

# Mme Cas9-CKO Strategy

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## **Project Overview**



**Project Name** 

Mme

**Project type** 

Cas9-CKO

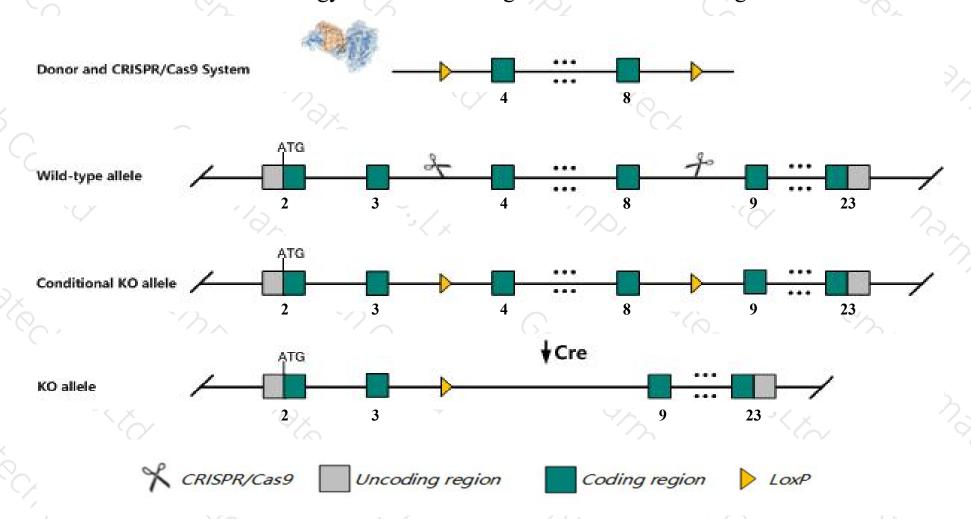
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



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



## Technical routes



- The *Mme* gene has 8 transcripts. According to the structure of *Mme* gene, exon4-exon8 of *Mme-205*(ENSMUST00000194134.5) transcript is recommended as the knockout region. The region contains 524bp coding sequence.

  Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Mme* 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 a knock-out allele exhibit enhanced allergic contact dermatitis responses, diffuse hepatic necrosis after LPS shock or treatment with a combination of TNF and interleukin-1 beta, and increased brain and plasma amyloid beta peptide levels.
- > The *Mme* gene is located on the Chr3. 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)



#### Mme membrane metallo endopeptidase [ Mus musculus (house mouse) ]

Gene ID: 17380, updated on 5-Nov-2019

#### Summary

☆ ?

Official Symbol Mme provided by MGI

Official Full Name membrane metallo endopeptidase provided by MGI

Primary source MGI:MGI:97004

See related Ensembl:ENSMUSG00000027820

Gene type protein coding
RefSeq status VALIDATED
Organism <u>Mus musculus</u>

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

Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus

Also known as NEP; SFE; CD10; CALLA; C85356; 6030454K05Rik

Expression Broad expression in limb E14.5 (RPKM 8.6), kidney adult (RPKM 7.9) and 18 other tissues See more

Orthologs human all

#### Genomic context



Location: 3 E1; 3 29.97 cM

See Mme in Genome Data Viewer

Exon count: 25

Status	Assembly		Location	
current	GRCm38.p6 (GCF_000001635.26)	3	NC_000069.6 (6329521163383713)	
previous assembly	MGSCv37 (GCF_000001635.18)	3	NC_000069.5 (6309979463186153)	
	current	current GRCm38.p6 (GCF_000001635.26)	current GRCm38.p6 (GCF_000001635.26) 3	current GRCm38.p6 (GCF_000001635.26) 3 NC_000069.6 (6329521163383713)

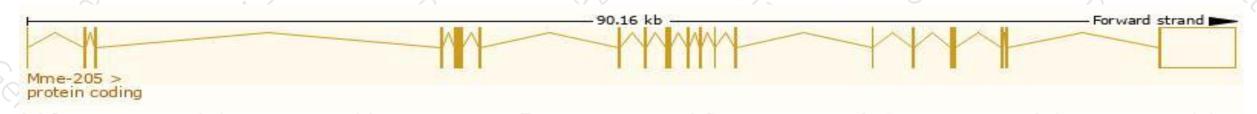
## Transcript information (Ensembl)



The gene has 8 transcripts, all transcripts are shown below:

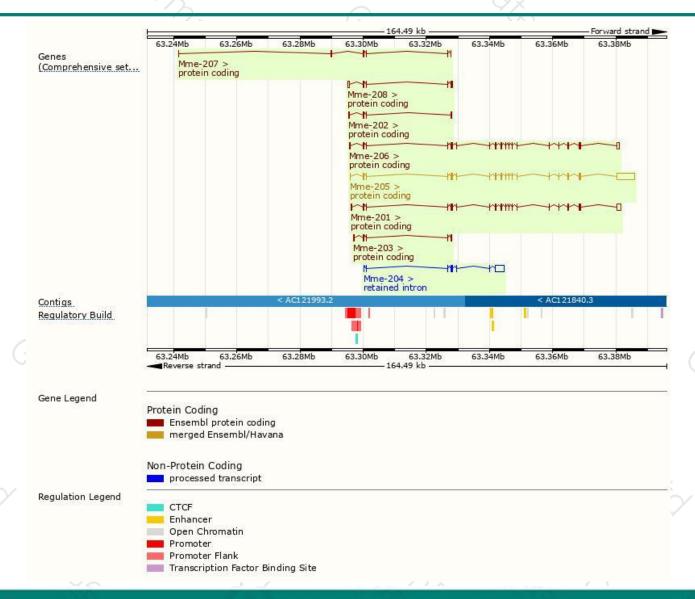
Name	Transcript ID	bp	Protein	Biotype	ccps	UniProt	Flags
Mme-205	ENSMUST00000194134.5	8039	750aa	Protein coding	CCDS17381	Q61391	TSL:1 GENCODE basic APPRIS P1
Mme-201	ENSMUST00000029400.6	3782	750aa	Protein coding	CCDS17381	Q61391	TSL:1 GENCODE basic APPRIS P1
Mme-206	ENSMUST00000194150.5	3345	750aa	Protein coding	CCDS17381	Q61391	TSL:1 GENCODE basic APPRIS P1
Mme-208	ENSMUST00000194836.5	921	<u>187aa</u>	Protein coding	725	A0A0A6YW93	CDS 3' incomplete TSL:3
Mme-203	ENSMUST00000192002.2	629	<u>178aa</u>	Protein coding	1.5	A0A0A6YWB9	CDS 3' incomplete TSL:3
Mme-207	ENSMUST00000194324.5	615	<u>133aa</u>	Protein coding	-	A0A0A6YY36	CDS 3' incomplete TSL:3
Mme-202	ENSMUST00000191633.5	569	<u>130aa</u>	Protein coding	120	A0A0A6YWA6	CDS 3' incomplete TSL:3
Mme-204	ENSMUST00000193805.1	3543	No protein	Retained intron	728	-	TSL:1

The strategy is based on the design of Mme-205 transcript, The transcription is shown below



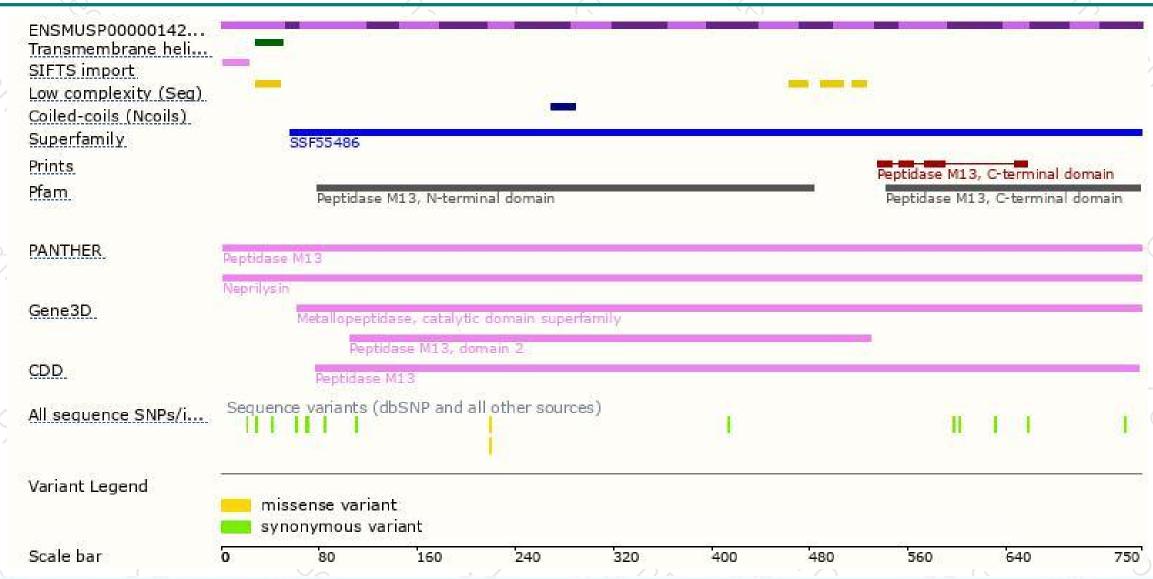
### Genomic location distribution





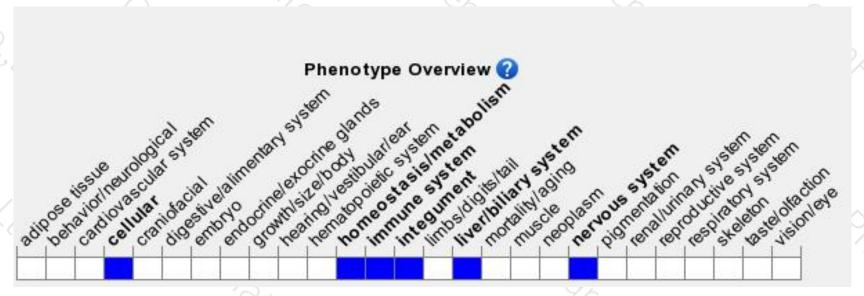
### Protein domain





## 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, Mice homozygous for a knock-out allele exhibit enhanced allergic contact dermatitis responses, diffuse hepatic necrosis after LPS shock or treatment with a combination of TNF and interleukin-1 beta, and increased brain and plasma amyloid beta peptide levels.



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





