

# Hspa8 Cas9-CKO Strategy

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#### Overview

#### Target Gene Name

• Hspa8

#### Project Type

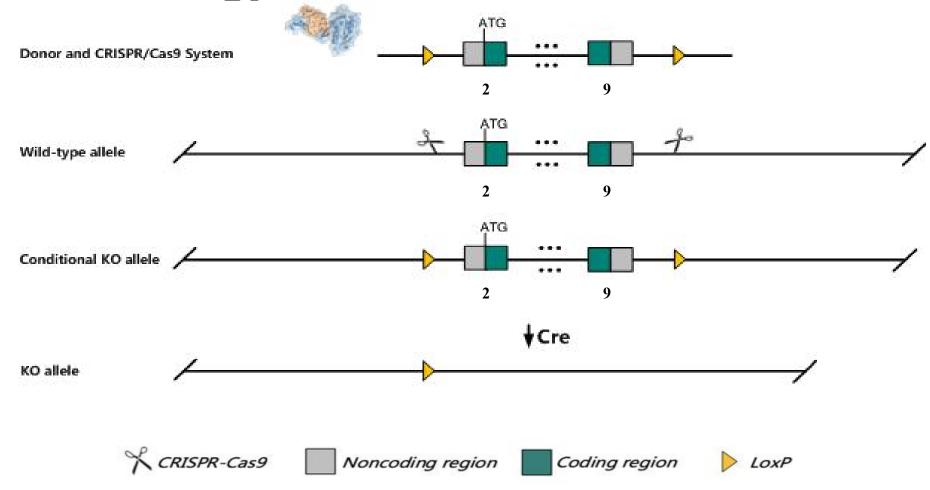
• Cas9-CKO

#### Genetic Background

• C57BL/6JGpt



### Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the Hspa8 gene.



#### **Technical Information**

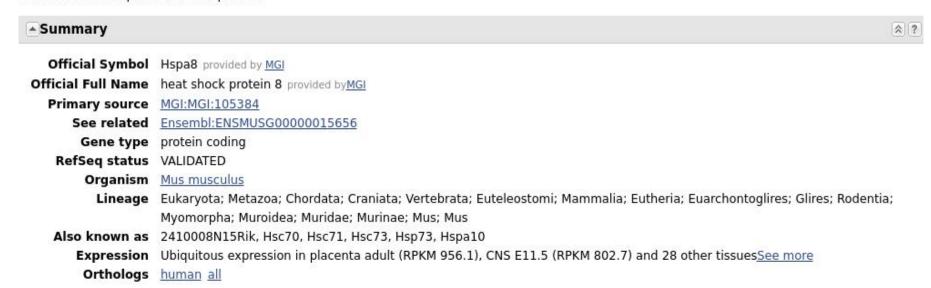
- The *Hspa8* gene has 10 transcripts. According to the structure of *Hspa8* gene, exon2-exon9 of *Hspa8*-201 (ENSMUST00000015800.16) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Hspa8* 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

#### Hspa8 heat shock protein 8 [Mus musculus (house mouse)]

Gene ID: 15481, updated on 12-Apr-2023



Source: https://www.ncbi.nlm.nih.gov/

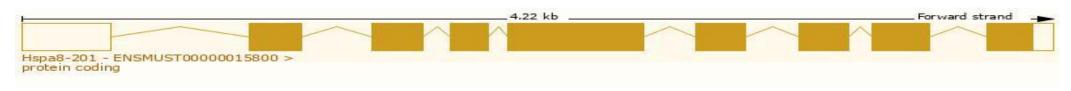


## Transcript Information

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

Transcript ID	Name ▲	bp 🌲	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000015800.16	Hspa8-201	2394	646aa	Protein coding	CCDS23083 ₽	P63017 ₺	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1
ENSMUST00000117557.8	Hspa8-202	2019	<u>627aa</u>	Protein coding		Q504P4	GENCODE basic TSL:1
ENSMUST00000117870.9	Hspa8-203	598	No protein	Protein coding CDS not defined		626	TSL:3
ENSMUST00000127699.2	Hspa8-204	456	No protein	Retained intron		-	TSL:1
ENSMUST00000133964.2	Hspa8-205	514	<u>116aa</u>	Protein coding		<u>D3Z5E2</u> ₺	TSL:2 CDS 3' incomplete
ENSMUST00000138895.2	Hspa8-206	920	No protein	Retained intron		12	TSL:1
ENSMUST00000140984.2	Hspa8-207	1152	No protein	Retained intron		-	TSL:5
ENSMUST00000149936.2	Hspa8-208	3158	No protein	Retained intron		-	TSL:1
ENSMUST00000153847.2	Hspa8-209	727	No protein	Protein coding CDS not defined		1-	TSL:5
ENSMUST00000215526.2	Hspa8-210	357	No protein	Protein coding CDS not defined		626	TSL:5

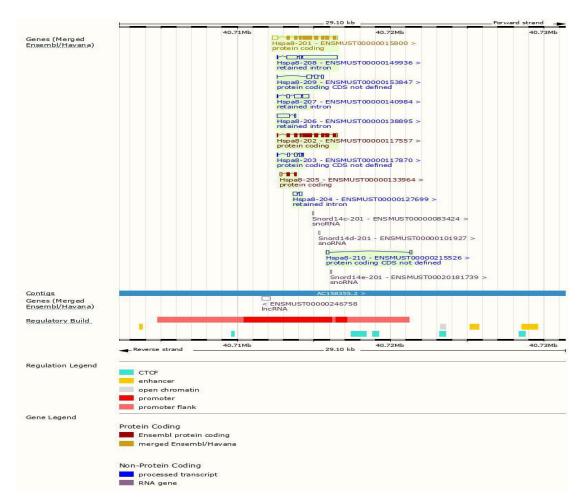
The strategy is based on the design of *Hspa8*-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org



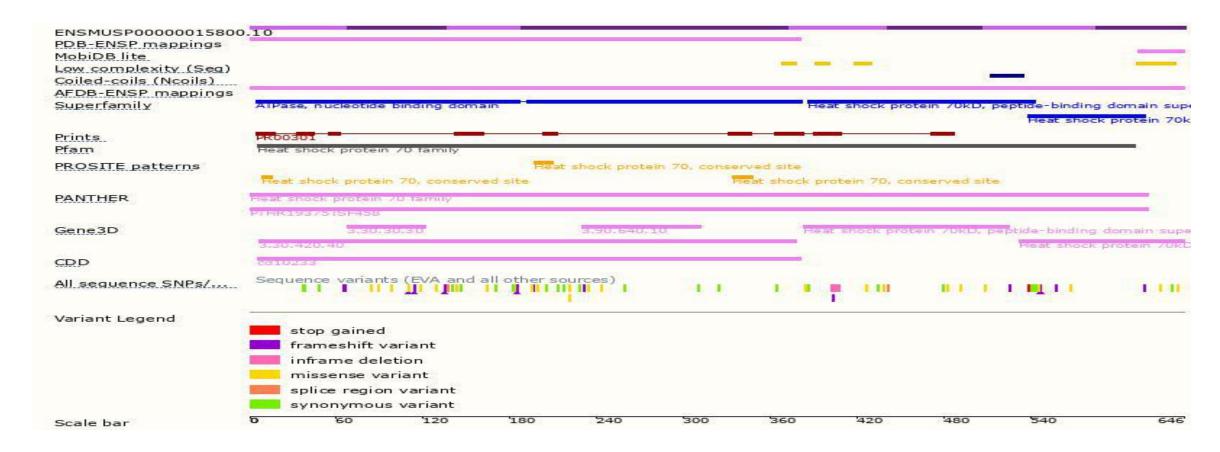
### Genomic Information





Source: : https://www.ensembl.org

#### Protein Information





Source: : https://www.ensembl.org

### Important Information

- *Hspa8* is located on Chr9. 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.
- The *Snord14c*, *Snord14d*, and *Snord14e* snoRNA genes overlap with the knockout region, and this strategy will knock out these genes.
- The insertion of 5'loxp may affect the normal splicing regulation function of *Hspa8* gene.
- This strategy may affect the 5-terminal regulatory function of the *Gm57042-01* gene.
- 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.

