

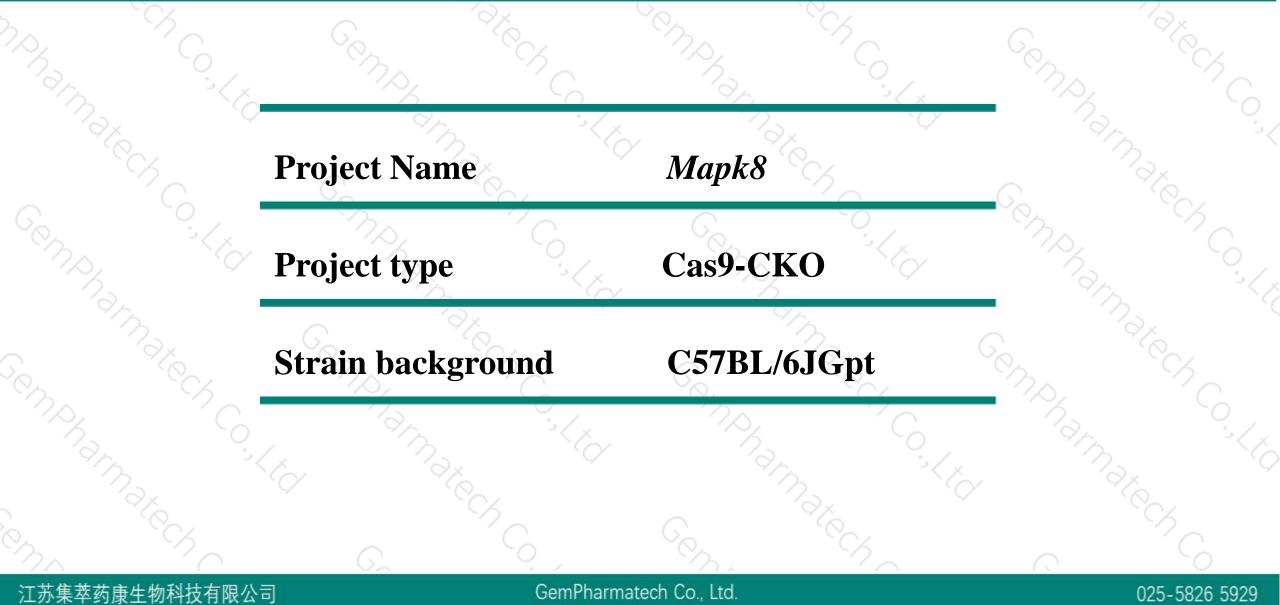
Mapk8 Cas9-CKO Strategy Enphamaten Co-1ty

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Designer: Yanhua Shen Design Date: 2019-08-07 harmatech

Project Overview



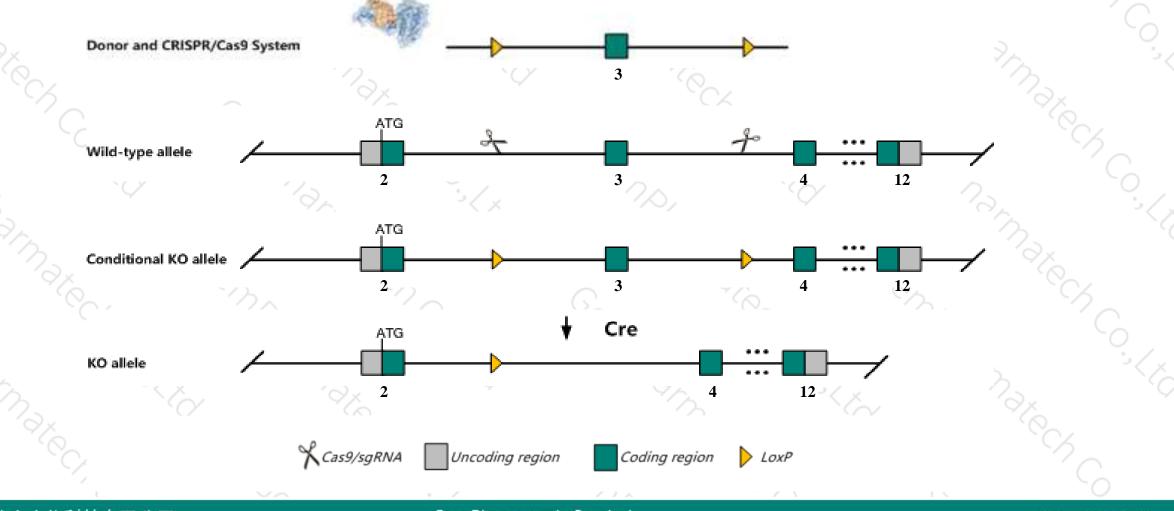


Conditional Knockout strategy



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This model will use CRISPR/Cas9 technology to edit the Mapk8 gene. The schematic diagram is as follows:



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- The Mapk8 gene has 11 transcripts. According to the structure of Mapk8 gene, exon3 of Mapk8-201 (ENSMUST0000022504.11) transcript is recommended as the knockout region. The region contains 130bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Mapk8* 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.



- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit abnormal T cell differentiation and proliferation, cardiac morphology and physiology, and chemically-induced tumorigenesis. Mice homozygous for another knock-out allele exhibit abnormal glucose homeostasis and T cell physiology.
- The Mapk8 gene is located on the Chr14. 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)



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Mapk8 mitogen-activated protein kinase 8 [Mus musculus (house mouse)]

Gene ID: 26419, updated on 19-Mar-2019

Summary

Official Symbol	Mapk8 provided by MGI							
Official Full Name	mitogen-activated protein kinase 8 provided by MGI							
Primary source	<u>MGI:MGI:1346861</u>							
See related	Ensembl:ENSMUSG00000021936							
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	Al849689, JNK, JNK1, Prkm8, SAPK1							
Expression	Broad expression in CNS E18 (RPKM 19.1), CNS E14 (RPKM 18.4) and 26 other tissuesSee more							
Orthologs	human all							

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Transcript information (Ensembl)



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The gene has 11 transcripts, all transcripts are shown below:

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Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mapk8-205	ENSMUST00000111945.8	5756	<u>384aa</u>	Protein coding	CCDS36869	Q544A0 Q91Y86	TSL:1 GENCODE basic
Mapk8-201	ENSMUST00000022504.11	2587	<u>427aa</u>	Protein coding	CCDS79293	<u>G3X8U9</u>	TSL:1 GENCODE basic APPRIS P5
Mapk8-204	ENSMUST00000111944.9	1528	<u>427aa</u>	Protein coding	CCDS79295	Q7TSJ7	TSL:1 GENCODE basic APPRIS ALT
Mapk8-203	ENSMUST00000111943.7	1310	<u>384aa</u>	Protein coding	CCDS84111	A6P3E4	TSL:1 GENCODE basic APPRIS ALT
Mapk8-202	ENSMUST00000111942.1	927	<u>308aa</u>	Protein coding	2	D3Z1Z4	TSL:5 GENCODE basic
Mapk8-210	ENSMUST00000226798.1	597	<u>40aa</u>	Nonsense mediated decay	•	A0A2I3BPL3	
Mapk8-209	ENSMUST00000150659.7	533	No protein	Processed transcript	÷.	19 - 0	TSL:5
Mapk8-211	ENSMUST00000227306.1	3529	No protein	Retained intron	29	121	
Mapk8-207	ENSMUST00000130539.7	2811	No protein	Retained intron	-2	353	TSL:1
Mapk8-208	ENSMUST00000132379.1	1619	No protein	Retained intron	•	1. 1	TSL:1
Mapk8-206	ENSMUST00000127143.1	647	No protein	Retained intron	.5	199	TSL:5
				()		. Vo	

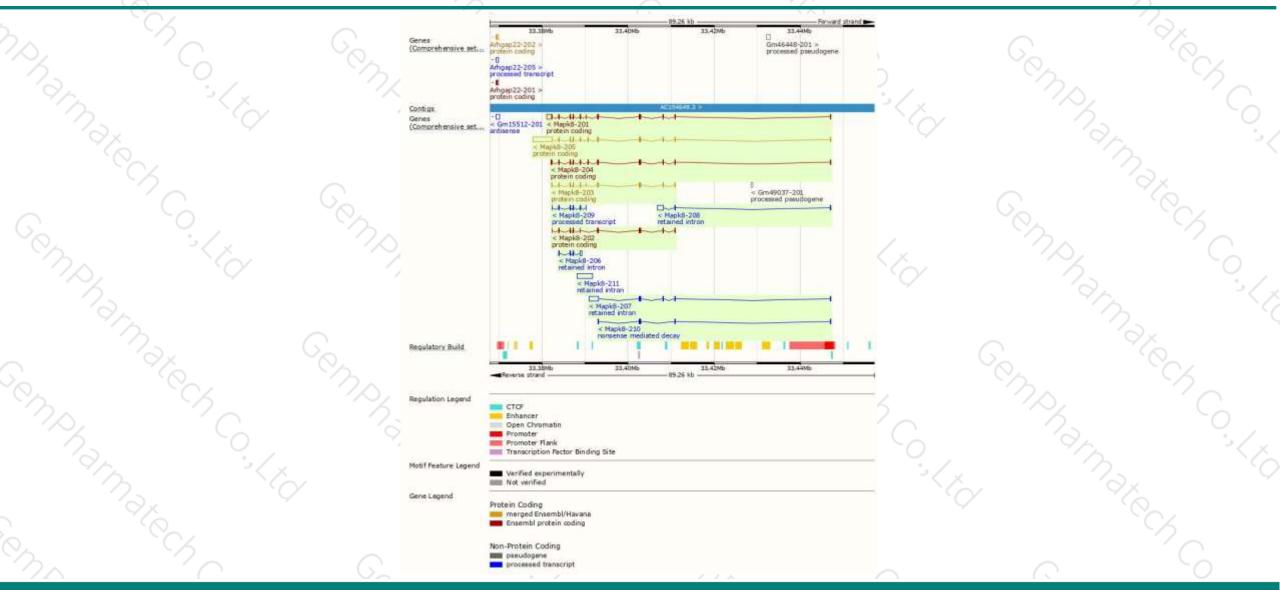
The strategy is based on the design of Mapk8-201 transcript, The transcription is shown below

< Mapk8-201 protein coding Reverse strand 66.10 kb

Genomic location distribution



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Protein domain

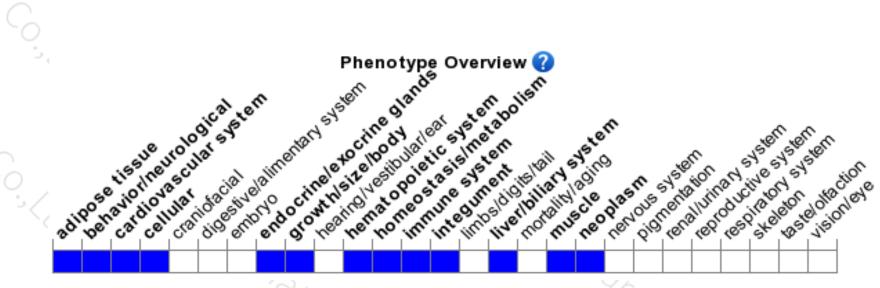


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Mouse phenotype description(MGI)





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 a knock-out allele exhibit abnormal T cell differentiation and proliferation, cardiac morphology and physiology, and chemically-induced tumorigenesis. Mice homozygous for another knock-out allele exhibit abnormal glucose homeostasis and T cell physiology.

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If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



