

Map3k1 Cas9-KO Strategy

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Overview

Target Gene Name

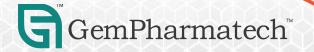
• Map3k1

Project Type

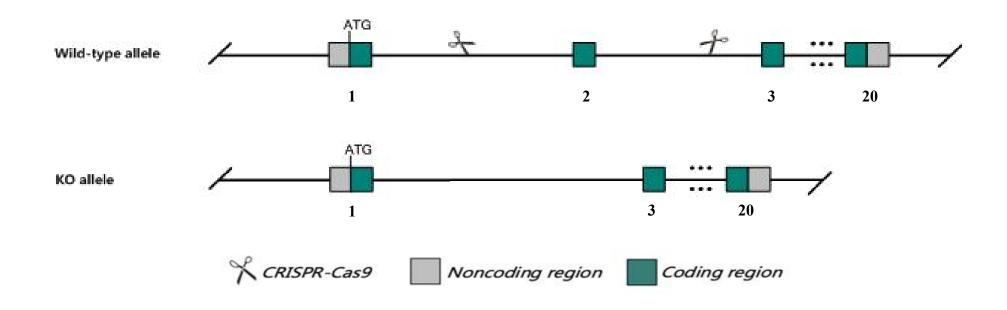
• Cas9-KO

Genetic Background

• C57BL/6JGpt



Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the Map3k1 gene.



Technical Information

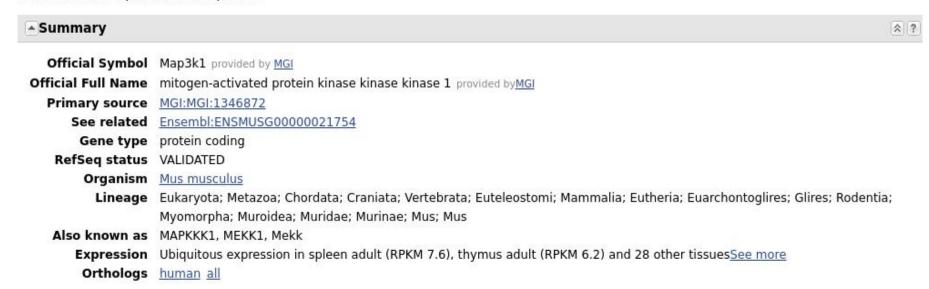
- The *Map3k1* gene has 5 transcripts. According to the structure of *Map3k1* gene, exon2 of *Map3k1*-201 (ENSMUST00000109267.9) transcript is recommended as the knockout region. The region contains 151bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Map3k1* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs 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.



Gene Information

Map3k1 mitogen-activated protein kinase kinase kinase 1 [Mus musculus (house mouse)]

Gene ID: 26401, updated on 12-Apr-2023



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

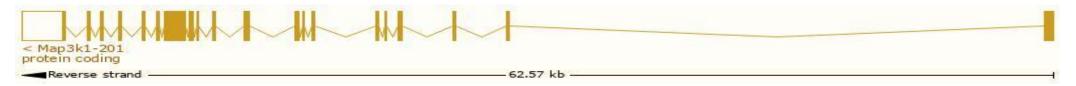


Transcript Information

The gene has 5 transcripts, all transcripts are shown below:

Name	Transcrip	ot ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Map3k1-201	ENSMUSTO0000	0109267.9	6978	<u>1493aa</u>	Protein coding	CCDS26771		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 INCBI and UniProt. This is defined in detail on http://www.ensembl.org/info/genome/genebuild/caronical.html Ensembl Caronical, The GENCODE set is the gene set for human and mouse. GENCODE basic, APPRIS P1, TSL2,
Map3k1-204	<u>ENSMUSTOOOO</u>	0145055.2	867	<u>263aa</u>	Protein coding			TSL-3 , COS 3 incomplete ,
Map3k1-203	ENSMUST00000	133364.2	443 N	No protein P	rotein coding CDS not defined			TSL3,
Map3k1-205	ENSMUSTO0000	0175936.2	544 1	No protein	Retained intron			TSL3,
Map3k1-202	ENSMUST00000	0130594.2	396 N	No protein	Retained intron			TSL5.

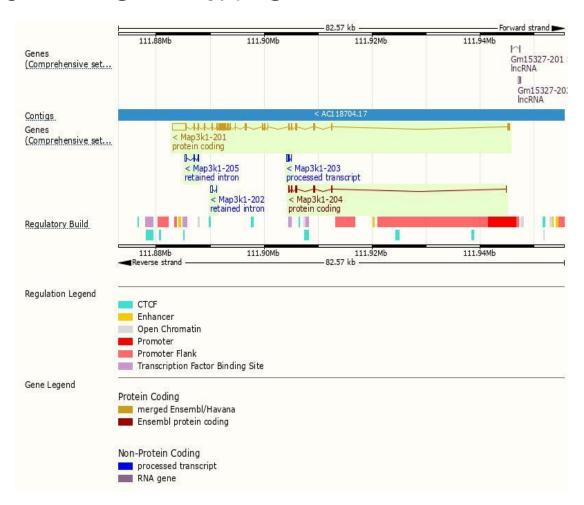
The strategy is based on the design of *Map3k1*-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org



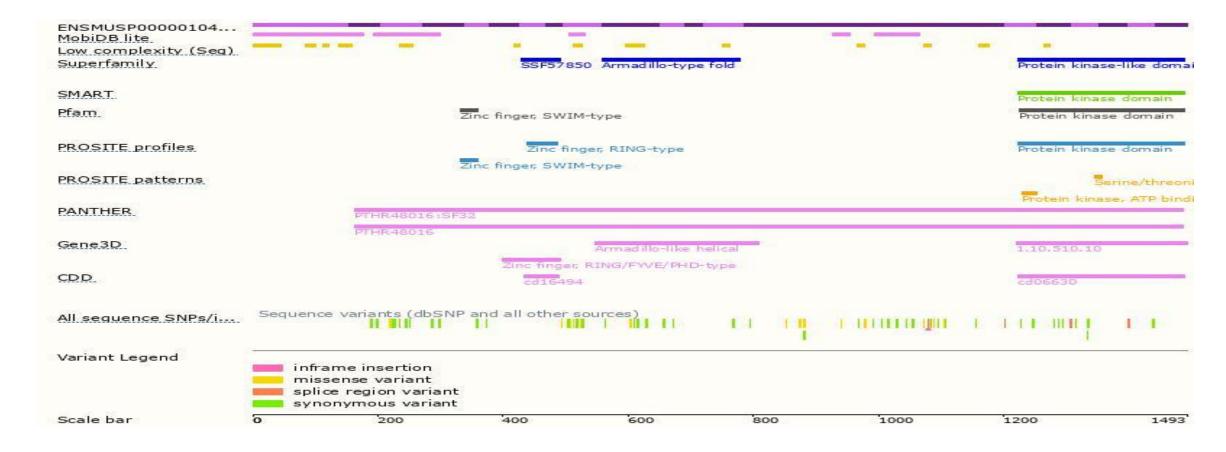
Genomic Information





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

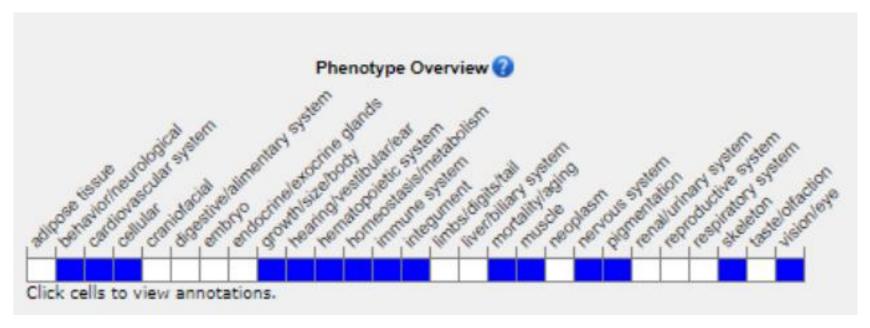
Protein Information





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

Mouse Phenotype Information (MGI)



• Mice homozygous for a spontaneous allele are born with one or both eyes open, defects in eye morphology, and defects in ear morphology and function. Mice homozygous for a knock-out allele are born with open eyes and exhibit increased response to aortic banding and blood vessel healing.



Source: https://www.informatics.jax.org

Important Information

- According to MGI information and breeding data, the gene knockout homozygote mice died at embryonic stage.
- Transcript Map3k1-202, Map3k1-203, Map3k1-205 may not be affected.
- *Map3k1* is located on Chr13. 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 risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

