

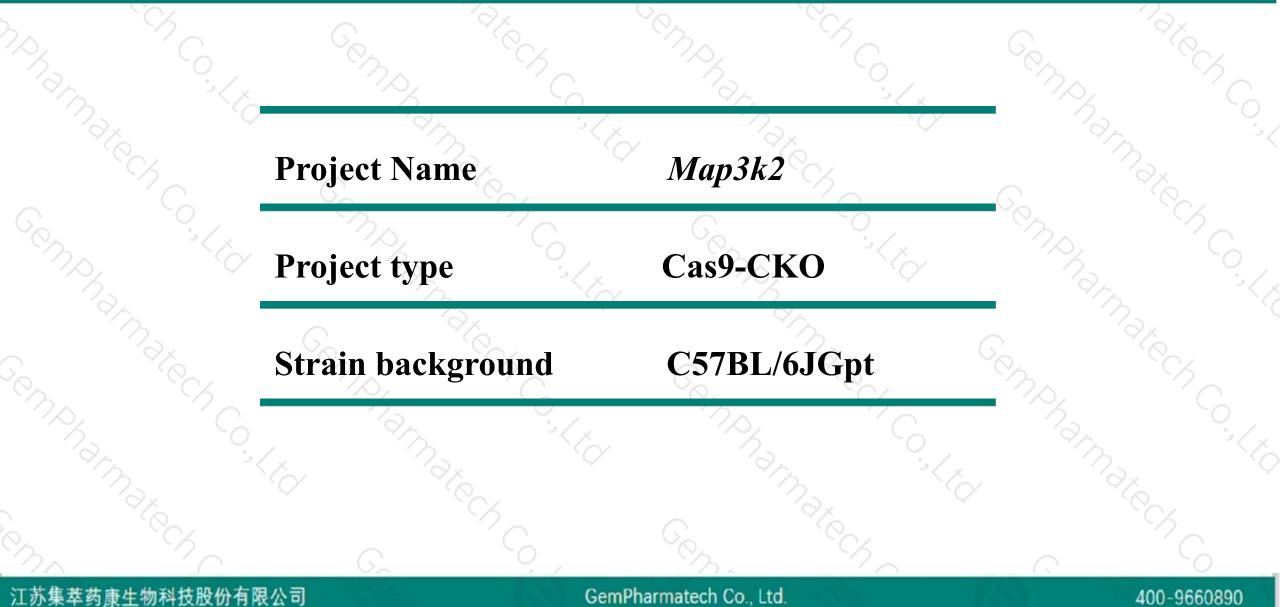
# Map3k2 Cas9-CKO Strategy

Designer: Reviewer: Design Date:

Yang Zeng Xueting Zhang 2019-10-28

# **Project Overview**



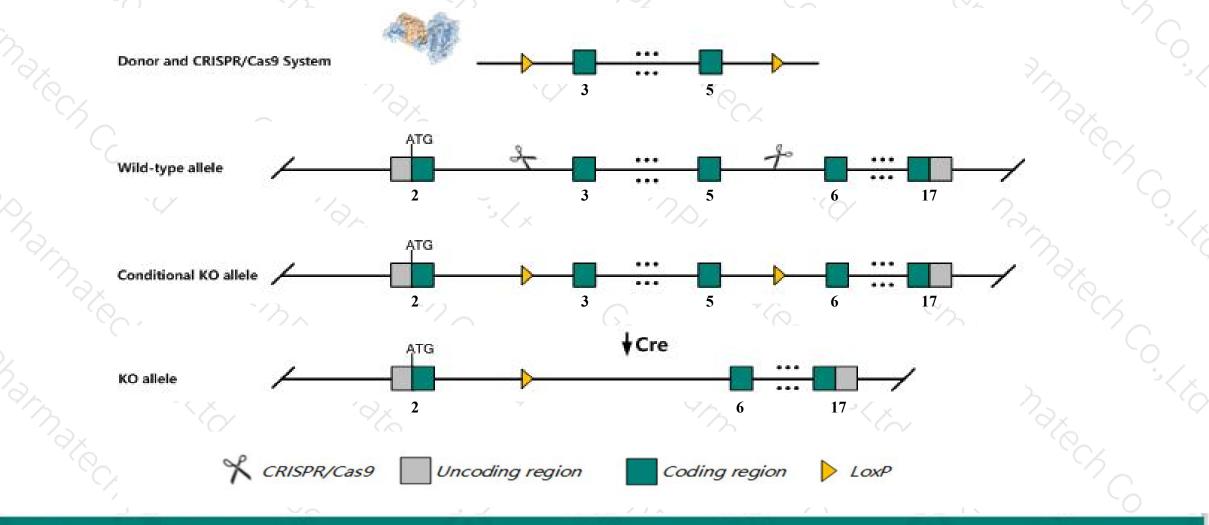


# **Conditional Knockout strategy**



400-9660890

This model will use CRISPR/Cas9 technology to edit the Map3k2 gene. The schematic diagram is as follows:



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The Map3k2 gene has 3 transcripts. According to the structure of Map3k2 gene, exon3-exon5 of Map3k2-201 (ENSMUST00000096575.4) transcript is recommended as the knockout region. The region contains 260bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Map3k2* 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 grossly normal and fertile.
- The Map3k2 gene is located on the Chr18. 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)



Map3k2 mitogen-activated protein kinase kinase kinase 2 [ Mus musculus (house mouse) ] Gene ID: 26405, updated on 10-Oct-2019 Summary ☆ ? Official Symbol Map3k2 provided by MGI Official Full Name mitogen-activated protein kinase kinase kinase 2 provided by MGI Primary source MGI:MGI:1346873 See related Ensembl:ENSMUSG0000024383 Gene type protein coding RefSeg 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 Mekk2; Mekk2b; Al585793; 9630061B06Rik Ubiquitous expression in CNS E18 (RPKM 3.3), placenta adult (RPKM 2.8) and 28 other tissues See more Expression Orthologs human all Chromosome 18 - NC\_000084.6 321 40 729 32435740 A830052D11Rik 🔶 Gm5969 Ercc3= Gm26717 Bin1 Gm34806

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# **Transcript information (Ensembl)**



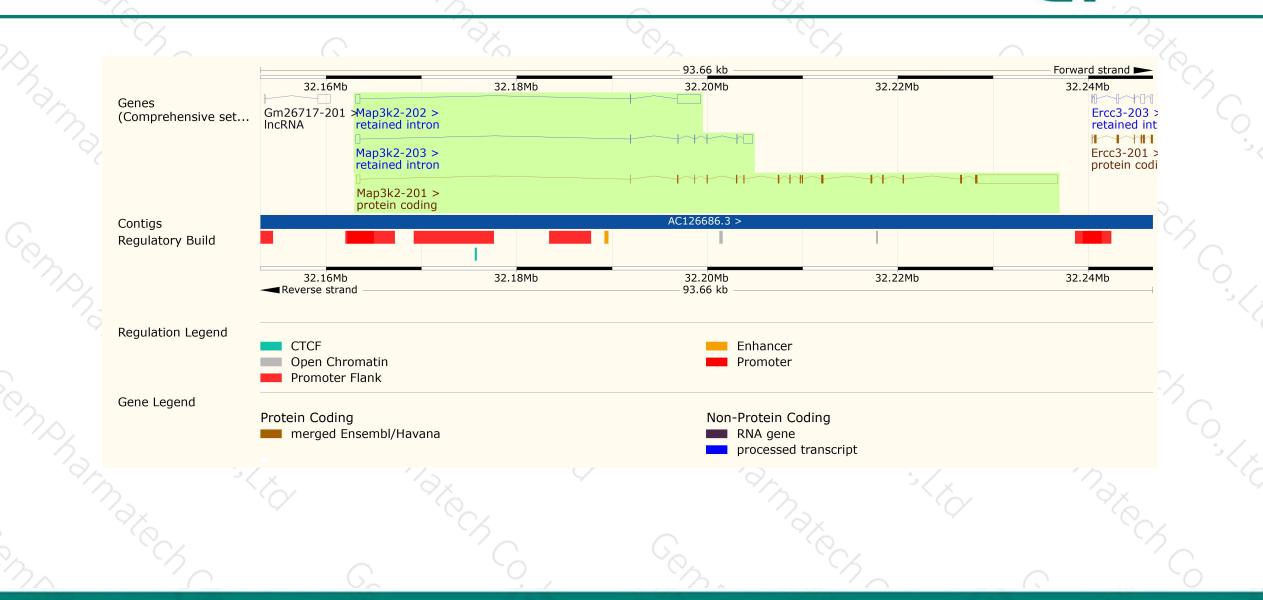
## The gene has 3 transcripts, all transcripts are shown below:

Name 💧	Transcript ID 💧	bp 🍦	Protein 🖕	Translation ID	Biotype 💧	CCDS 🖕	UniProt 🖕	Flags 🍦		
Map3k2-201	ENSMUST0000096575.4	10684	<u>619aa</u>	ENSMUSP0000094326.3	Protein coding	<u>CCDS29117</u> &	<u>G5E8L8</u>	TSL:1 GENCODE basic APPRIS P1		
Map3k2-202	ENSMUST00000234576.1	2989	No protein	-	Retained intron	-	-	-		
Map3k2-203	ENSMUST00000234611.1	1852	No protein		Retained intron	0 <del>.0</del>	-	-		

The strategy is based on the design of Map3k2-201 transcript, The transcription is shown below

73.56 kb Forward strand Map3k2-201 > protein coding

# **Genomic location distribution**



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# **Protein domain**



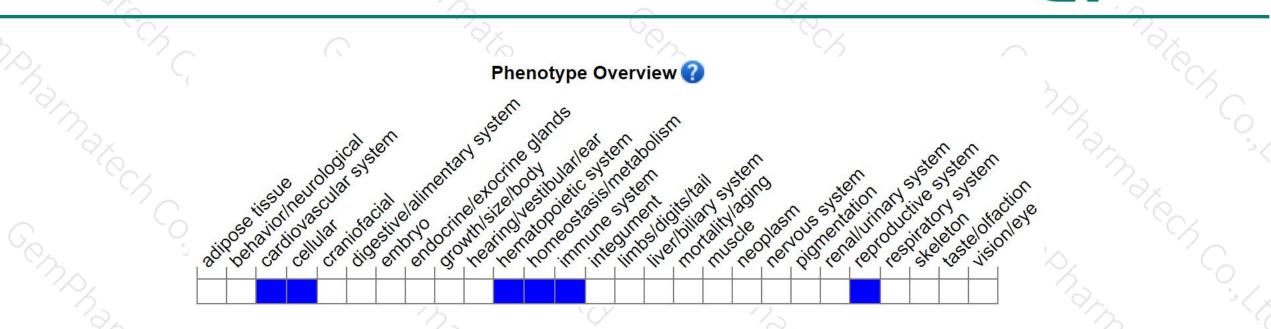
	°Ч с	G	d x	Con a	°C/	(	2	12	X Ca			
	ENSMUSP00000094 MobiDB lite Low complexity (Seg) Superfamily	SSF54277			Protein kinase-like domain s	uperfamily			C C			
	SMART	PB1 domain			Protein kinase domain							
	Pfam	PB1 domain			Protein kinase domain							
	PROSITE profiles	PB1 domain			Protein kinase domain			(	-7 <sub>0</sub>			
	PROSITE patterns PANTHER	PTHR24361:SF342			Protein kinase, ATP bindir	ng site			0			
9	Gene3D	PTHR24361 3.10.20.90			1.10.510.10							
	CDD	Mitogen-activated protein kinase l	kinase kinase 2/3, PB1 domain		cd06652							
	All sequence SNPs/i	Sequence variants (dbSNP and all other so	ources)	I I		1 1	I I	ĺ.	0			
	Variant Legend	missense variant synonymous variant										
	Scale bar	0 60 120	180 240	300	360 420	480	540	619				

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# 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 grossly normal and fertile.



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



