

Hspa5 Cas9-CKO Strategy

Designer:

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Design Date:

2019-9-30

Project Overview



Project Name

Hspa5

Project type

Cas9-CKO

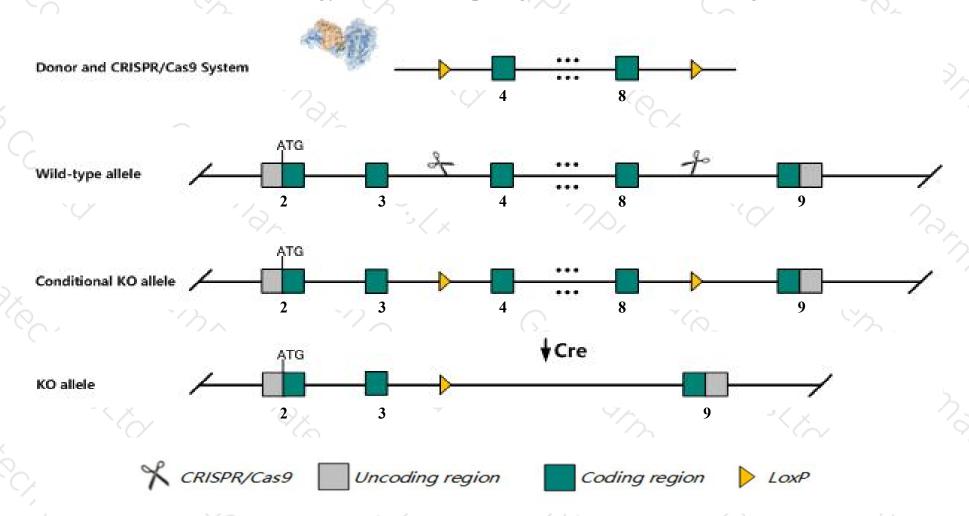
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Hspa5* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Hspa5* gene has 6 transcripts. According to the structure of *Hspa5* gene, exon4-exon8 of *Hspa5-201* (ENSMUST00000028222.12) transcript is recommended as the knockout region. The region contains 1048bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Hspa5* 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.

Notice



- ➤ According to the existing MGI data, Nullizygous embryos die around implantation. Neonates homozygous for a knock-in allele die of respiratory failure. Mice homozygous for an ENU-induced mutation exhibit abnormal thalamocortical axon patterning, small kidneys, cleft palate, respiratory distress, and postnatal lethality.
- > The *Hspa5* gene is located on the Chr2. 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)



Hspa5 heat shock protein 5 [Mus musculus (house mouse)]

Gene ID: 14828, updated on 7-Apr-2019

Summary

↑ ?

Official Symbol Hspa5 provided by MGI

Official Full Name heat shock protein 5 provided by MGI

Primary source MGI:MGI:95835

See related Ensembl:ENSMUSG00000026864

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 AL022860, AU019543, Bip, D2Wsu141e, D2Wsu17e, Grp78, Hsce70, SEZ-7, Sez7, baffled, mBiP

Expression Ubiquitous expression in placenta adult (RPKM 321.6), genital fat pad adult (RPKM 154.1) and 28 other tissuesSee more

Orthologs <u>human</u> all

Transcript information (Ensembl)



The gene has 6 transcripts, all transcripts are shown below:

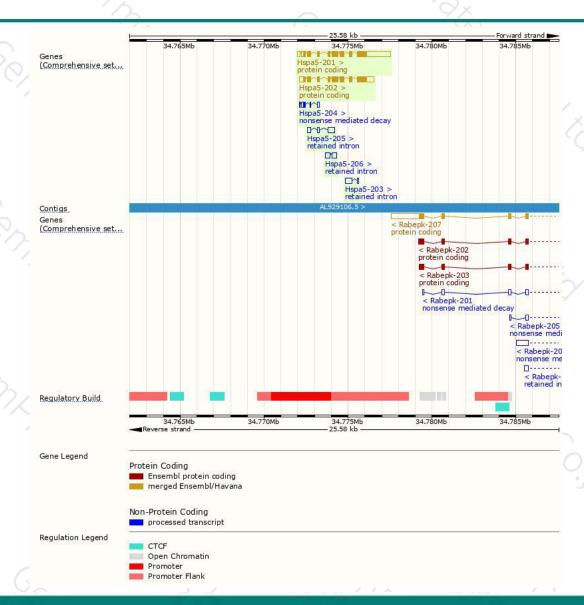
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Hspa5-201	ENSMUST00000028222.12	3672	655aa	Protein coding	CCDS15950	P20029	TSL:1 GENCODE basic APPRIS P1
Hspa5-202	ENSMUST00000100171.2	2613	<u>655aa</u>	Protein coding	CCDS15950	P20029	TSL:1 GENCODE basic APPRIS P1
Hspa5-204	ENSMUST00000137145.7	477	<u>42aa</u>	Nonsense mediated decay	929	A0A0A6YXF5	TSL:5
Hspa5-205	ENSMUST00000145466.1	720	No protein	Retained intron	3523	2	TSL:1
Hspa5-206	ENSMUST00000155595.1	574	No protein	Retained intron	187		TSL:2
Hspa5-203	ENSMUST00000129333.1	452	No protein	Retained intron	163	-	TSL:2

The strategy is based on the design of *Hspa5-201* transcript, The transcription is shown below



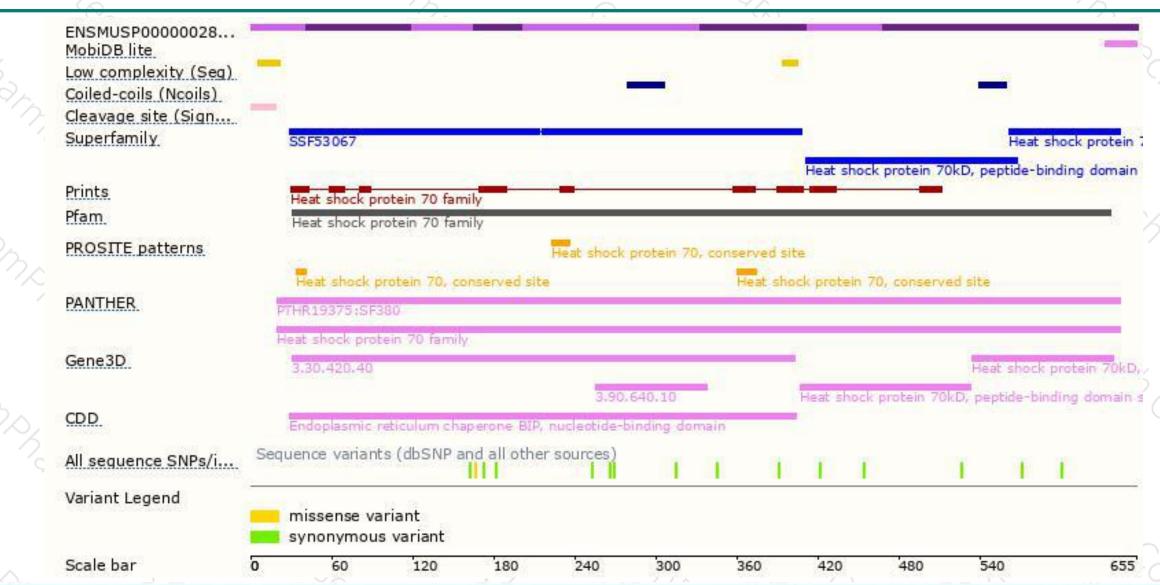
Genomic location distribution





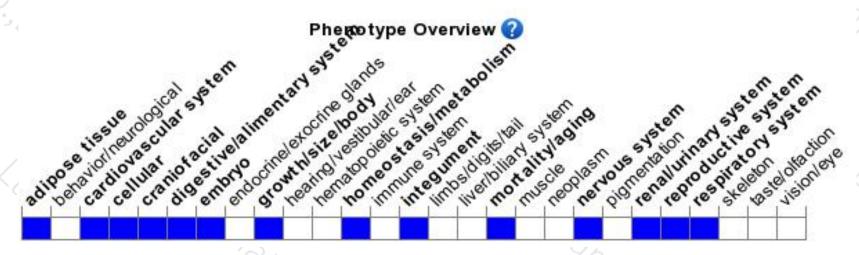
Protein domain





Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

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If you have any questions, you are welcome to inquire. Tel: 400-9660890





