

# Map3k6 Cas9-CKO Strategy

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**Reviewer:** Jing Jin

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# **Project Overview**



**Project Name** 

Map3k6

**Project type** 

Cas9-CKO

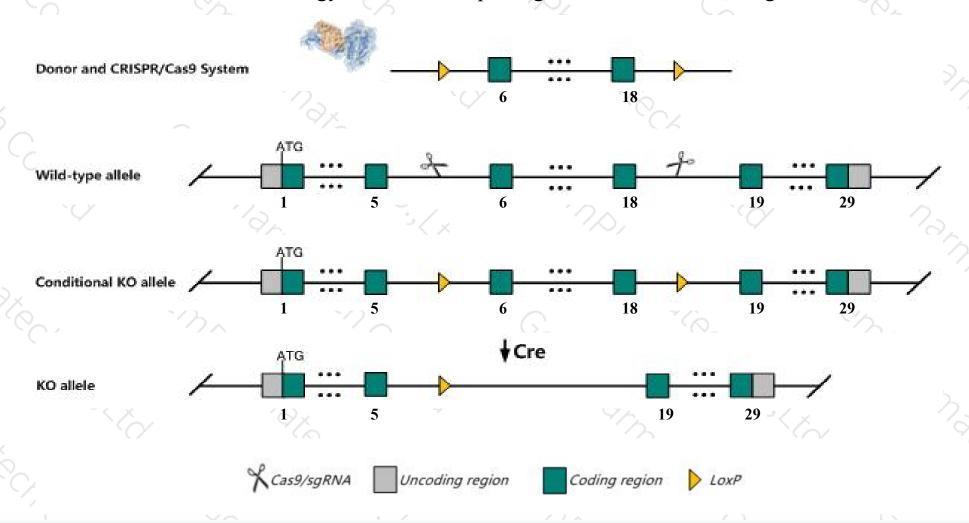
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Map3k6 gene. The schematic diagram is as follows:



### Technical routes



- The *Map3k6* gene has 4 transcripts. According to the structure of *Map3k6* gene, exon6-exon18 of *Map3k6-201* (ENSMUST00000030677.6) transcript is recommended as the knockout region. The region contains 1561bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Map3k6* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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, Homozygous and heterozygous null mice display an increased incidence of chemically induced skin tumors and homozygous mice also show resistance to induced apoptosis.
- ➤ Transcript *Map3k6-204* lncRNA may not be affected.
- The *Map3k6* gene is located on the Chr4. 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)



#### Map3k6 mitogen-activated protein kinase kinase kinase 6 [ Mus musculus (house mouse) ]

Gene ID: 53608, updated on 14-Sep-2019

#### Summary

2 7

Official Symbol Map3k6 provided by MGI

Official Full Name mitogen-activated protein kinase kinase kinase 6 provided by MGI

Primary source MGI:MGI:1855691

See related Ensembl:ENSMUSG00000028862

Gene type protein coding
RefSeq status VALIDATED
Organism Mus musculus

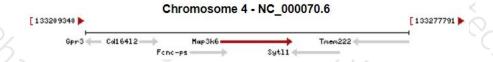
Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;

Muroidea; Muridae; Murinae; Mus; Mus

Also known as Ask2; MEKK6; MAPKKK6

Expression Broad expression in large intestine adult (RPKM 10.4), genital fat pad adult (RPKM 9.5) and 21 other tissues See more

Orthologs human all



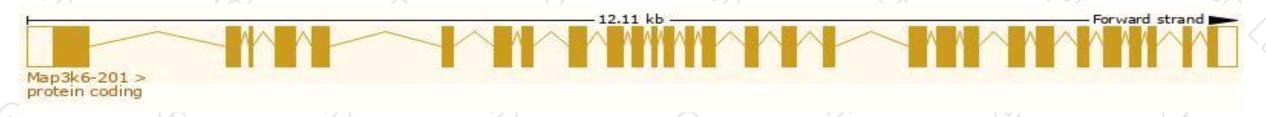
# Transcript information (Ensembl)



The gene has 4 transcripts, all transcripts are shown below:

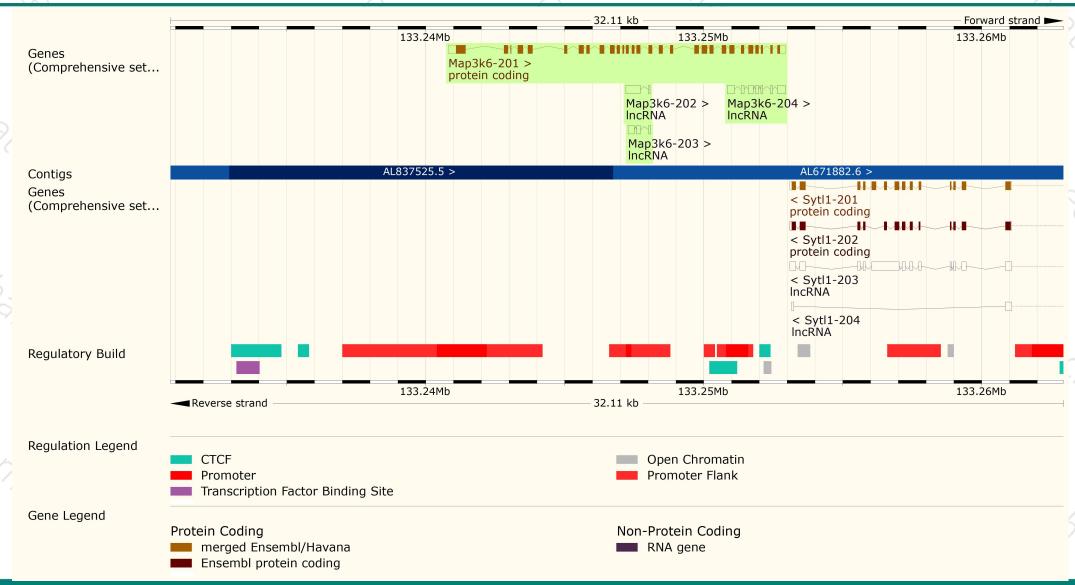
| Name 🍦     | Transcript ID 🖕      | bp 🌲 | Protein       | Translation ID       | Biotype 🍦      | CCDS 🍦             | UniProt 🍦   | Flags                         |
|------------|----------------------|------|---------------|----------------------|----------------|--------------------|-------------|-------------------------------|
| Map3k6-201 | ENSMUST00000030677.6 | 4334 | <u>1291aa</u> | ENSMUSP00000030677.6 | Protein coding | CCDS18744₽         | A0A0A0MQ82₽ | TSL:1 GENCODE basic APPRIS P1 |
| Map3k6-204 | ENSMUST00000134895.1 | 1062 | No protein    | -                    | IncRNA         | 1343               | (4)         | TSL:1                         |
| Map3k6-202 | ENSMUST00000123612.1 | 570  | No protein    | -                    | IncRNA         | 19 <del>4</del> 31 | (4)         | TSL:3                         |
| Map3k6-203 | ENSMUST00000127681.1 | 443  | No protein    | -                    | IncRNA         | 1341               | (4)         | TSL:2                         |

The strategy is based on the design of Map3k6-201 transcript, The transcription is shown below



### Genomic location distribution





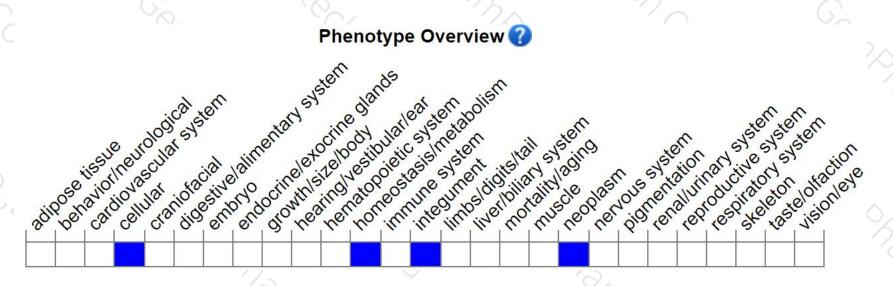
### Protein domain





# Mouse phenotype description(MGI)





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

According to the existing MGI data, Homozygous and heterozygous null mice display an increased incidence of chemically induced skin tumors and homozygous mice also show resistance to induced apoptosis.



If you have any questions, you are welcome to inquire.

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