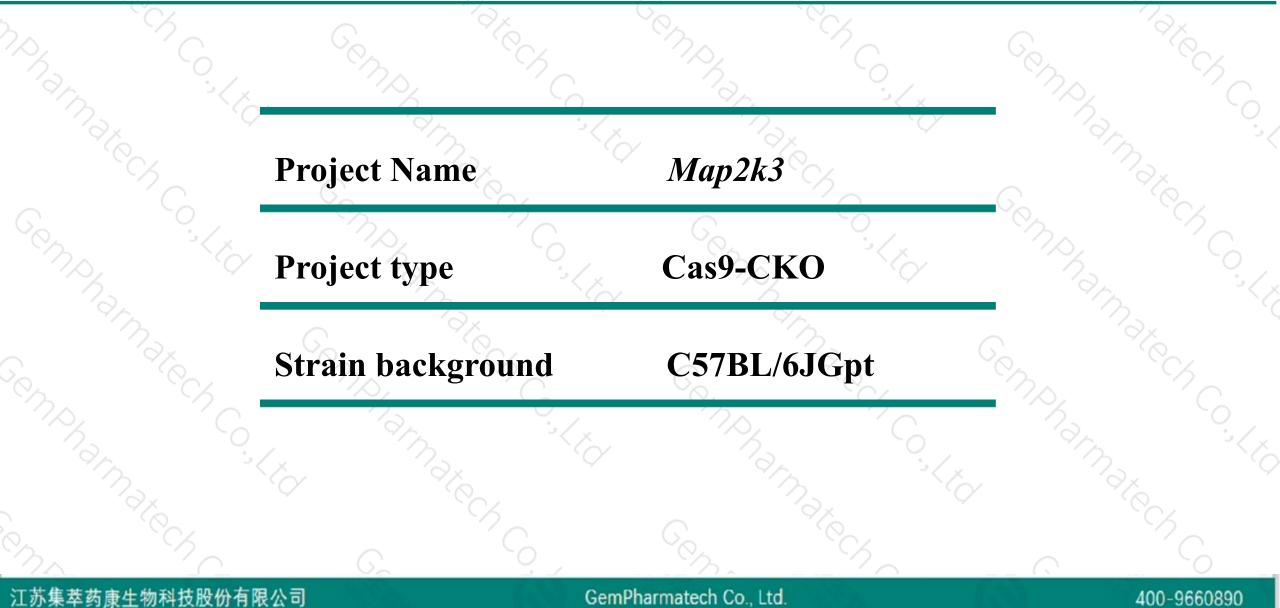


Map2k3 Cas9-CKO Strategy

Designer: Yanhua Shen Reviewer: Xueting Zhang Design Date: 2019-09-03

Project Overview



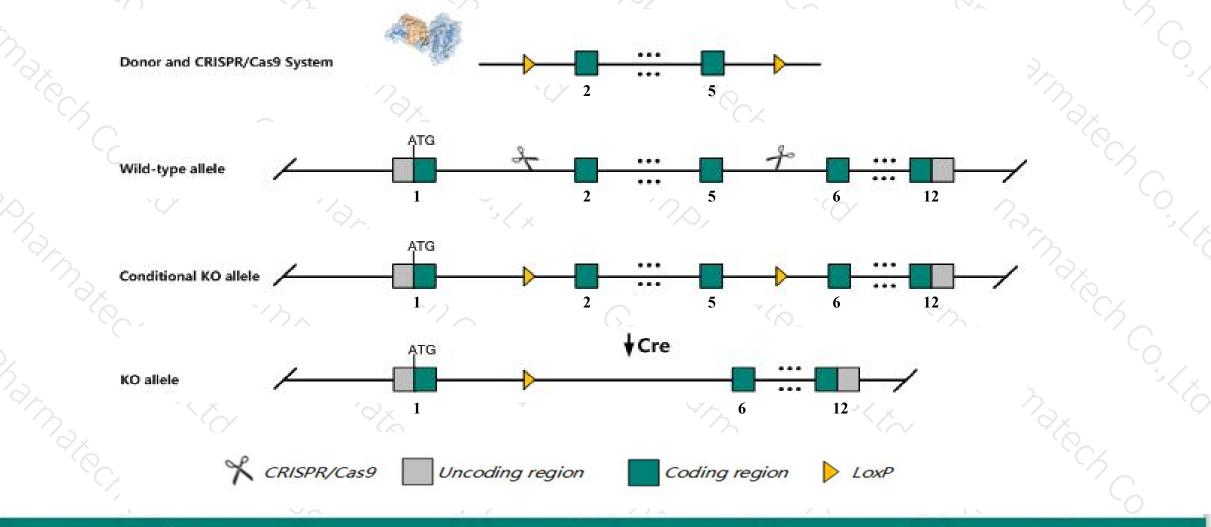


Conditional Knockout strategy



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This model will use CRISPR/Cas9 technology to edit the *Map2k3* gene. The schematic diagram is as follows:



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The Map2k3 gene has 6 transcripts. According to the structure of Map2k3 gene, exon2-exon5 of Map2k3-201 (ENSMUST00000019076.9) transcript is recommended as the knockout region. The region contains 350bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Map2k3* 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, Mice homozygous for disruptions in this gene are viable and fertile but display abnormalities in cytokine production.

➤ Transcript 204 is unaffected.

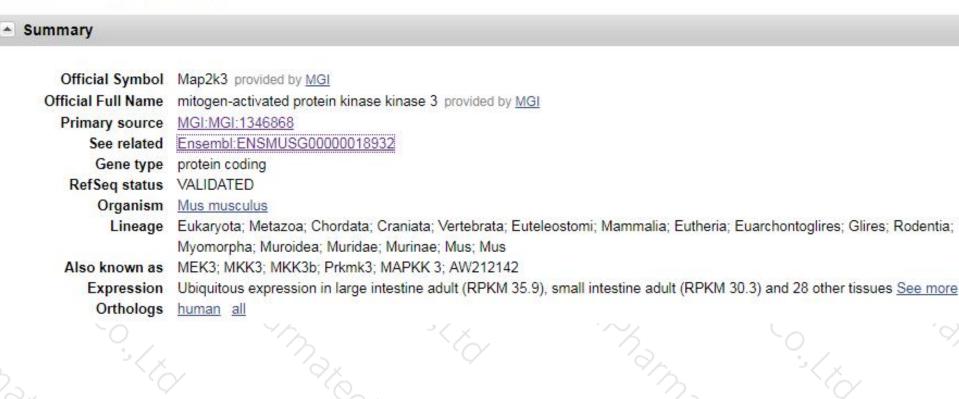
The Map2k3 gene is located on the Chr11. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.

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)

Gene ID: 26397, updated on 12-Aug-2019

Map2k3 mitogen-activated protein kinase kinase 3 [Mus musculus (house mouse)]





| < ?

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

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|------------|----------------------|------|--------------|-------------------------|---------------|---------------|-------------------------------|
| Map2k3-201 | ENSMUST0000019076.9 | 2198 | <u>347aa</u> | Protein coding | CCDS24804 | 009110 Q5SWN9 | TSL:1 GENCODE basic APPRIS P1 |
| Map2k3-203 | ENSMUST00000130269.1 | 2096 | <u>173aa</u> | Nonsense mediated decay | 1.4 | A0A0R4J1Q6 | TSL:1 |
| Map2k3-206 | ENSMUST00000145828.7 | 1346 | No protein | Retained intron | 620 | 12 | TSL:1 |
| Map2k3-204 | ENSMUST00000137609.1 | 600 | No protein | Retained intron | 35 <u>4</u> 3 | 20 | TSL:2 |
| Map2k3-205 | ENSMUST00000137739.1 | 455 | No protein | Retained intron | | 50 | TSL:2 |
| Map2k3-202 | ENSMUST00000126043.1 | 694 | No protein | IncRNA | 14.3 | | TSL:5 |

20.78 kb

The strategy is based on the design of Map2k3-201 transcript, The transcription is shown below

Map2k3-201 > protein coding

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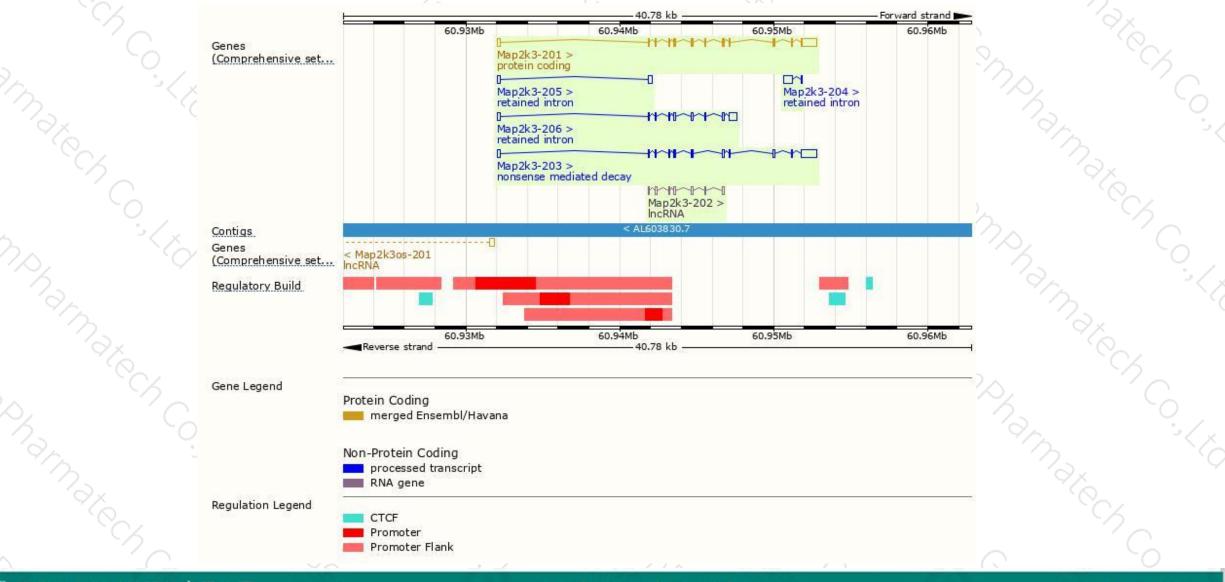
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Forward strand

Genomic location distribution



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Protein domain

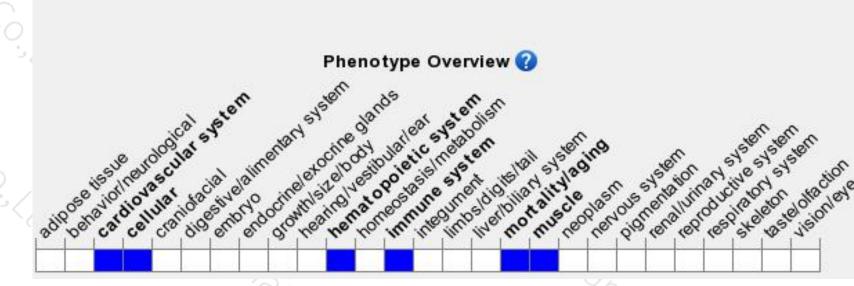
江苏集团



| | ENSMUSP00000019 SIFTS import MobiDB lite | | i e c s | | | | | | | |
|---|--|---|------------------|--|--|--|--|--|--|--|
| | Low complexity (Seg) Superfamily | Protein kinase-like domain superfamily | | | | | | | | |
| 0 | SMART | Protein kinase domain | | | | | | | | |
| | Pfam | Protein kinase domain | | | | | | | | |
| | PROSITE profiles | Protein kinase domain | | | | | | | | |
| | PROSITE patterns | Protein kinase, ATP binding site Serine/threonine-protein kinase, active site | 5 | | | | | | | |
| 2 | PIRSF | PIR:SF000654 | | | | | | | | |
| | PANTHER | PTHR44305:SF7 | | | | | | | | |
| | Gene3D | PTHR44305 3.30.200.20 1.10.510.10 | - 7 | | | | | | | |
| | CDD | cd06617 | <u> </u> | | | | | | | |
| $\langle \mathbf{P}_{\mathbf{r}} \rangle$ | All sequence SNPs/i | Sequence variants (dbSNP and all other sources) | ~ < ₍ | | | | | | | |
| | Variant Legend | missense variant synonymous variant | | | | | | | | |
| | Scale bar | 0 40 80 120 160 200 240 280 | 347 | | | | | | | |

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, Mice homozygous for disruptions in this gene are viable and fertile but display abnormalities in cytokine production.



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



