

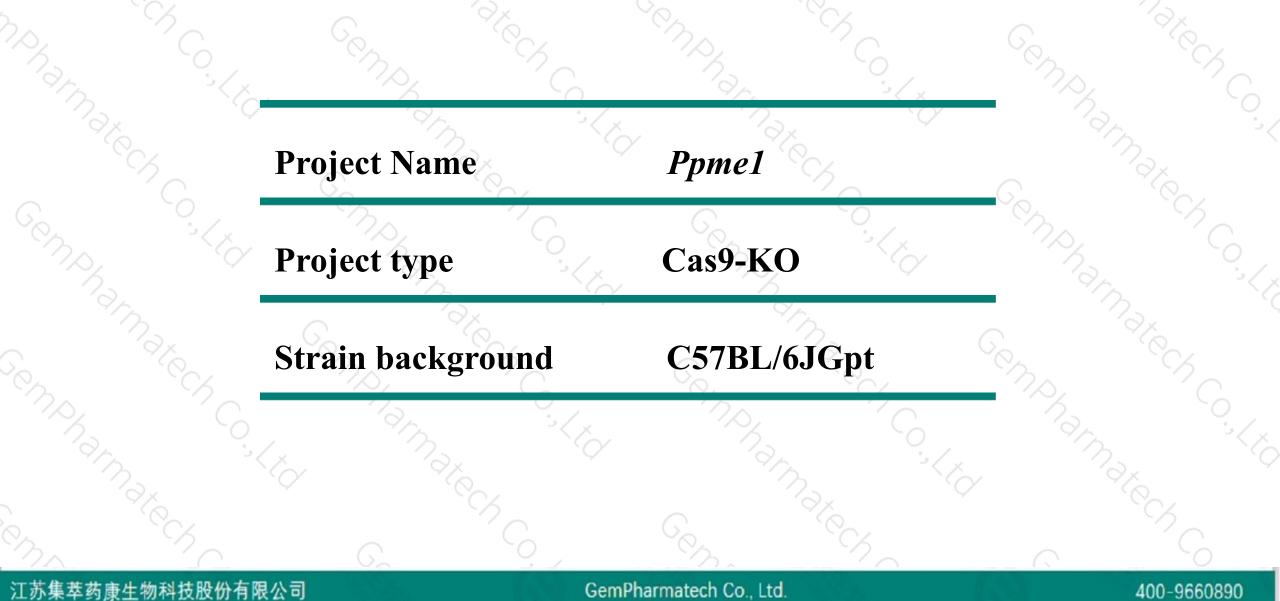
Ppmel Cas9-KO Strategy

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Designer: Xueting Zhang Reviewer:Yanhua Shen Date:2020-03-11

Project Overview

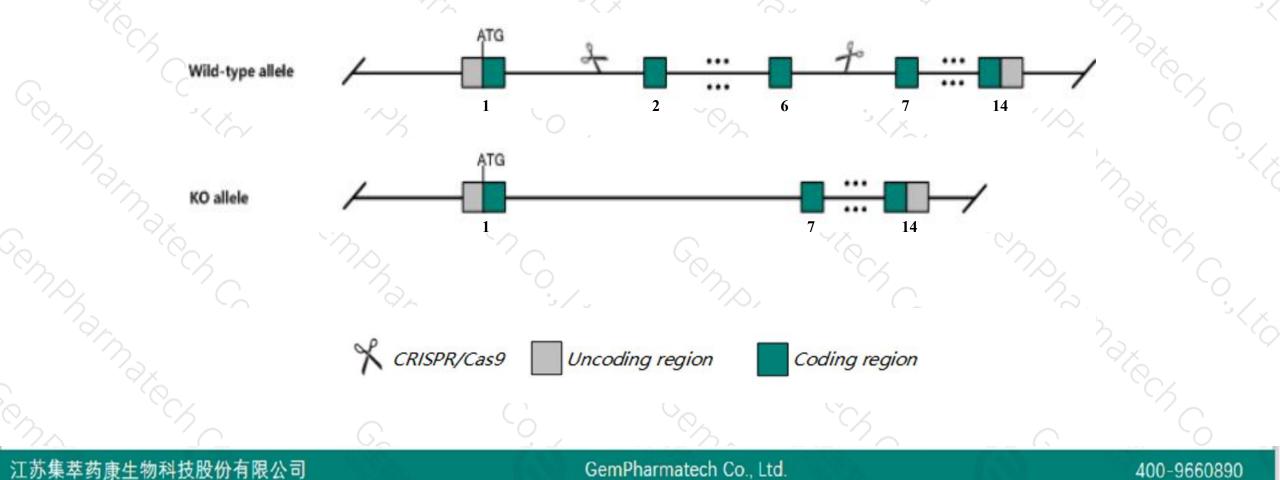




Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Ppme1* gene. The schematic diagram is as follows:





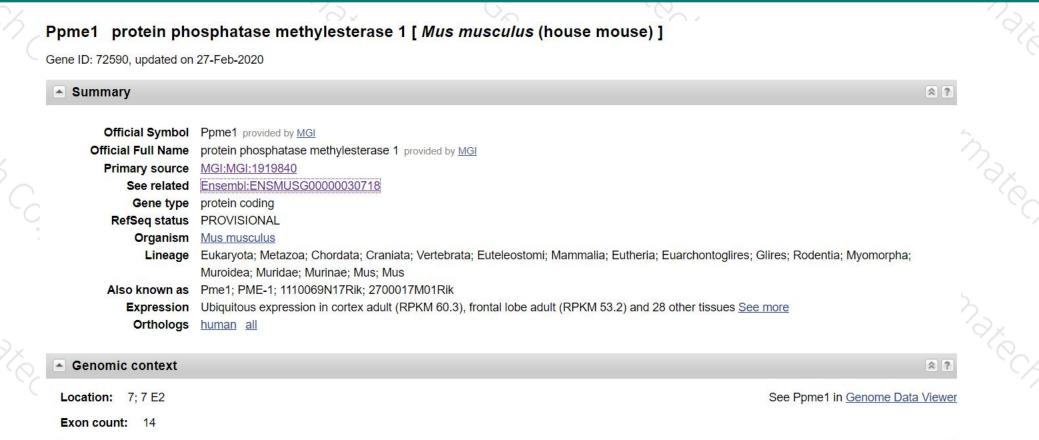
- The Ppme1 gene has 5 transcripts. According to the structure of Ppme1 gene, exon2-exon6 of Ppme1-201 (ENSMUST00000032963.9) transcript is recommended as the knockout region. The region contains 452bp coding sequence. Knock out the region will result in disruption of protein function.
- > In this project we use CRISPR/Cas9 technology to modify *Ppme1* gene. The brief process is as follows: CRISPR/Cas9 system

- According to the existing MGI data, Targeted disruption of this gene causes virtual loss of the demethylated form of phosphoprotein phosphatase 2A in the nervous system and peripheral tissues. Homozygous null mice fail to initiate normal breathing or suckling behavior and die within the first day of life.
- > The effect on transcript Ppme1-204 is unknown.
- ➤ Transcript *Ppme1*-203 may not be affected.
- The *Ppme1* gene is located on the Chr7. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Notice

Gene information (NCBI)





| Annotation release | Status | Assembly | Chr | Location |
|--------------------|-------------------|------------------------------|-----|--|
| <u>108</u> | current | GRCm38.p6 (GCF_000001635.26) | 7 | NC_000073.6 (100326737100371896, complement) |
| Build 37.2 | previous assembly | MGSCv37 (GCF_000001635.18) | 7 | NC_000073.5 (107475247107520406, complement) |

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Transcript information (Ensembl)



The gene has 5 transcripts, all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags | |
|-----------|----------------------|------|--------------|-----------------|-----------|---------------|-------------------------------|--|
| Ppme1-201 | ENSMUST0000032963.9 | 2772 | <u>386aa</u> | Protein coding | CCDS40036 | <u>Q8BVQ5</u> | TSL:1 GENCODE basic APPRIS P1 | |
| Ppme1-204 | ENSMUST00000207634.1 | 673 | <u>182aa</u> | Protein coding | - | A0A140LI84 | CDS 5' incomplete TSL:5 | |
| Ppme1-203 | ENSMUST00000207622.1 | 942 | No protein | Retained intron | 84 | 1 | TSL:2 | |
| Ppme1-205 | ENSMUST00000208168.1 | 474 | No protein | Retained intron | 62 | 2 | TSL:2 | |
| Ppme1-202 | ENSMUST00000207092.1 | 754 | No protein | IncRNA | 15 | - | TSL:3 | |

The strategy is based on the design of *Ppme1-201* transcript, The transcription is shown below

< Ppme1-201 protein coding

Reverse strand -

- 45.57 kb -

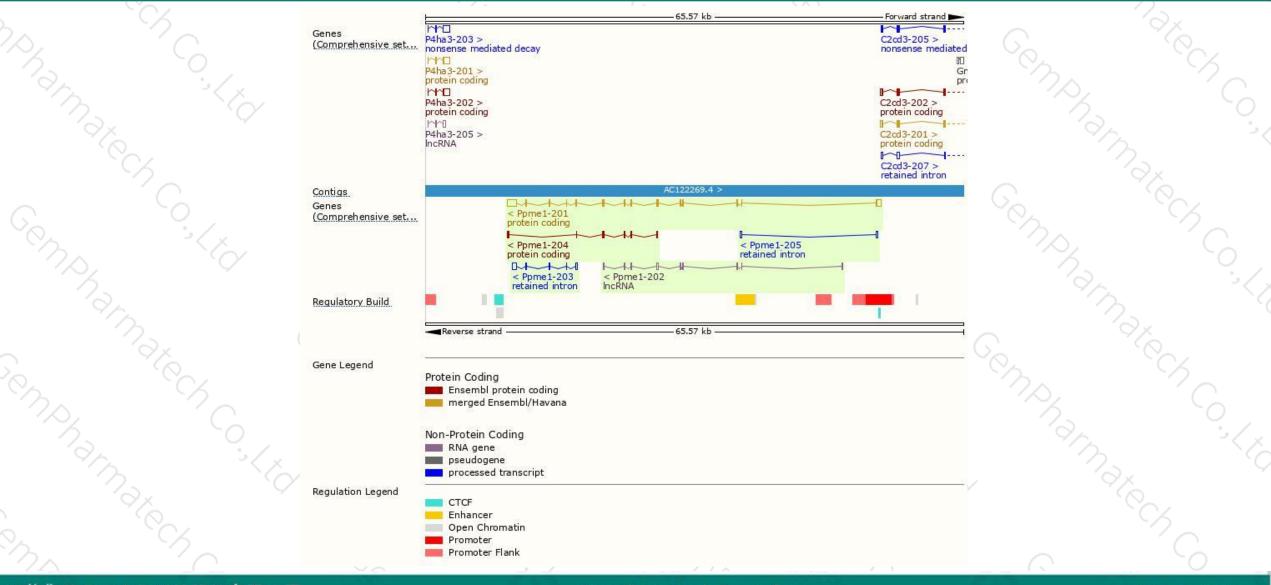
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Genomic location distribution



400-9660890



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Protein domain



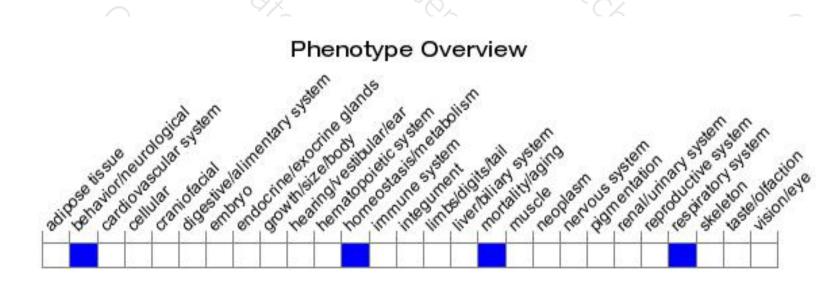
| , C | | ° CA | ns, | | | 2000 · | °°G |
|--------------------------------|--|---------------------------------------|--|---------|-----|--------|------|
| ENSMUSP00000032 MobiDB lite | | | | | | | |
| Low complexity (Seg) | | | | | | | |
| Superfamily | | Alpha/Beta hydrolase fold | and the state | | | | |
| Prints | | | ydrolase fold-1 | | | | |
| Pfam. | | Epoxide hydro Alpha/beta hydrolase | and the second | | | | - C |
| PIRSF | Protein phosphatase n | nethylesterase, eukaryotic | lense levelse i | | | | |
| PANTHER | Protein phosphata | ise methylesterase, eukaryo | otic | | | | |
| All sequence SNPs/i | Sequence variants (| (dbSNP and all other sou | irces) | í - | | 1 | (II) |
| Variant Legend | missense varia splice region v synonymous vi | /ariant | | | | | |
| Scale bar | 0 40 | 80 120 | 160 | 200 240 | 280 | 320 | 386 |
| | | | | | | | ° Ch |
| | | | | | | | |

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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, Targeted disruption of this gene causes virtual loss of the demethylated form of phosphoprotein phosphatase 2A in the nervous system and peripheral tissues. Homozygous null mice fail to initiate normal breathing or suckling behavior and die within the first day of life.

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



