

# Ing4 Cas9-CKO Strategy

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### Overview

#### Target Gene Name

• *Ing4* 

### Project Type

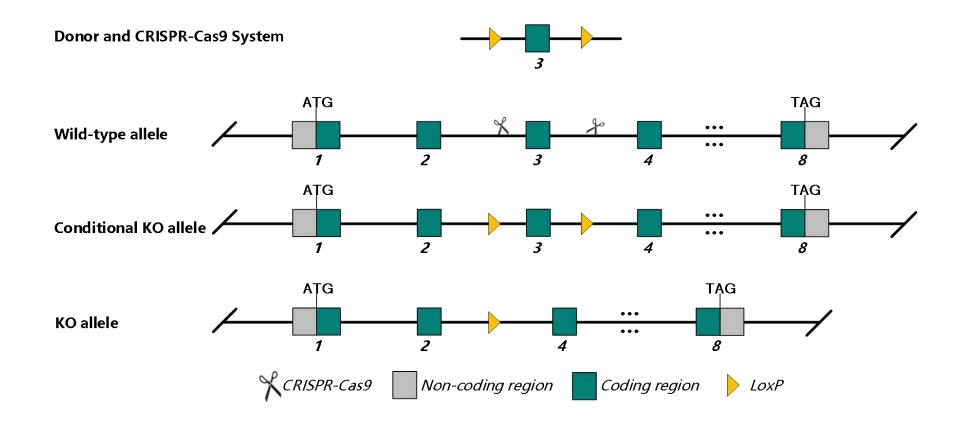
• Cas9-CKO

#### Genetic Background

• C57BL/6JGpt



# Strain Strategy

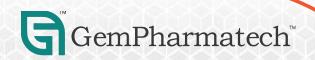


Schematic representation of CRISPR-Cas9 engineering used to edit the Ing4 gene.



#### **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 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 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.
- 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.



#### Gene Information

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

**≛** Download Datasets

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



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

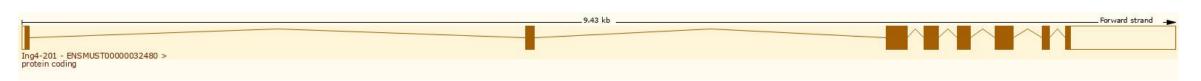


# Transcript Information

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

Transcript ID	Name 🍦	bp 🍦	Protein ▼	Biotype	CCDS 🍦	UniProt Match 🍦	Flags
ENSMUST00000140131.8	Ing4-205	1446	249aa	Protein coding		Q8C0D7₽	Ensembl Canonical GENCODE basic APPRIS ALT1 TSL:
ENSMUST00000032480.14	Ing4-201	1629	248aa	Protein coding	CCDS39632 ₽	Q8C0D7-2 ₽	GENCODE basic   APPRIS P4   TSL:1
ENSMUST00000112417.9	Ing4-202	1487	<u>168aa</u>	Nonsense mediated decay		Q8C0D7-5 ₽	TSL:1
ENSMUST00000133695.2	Ing4-204	3458	No protein	Retained intron		-	TSL1
ENSMUST00000128277.2	Ing4-203	2294	No protein	Retained intron		-	TSL1
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		-	TSL:2

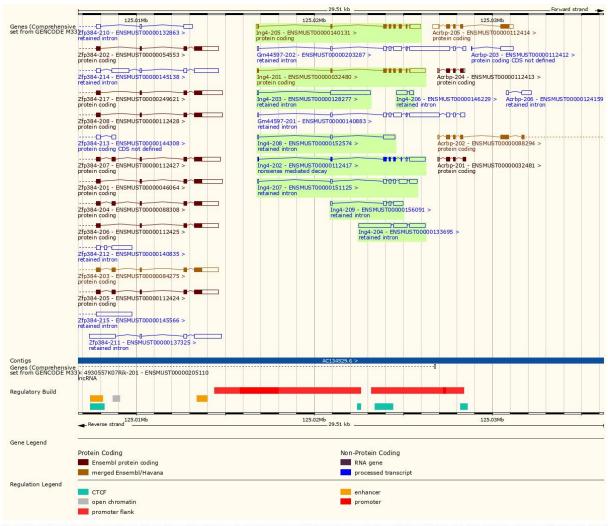
The strategy is based on the design of *Ing4*-201 transcript, the transcription is shown below:





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

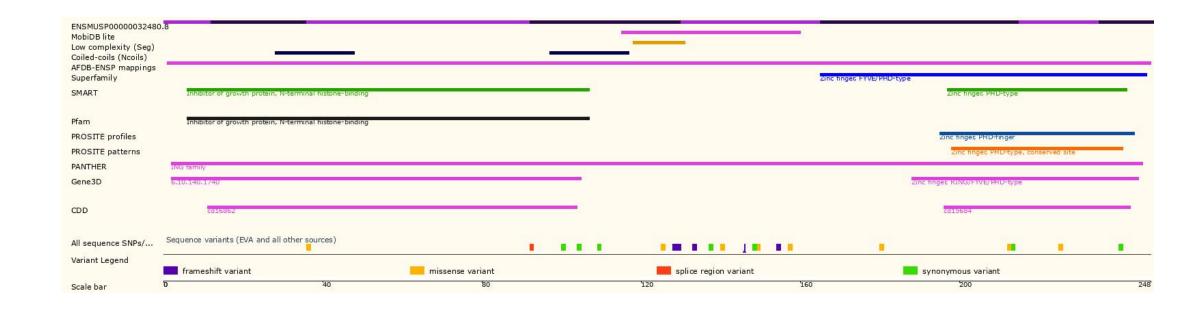
### Genomic Information





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

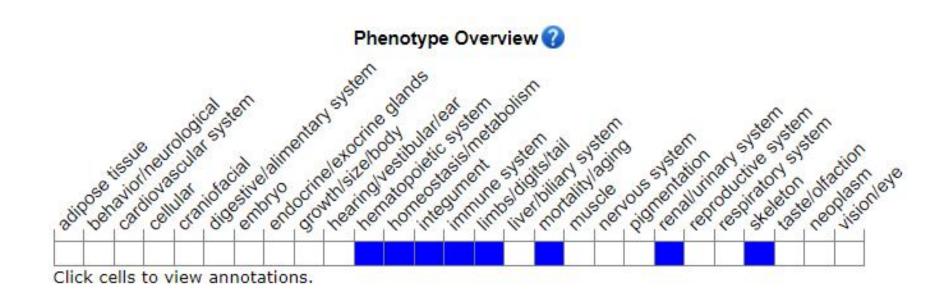
### **Protein Information**



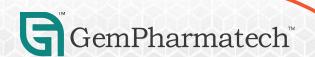


Source: https://www.ensembl.org

# 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

## **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.
- The intron 3-4 of *Ing4*-201 is 140 bp, the loxp insertion may affect the regulation of this gene.
- *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 of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

