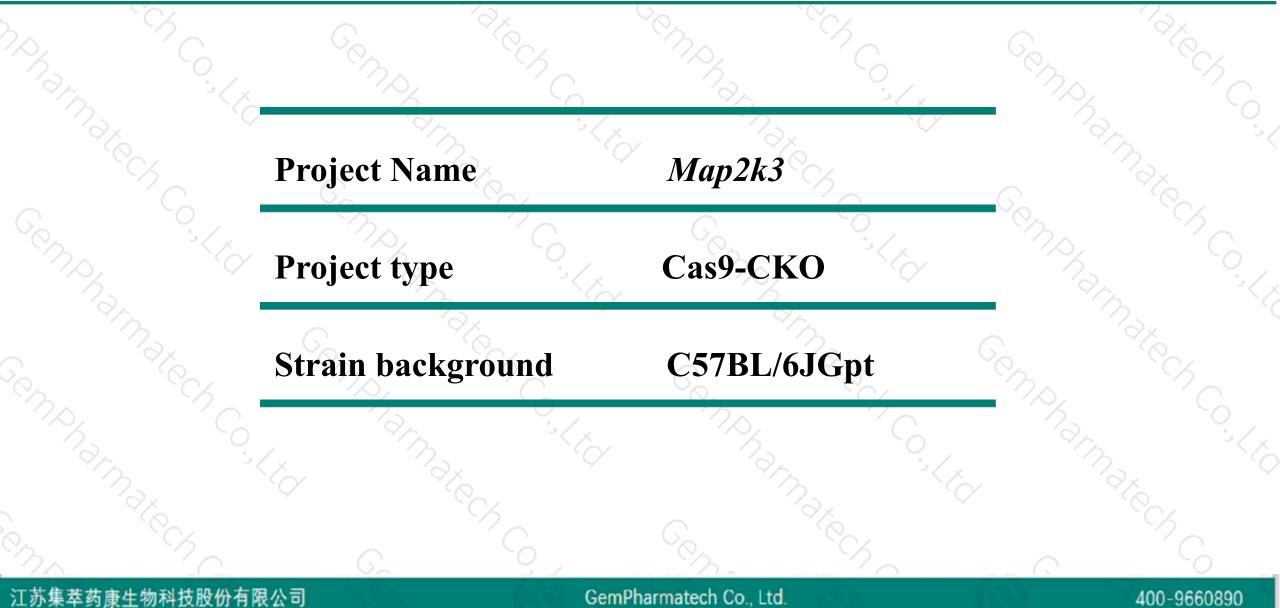


Map2k3 Cas9-CKO Strategy

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

Project Overview



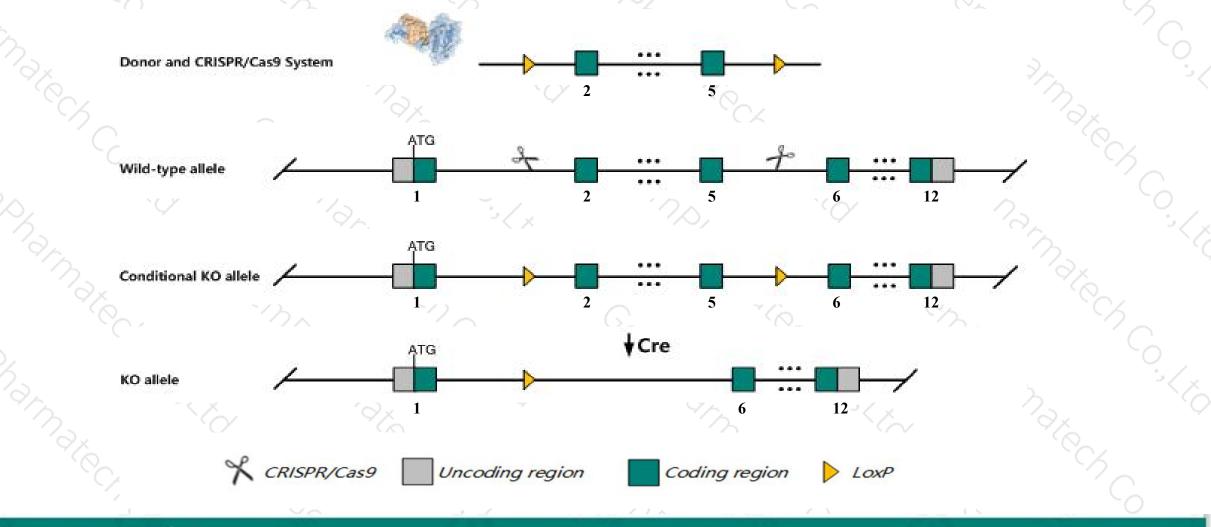


Conditional Knockout strategy



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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.

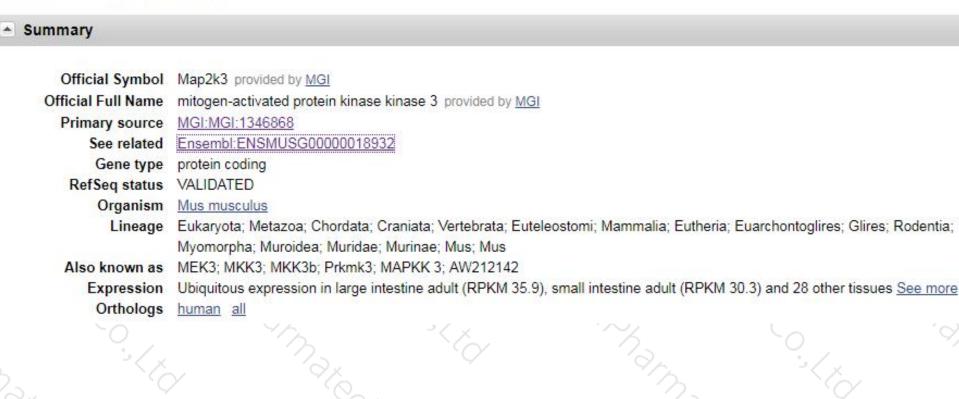
The Map2k3 gene is located on the Chr11. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.

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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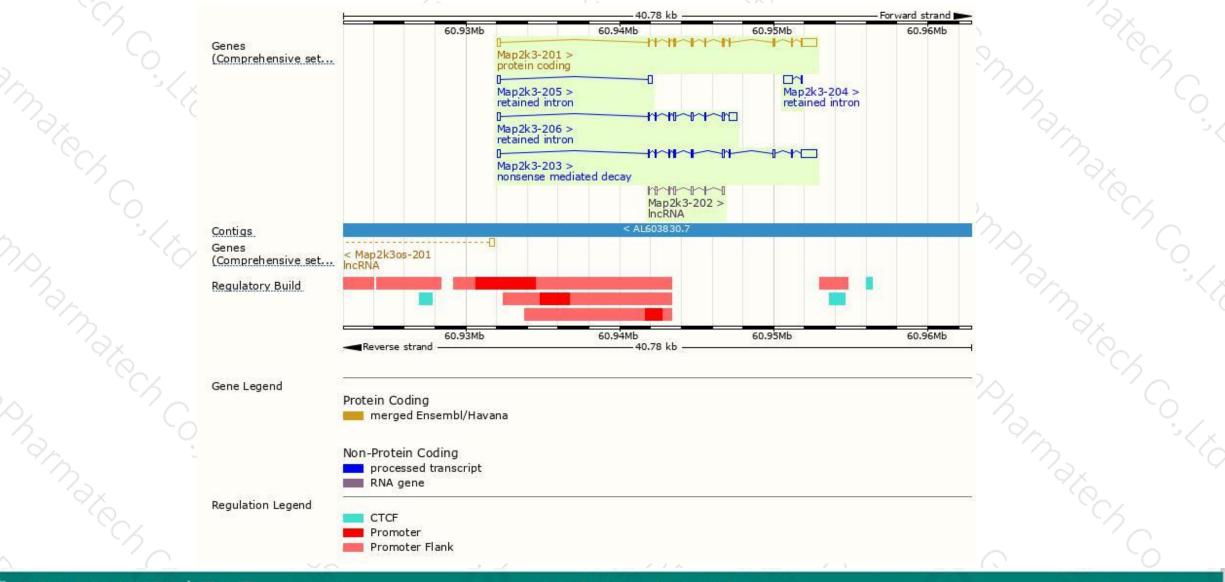
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Forward strand

Genomic location distribution



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Protein domain

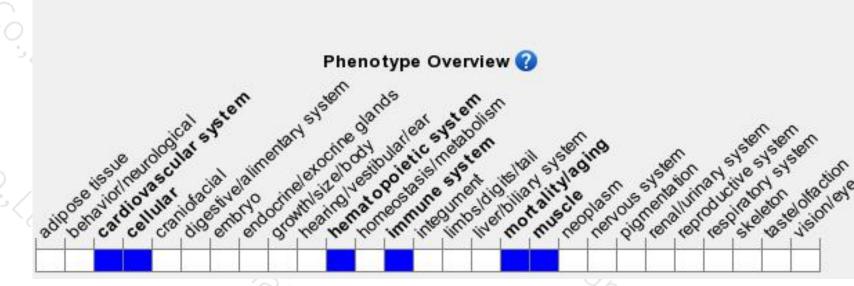
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	ENSMUSP00000019 SIFTS import MobiDB lite		i e c s							
	Low complexity (Seg) Superfamily	Protein kinase-like domain superfamily								
0	SMART	Protein kinase domain								
	Pfam	Protein kinase domain								
	PROSITE profiles	Protein kinase domain								
	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



