

# Mcm5 Cas9-KO Strategy

Designer: Lingyan Wu

Reviewer: Miaomiao Cui

**Design Date: 2021-5-26** 

# **Project Overview**

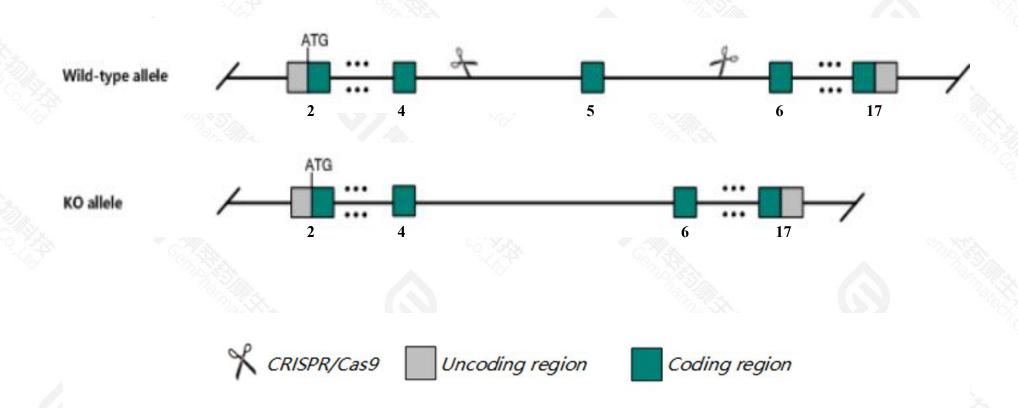


Project Name	Mcm5		
Project type	Cas9-KO		
Strain background	C57BL/6JGpt		

# **Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the *Mcm5* gene. The schematic diagram is as follows:



### **Technical routes**



- ➤ The *Mcm5* gene has 5 transcripts. According to the structure of *Mcm5* gene, exon5 of *Mcm5*201(ENSMUST00000164309.3) transcript is recommended as the knockout region. The region contains 173bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Mcm5* gene. The brief process is as follows: CRISPR/Cas9 system 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.

### **Notice**



- > According to the existing MGI data, mice homozygous for an ENU-induced allele exhibit embryonic lethality.
- > The *Mcm5* gene is located on the Chr8. 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.

### Gene information (NCBI)



#### Mcm5 minichromosome maintenance complex component 5 [Mus musculus (house mouse)]

Gene ID: 17218, updated on 13-Mar-2020

#### Summary

↑ ?

Official Symbol Mcm5 provided by MGI

Official Full Name minichromosome maintenance complex component 5 provided by MGI

Primary source MGI:MGI:103197

See related Ensembl: ENSMUSG00000005410

Gene type protein coding
RefSeq status REVIEWED
Organism Mus musculus

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

Muroidea; Muridae; Murinae; Mus; Mus

Also known as AA617332, Al324988, AL033333, Cdc46, Mcmd5, P1-CDC46

Summary The protein encoded by this gene is a member of the oligameric minichromosome maintenance protein complex. During DNA replication, the

complex loads onto chromatin in early G1 and is converted into an active replicative helicase during S phase. It functions to limit DNA

synthesis to once per cell cycle. During embryogenesis, the encoded protein is negatively regulated through expression of paired box protein

Pax 3. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Oct 2014]

Expression Broad expression in liver E14.5 (RPKM 77.8), liver E14 (RPKM 74.7) and 18 other tissuesSee more

Orthologs human all

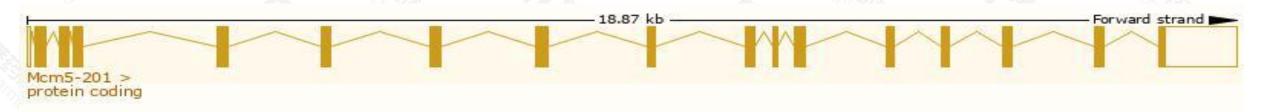
# Transcript information (Ensembl)



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

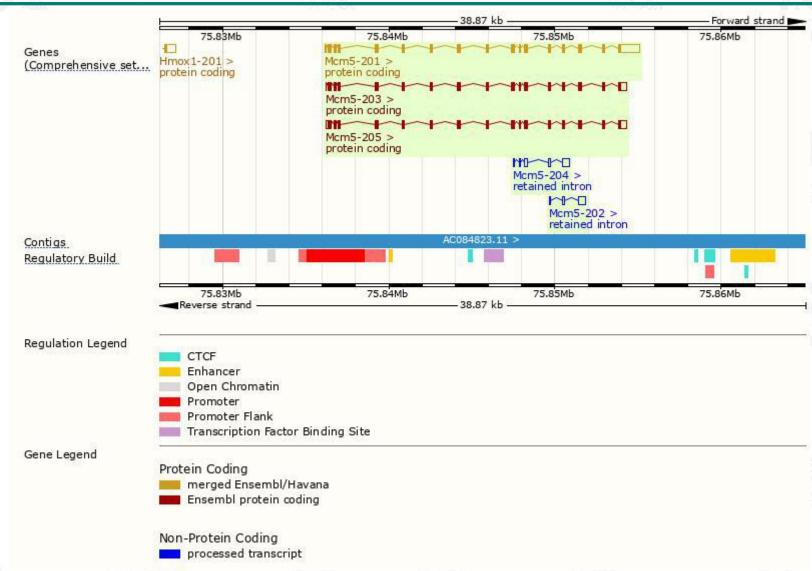
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mcm5-201	ENSMUST00000164309.2	3381	<u>734aa</u>	Protein coding	CCDS22424	P49718	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Mcm5-205	ENSMUST00000212811.1	2653	734aa	Protein coding	CCDS22424	P49718	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Mcm5-203	ENSMUST00000212426.1	2604	734aa	Protein coding	CCDS22424	P49718	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Mcm5-204	ENSMUST00000212648.1	760	No protein	Retained intron	-	0	TSL:2
Mcm5-202	ENSMUST00000211912.1	595	No protein	Retained intron	5	-	TSL:3

The strategy is based on the design of *Mcm5-201* transcript, the transcription is shown below:



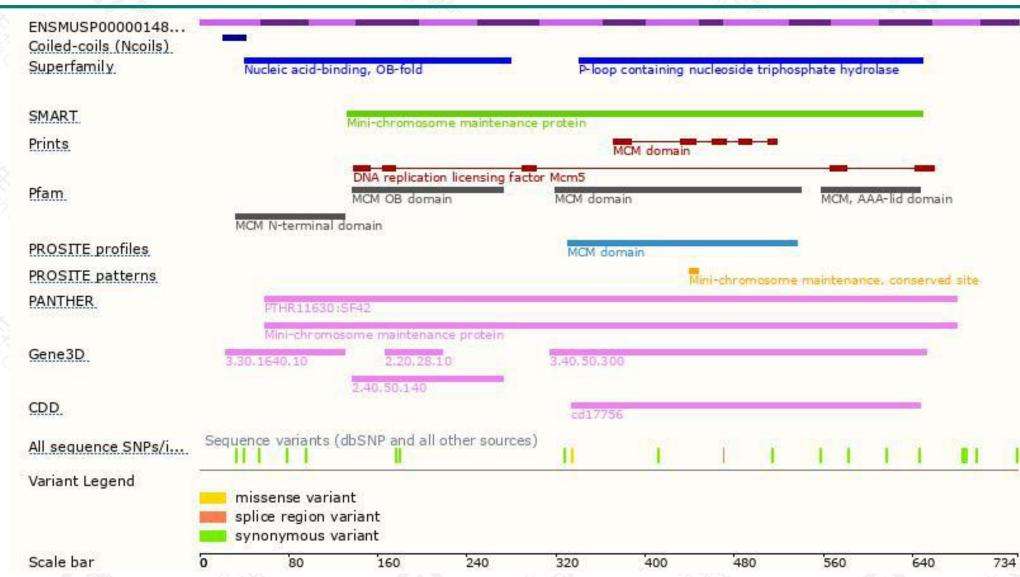
### Genomic location distribution





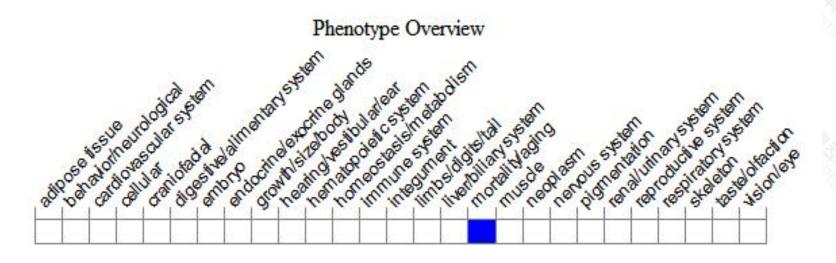
### Protein domain





# 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 an ENU-induced allele exhibit embryonic lethality.



If you have any questions, you are welcome to inquire.

Tel: 400-9660890





