

H2-Ke6 Cas9-CKO Strategy

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Overview

Target Gene Name

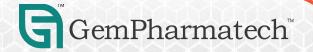
• H2-Ke6

Project Type

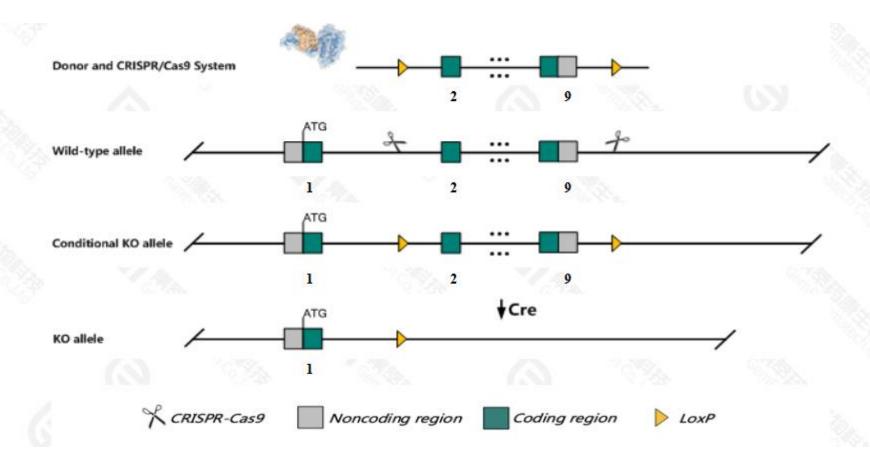
• Cas9-CKO

Genetic Background

• C57BL/6JGpt



Strain Strategy

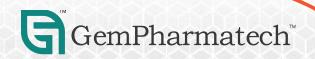


Schematic representation of CRISPR-Cas9 engineering used to edit the *H2-Ke6* gene.



Technical Information

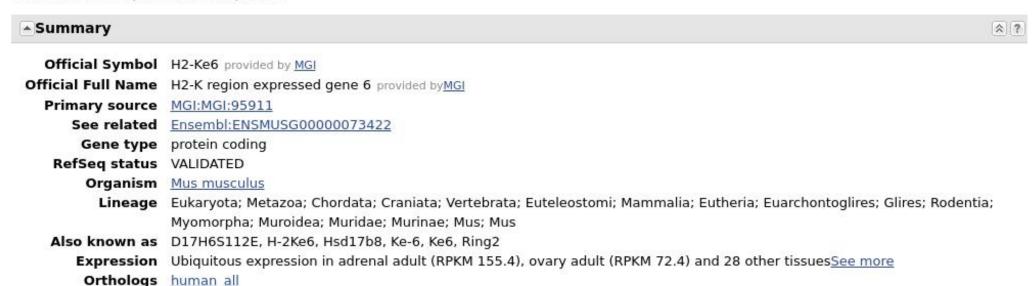
- The *H2-Ke6* gene has 12 transcripts. According to the structure of *H2-Ke6* gene, exon2-exon9 of *H2-Ke6-201*(ENSMUST00000045467.14) transcript is recommended as the knockout region. The region contains 734bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *H2-Ke6* 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

H2-Ke6 H2-K region expressed gene 6 [Mus musculus (house mouse)]

Gene ID: 14979, updated on 24-Apr-2022



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

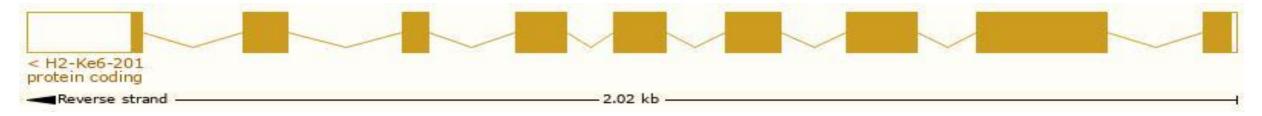


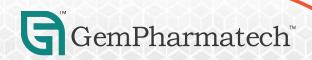
Transcript Information

The gene has 12 transcripts, all transcripts are shown below:

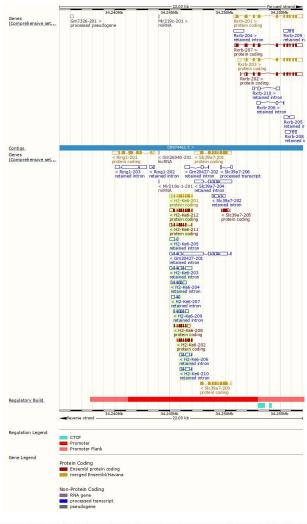
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
H2-Ke6-201	ENSMUST00000045467.14	965	259aa	Protein coding	CCDS50071		TSL:1, GENCODE basic, APPRIS P1
H2-Ke6-212	ENSMUST00000237759.2	1133	274aa	Protein coding	=		GENCODE basic ,
H2-Ke6-211	ENSMUST00000236627.2	733	<u>147aa</u>	Protein coding	12		GENCODE basic ,
H2-Ke6-208	ENSMUST00000174399.8	728	138aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
H2-Ke6-202	ENSMUST00000114303.4	716	234aa	Protein coding			CDS 3' incomplete , TSL:5 ,
H2-Ke6-203	ENSMUST00000170644.8	1293	No protein	Retained intron	37		TSL:5,
H2-Ke6-206	ENSMUST00000173894.2	753	No protein	Retained intron	-		TSL:3,
H2-Ke6-209	ENSMUST00000235579.2	745	No protein	Retained intron	32		
H2-Ke6-210	ENSMUST00000236164.2	731	No protein	Retained intron	-		
H2-Ke6-204	ENSMUST00000173425.9	709	No protein	Retained intron	=		TSL:3,
H2-Ke6-205	ENSMUST00000173616.8	534	No protein	Retained intron	-		TSL:3,
H2-Ke6-207	ENSMUST00000174029.8	459	No protein	Retained intron	-		TSL:2,

The strategy is based on the design of H2-Ke6-201 transcript, the transcription is shown below:





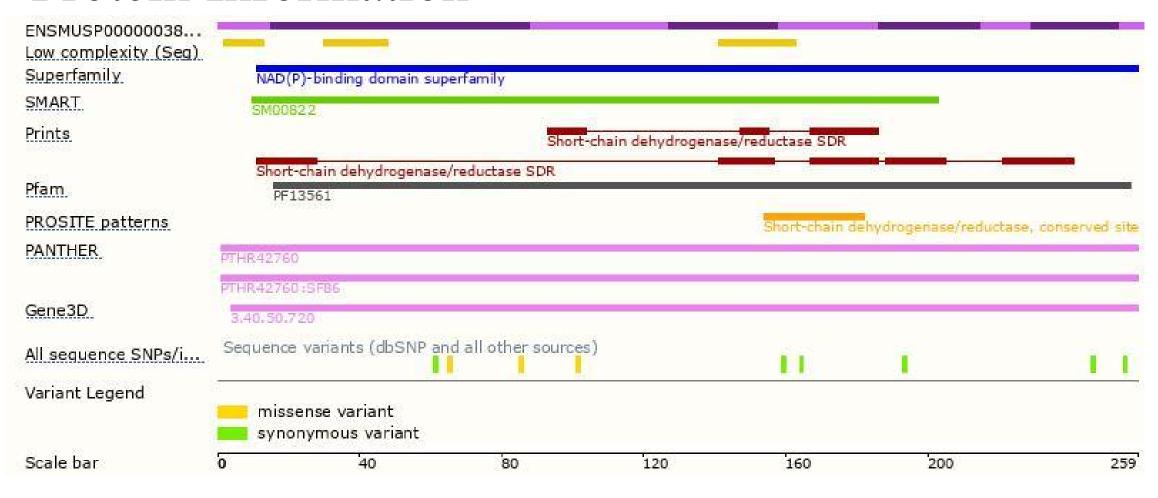
Genomic Information





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

Protein Information





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

Important Information

- The KO region contains functional region of the H2-Ke6 gene. Knockout the region may affect the function of Ring1 \, Gm26940 and Gm20427 gene.
- The intron1 is only 161bp,loxp insertion may affect mRNA splicing.
- The *H2-Ke6* gene is located on the Chr17. 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 the existing technology level.

