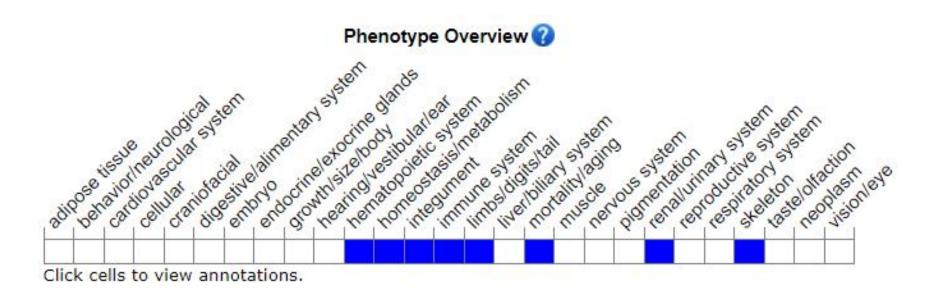
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ENSMUSP00000032480 MobiDB lite Low complexity (Seg) Coiled-coils (Ncoils) AFDB-ENSP mappings Superfamily						_	8			Zinc tinger, HYVE/VHD-type				_
SMART	Inhibitor of growth protein, N-terminal histone-binding			-							Zinc finger, PHD-type			_
Pfam	Inhibitor of growth protein, N-terminal histone-binding													
PROSITE profiles											Zinc finger, PHD-finger			
PROSITE patterns											Zinc finger, PHD-typ	e, con	served site	
PANTHER	ING family													
Gene3D	6.10.140.1740			1							Zinc finger, RING/FYVE/PHD-typ	)e		
CDD	cd16862		_								cd15684			
All sequence SNPs/	Sequence variants (EVA and all other sources)				2			1.		( <b>.</b>				
Variant Legend	frameshift variant		splice region variant						synonymous variant					
Scale bar	b '40	80				120	19 m li		У	.60	200			248'

Source: https://www.ensembl.org

## Mouse Phenotype Information (MGI)



Mice homozygous for a gene trapped allele are hypersensitive to LPS challenge and exhibit elevated cytokine responses.

Source: https://www.informatics.jax.org

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### **Important Information**

- The knockout region is about 2.5 kb away from the 5' of the *Acrbp* gene, which may affect the regulation of this gene.
- The knockout region overlaps with *Gm44597* and *4930557K07* gene, which may affect the function of this gene.
- This stratergy may not affect *Ing4*-203 and *Ing4*-206 non-coding transcript.
- *Ing4* is located on Chr 6. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risk on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

