

Usp10 Cas9-CKO Strategy

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Overview

Target Gene Name

- Usp10

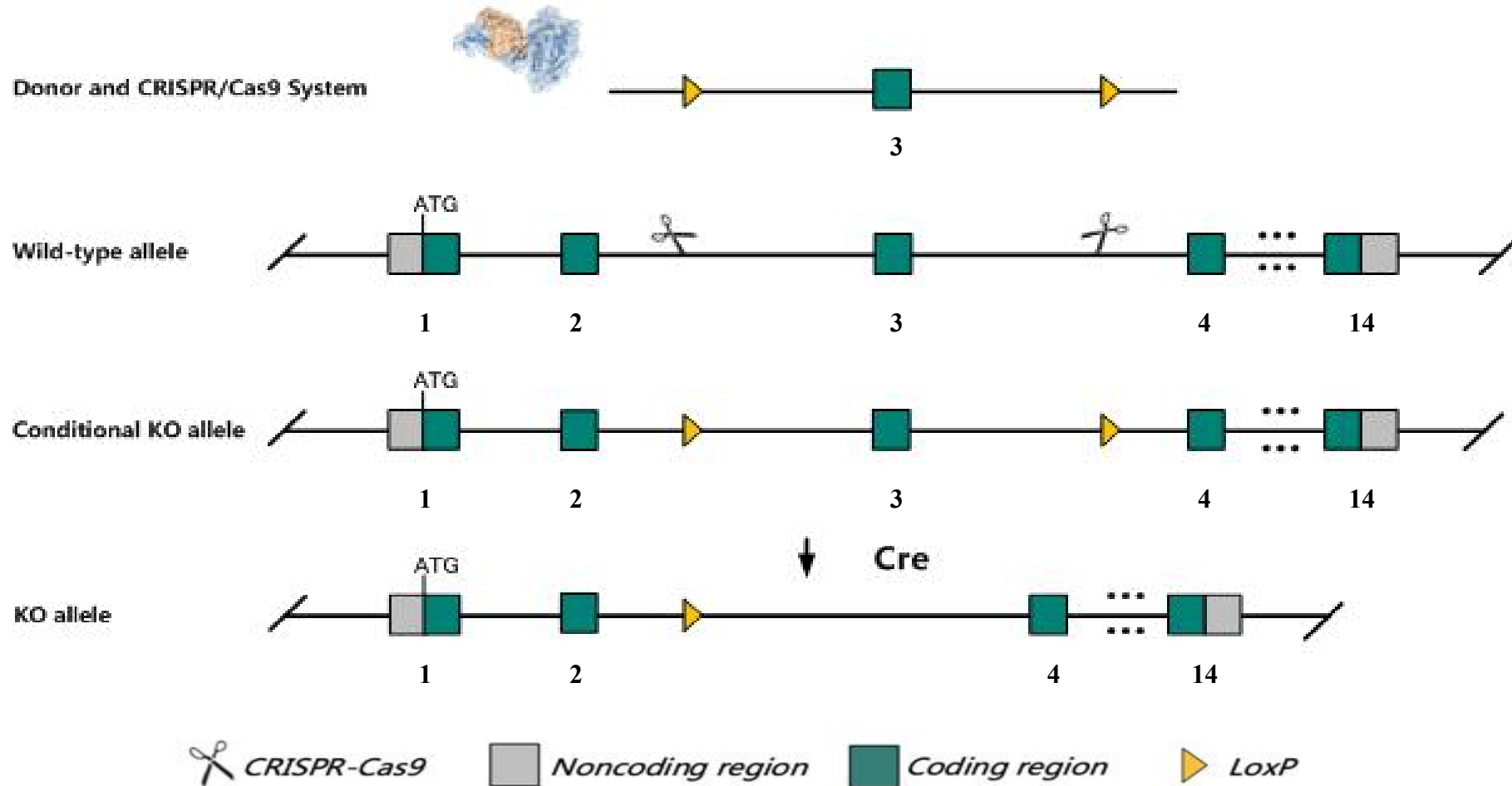
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Usp10* gene.

Technical Information

- The *Usp10* gene has 13 transcripts. According to the structure of *Usp10* gene, exon3 of *Usp10*-210 (ENSMUST00000144458.8) transcript is recommended as the knockout region. The region contains 58bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Usp10* 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

Usp10 ubiquitin specific peptidase 10 [Mus musculus (house mouse)]

Gene ID: 22224, updated on 21-May-2023

Summary

Official Symbol	Usp10 provided by MGI
Official Full Name	ubiquitin specific peptidase 10 provided by MGI
Primary source	MGI:MGI:894652
See related	Ensembl:ENSMUSG00000031826
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	2610014N07Rik, UBPO, Uchrp, mKIAA0190
Summary	Predicted to enable cysteine-type peptidase activity; p53 binding activity; and transmembrane transporter binding activity. Predicted to be involved in several processes, including DNA damage response, signal transduction by p53 class mediator; cellular response to interleukin-1; and negative regulation of I-kappaB kinase/NF-kappaB signaling. Predicted to act upstream of or within DNA repair and autophagy. Predicted to be located in cytoplasm; intermediate filament cytoskeleton; and nucleoplasm. Predicted to be part of protein-containing complex. Predicted to be active in cytosol; early endosome; and nucleus. Is expressed in several structures, including alimentary system; brain; genitourinary system; hemolymphoid system gland; and liver and biliary system. Orthologous to human USP10 (ubiquitin specific peptidase 10). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Ubiquitous expression in CNS E11.5 (RPKM 13.5), limb E14.5 (RPKM 11.2) and 28 other tissues See more
Orthologs	human all

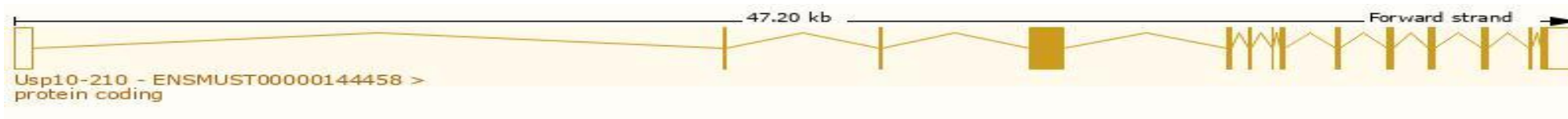
Source: <https://www.ncbi.nlm.nih.gov/>

Transcript Information

The gene has 13 transcripts, all transcripts are shown below:

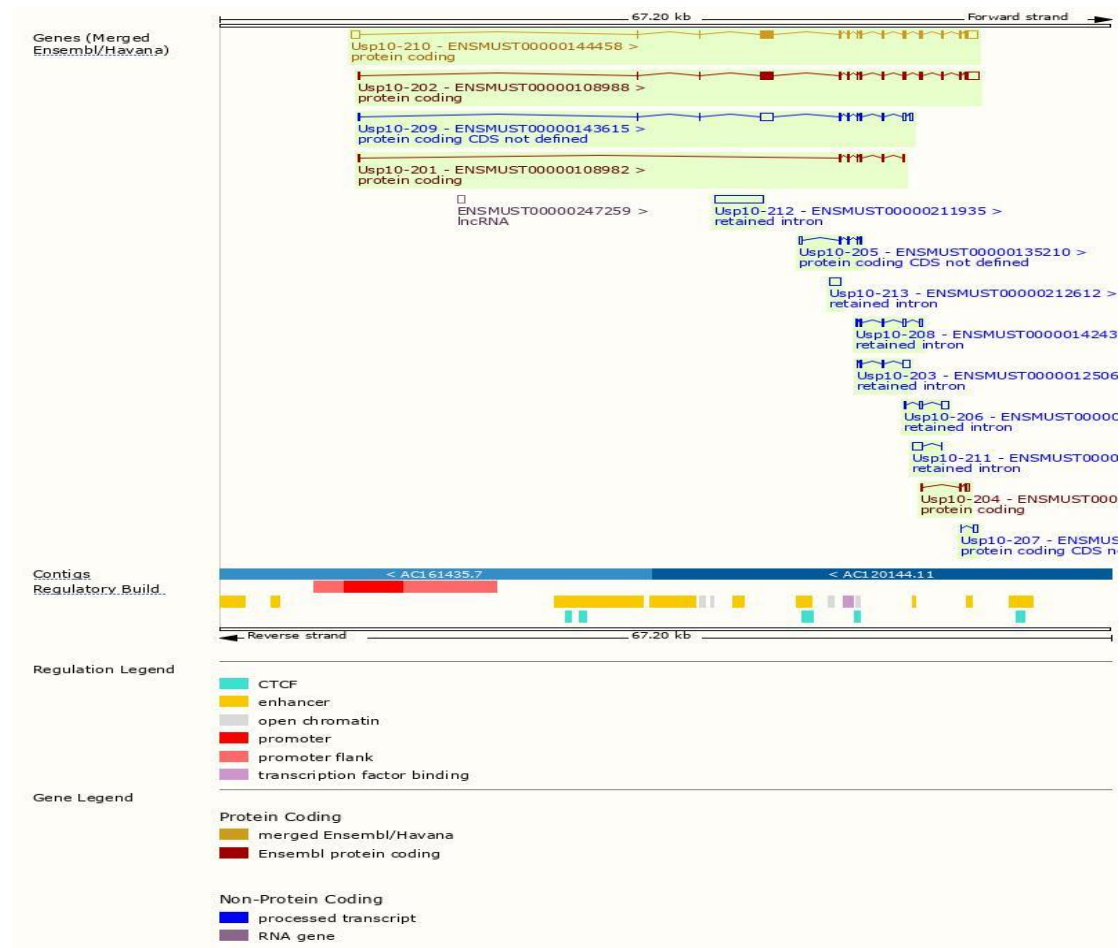
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Usp10-210	ENSMUST00000144458.8	3726	793aa	Protein coding	CCDS40495		A single transcript chosen for a gene which is the most conserved, most highly expressed, has the longest coding sequence and is represented in other key resources, such as NCBI and UniProt. This is defined in detail on http://www.ensembl.org/info/genome/genebuild/canonical.html Ensembl Canonical, The GENCODE set is the gene set for human and mouse. GENCODE basic, APPRIS P4, TSL:1,
Usp10-202	ENSMUST00000108989.9	3274	792aa	Protein coding	CCDS80938		The GENCODE set is the gene set for human and mouse. GENCODE basic, APPRIS ALT2, TSL:1,
Usp10-201	ENSMUST00000108982.11	559	114aa	Protein coding			TSL:5, CDS 3' incomplete,
Usp10-204	ENSMUST00000134729.2	424	88aa	Protein coding			TSL:3, CDS 5' incomplete,
Usp10-209	ENSMUST00000143615.8	2129	No protein	Protein coding CDS not defined			TSL:1,
Usp10-205	ENSMUST00000135210.2	467	No protein	Protein coding CDS not defined			TSL:5,
Usp10-207	ENSMUST00000139648.2	459	No protein	Protein coding CDS not defined			TSL:5,
Usp10-212	ENSMUST00000211935.2	3723	No protein	Retained intron			TSL:NA,
Usp10-206	ENSMUST00000137310.2	831	No protein	Retained intron			TSL:2,
Usp10-213	ENSMUST00000212612.2	761	No protein	Retained intron			TSL:NA,
Usp10-203	ENSMUST00000125066.2	747	No protein	Retained intron			TSL:3,
Usp10-211	ENSMUST00000148767.2	717	No protein	Retained intron			TSL:3,
Usp10-208	ENSMUST00000142432.8	665	No protein	Retained intron			TSL:2,

The strategy is based on the design of *Usp10-210* transcript, the transcription is shown below:



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

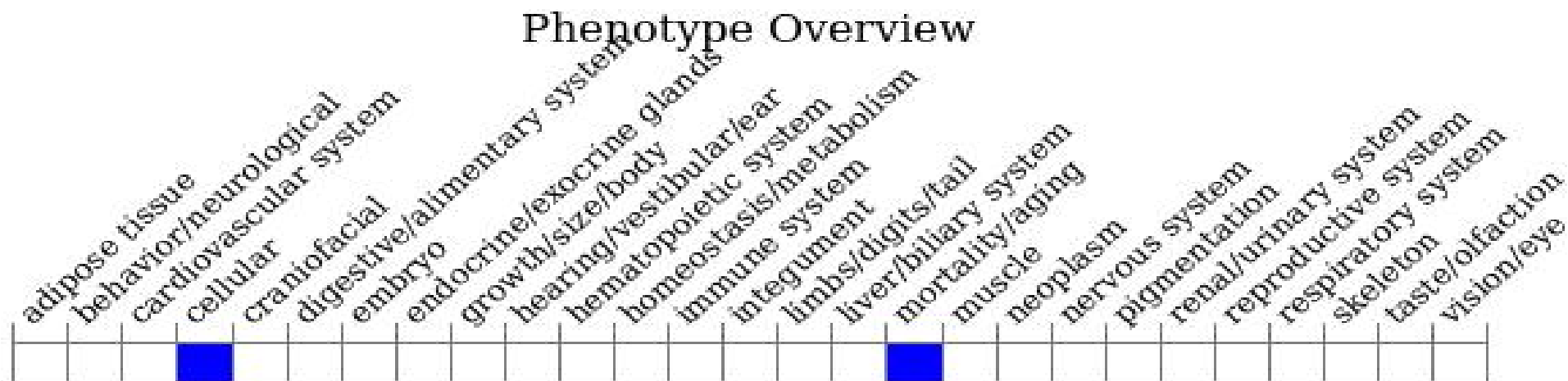
Genomic Information



Protein Information



Mouse Phenotype Information (MGI)



- Homozygous inactivation of this gene leads to alterations in arsenite-induced stress granule formation, reactive oxygen species (ROS) production, and ROS-dependent apoptosis in mouse embryonic fibroblasts.

Important Information

- According to MGI, homozygous mice that knock out this gene completely die before weaning.
- This strategy may not affect *Usp10*-203~208 and *Usp10*-211~*Usp10*-213 transcript.
- *Usp10* is located on Chr8. 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.

Reference

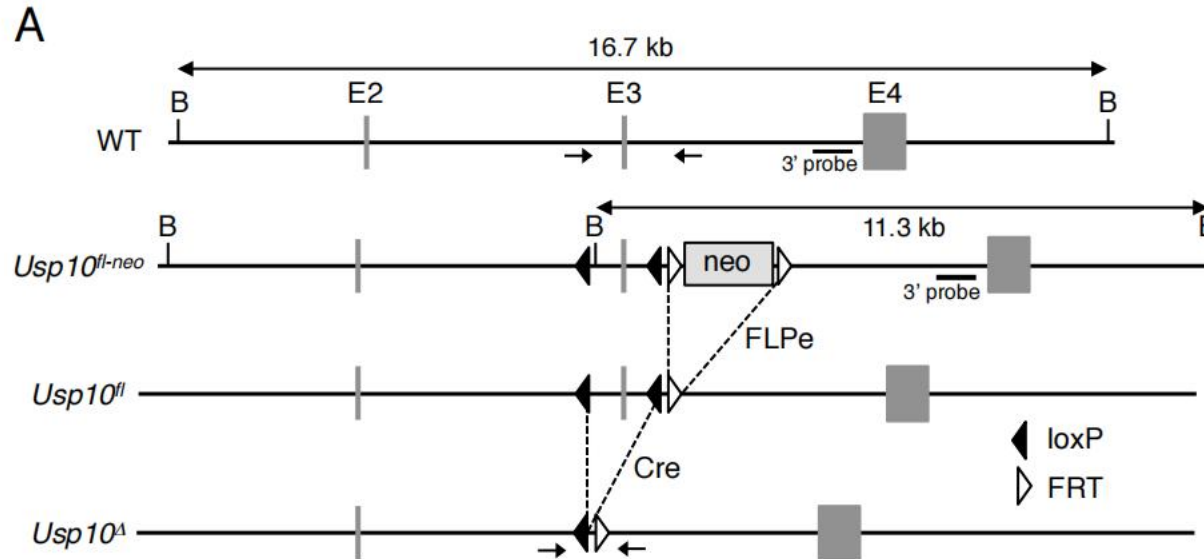


Figure S1 related to figure 1. Generation of USP10-KO mice. (A) Genomic structure of mouse *Usp10*. Grey rectangles (E2, E3, E4) represent *Usp10* exons. Neo indicates the Neomycin selection cassette flanked by FRT recombination sites (white triangles), inserted between exon 3 and 4. Black triangles represent the loxP sites, flanking exon 3. Removal of the Neomycin cassette by crossing with *CAG-FLPe* mice resulted in the generation of the conditional *Usp10^{fl}* allele. The conditional *Usp10^{fl}* allele was converted to the recombined *Usp10^Δ* allele by crossing with *TLCN-Cre* mice. Positions of primers used for genotyping are indicated as small arrows. The Bgl II recognition sites were indicated as B. (B) Southern blot analysis of the tail DNA derived from *Usp10^{fl-neo/+}* heterozygous mice. (C) PCR genotyping of USP10-KO mice. The primer sequences are 5'-GGTGTGTTGGGGCTCGGTTCTGTCA-3' and 5'-TGGCAGTTGTGGTGGTTTGAGTATG-3'. (D) USP10 protein expression in USP10-WT and KO MEFs. Total cell lysates were prepared from USP10-WT and KO MEFs and probed with anti-USP10 or anti-eIF4E antibody.

Higuchi M, Kawamura H, Matsuki H, Hara T, Takahashi M, Saito S, Saito K, Jiang S, Naito M, Kiyonari H, Fujii M. USP10 Is an Essential Deubiquitinase for Hematopoiesis and Inhibits Apoptosis of Long-Term Hematopoietic Stem Cells. *Stem Cell Reports*. 2016 Dec 13;7(6):1116-1129.