Mcm8 Cas9-CKO Strategy

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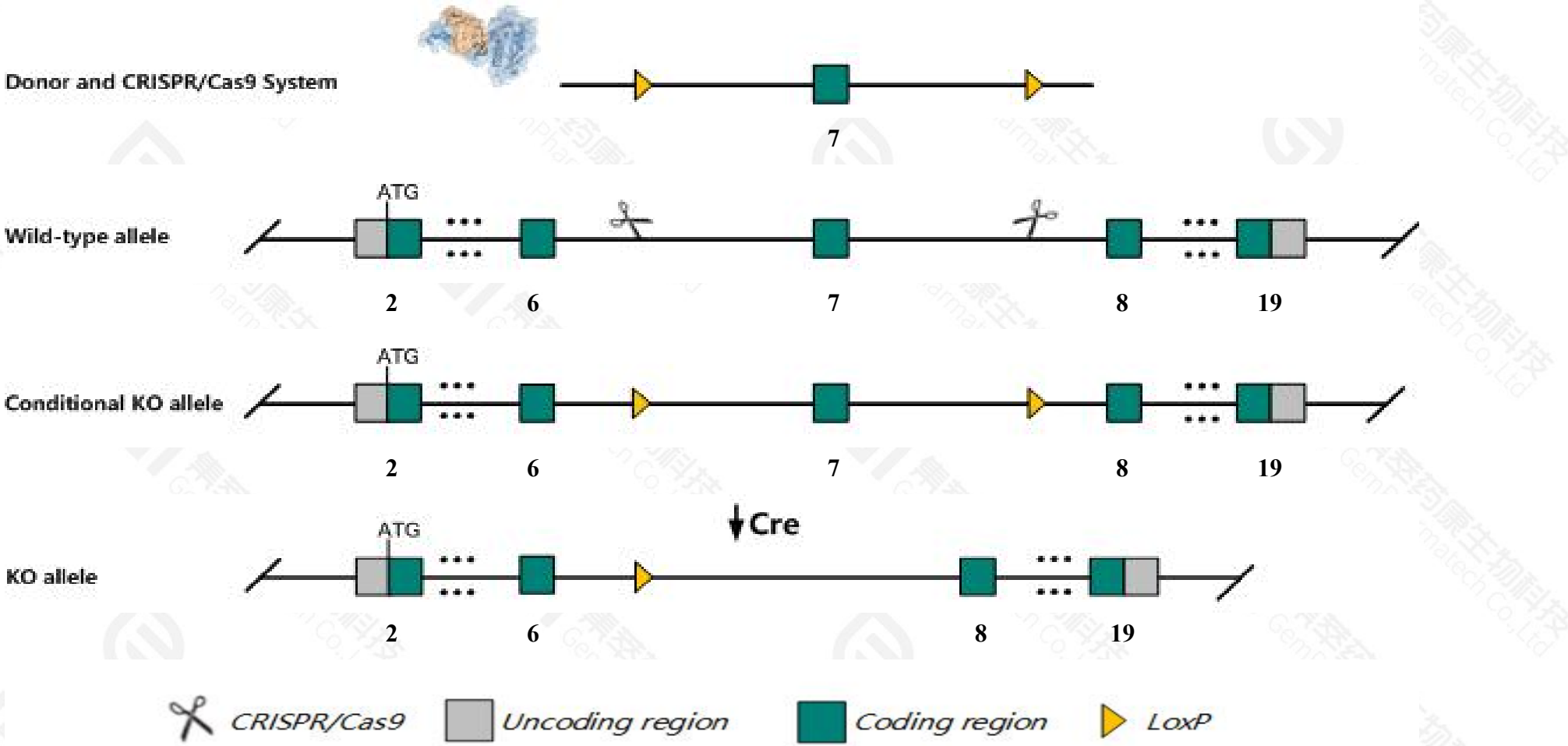
Project Overview

Project Name	***Mcm8***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mcm8* gene. The schematic diagram is as follows:



Technical routes

➢ The *Mcm8* gene has 3 transcripts. According to the structure of *Mcm8* gene, exon7 of *Mcm8-201*(ENSMUST00000028831.15) transcript is recommended as the knockout region.The region contains 199bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Mcm8* 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 female and male infertility associated with impaired ovarian development and arrested male meiosis, and impaired sensitivity to homologous recombination double-strand break repair.

➢ The *Mcm8* gene is located on the Chr2. 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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Mcm8 minichromosome maintenance 8 homologous recombination repair factor [Mus musculus (house mouse)]

Gene ID: 66634, updated on 25-Sep-2020

Summary

Official Symbol	Mcm8 provided by MGI
Official Full Name	minichromosome maintenance 8 homologous recombination repair factor provided by MGI
Primary source	MGI:MGI:1913884
See related	Ensembl:ENSMUSG00000027353
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	5730432L01Rik
Expression	Broad expression in CNS E11.5 (RPKM 5.2), liver E14 (RPKM 3.0) and 23 other tissues See more
Orthologs	human all

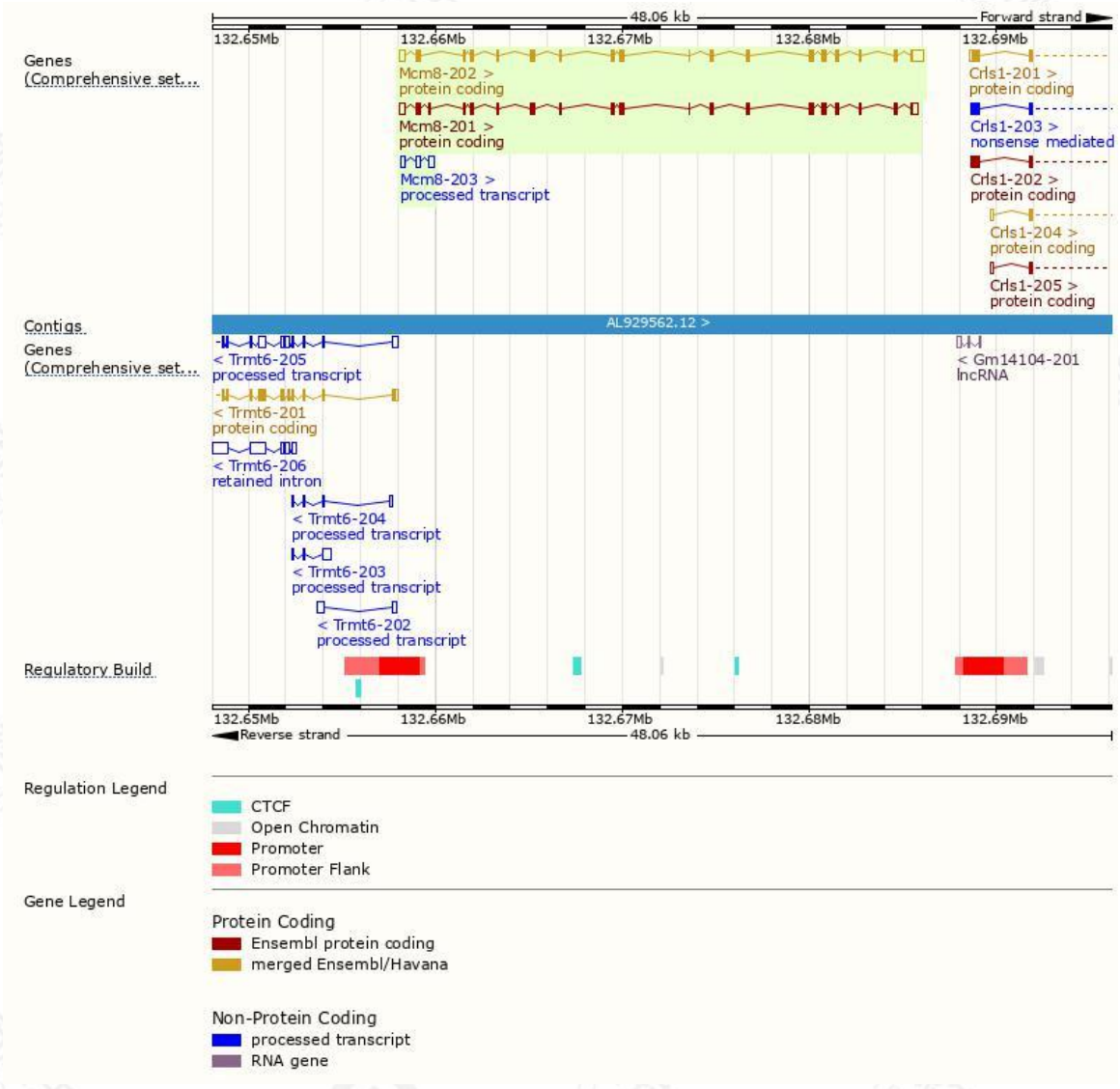
Transcript information（Ensembl）

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

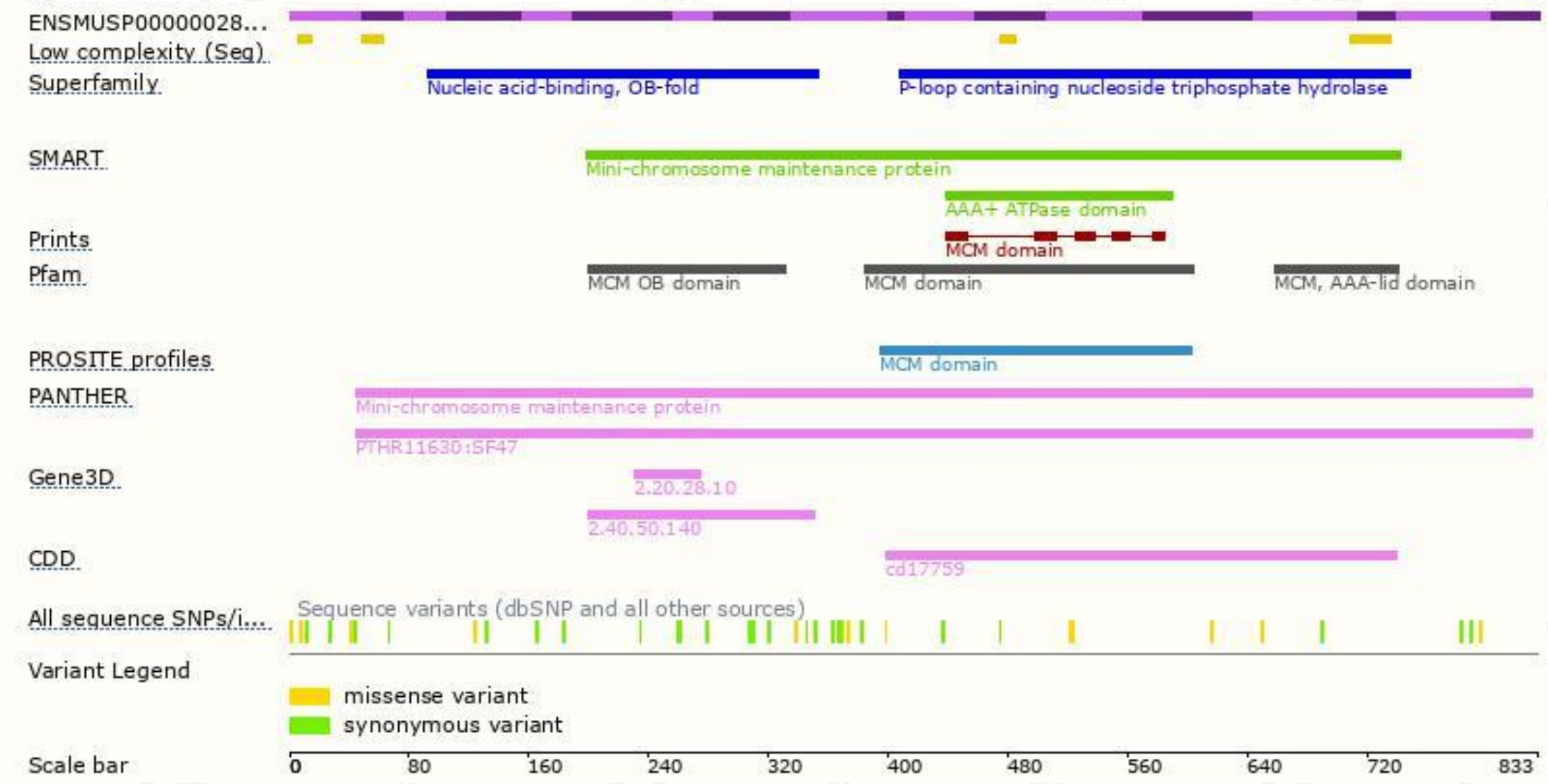
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mcm8-202	ENSMUST00000066559.6	3397	805aa	Protein coding	CCDS16778		TSL:1 , GENCODE basic , APPRIS P3 ,
Mcm8-201	ENSMUST00000028831.15	3179	833aa	Protein coding	CCDS71152		TSL:1 , GENCODE basic , APPRIS ALT2 ,
Mcm8-203	ENSMUST00000135685.2	735	No protein	Processed transcript	-		TSL:2 ,

The strategy is based on the design of *Mcm8-201* transcript,the transcription is shown below:

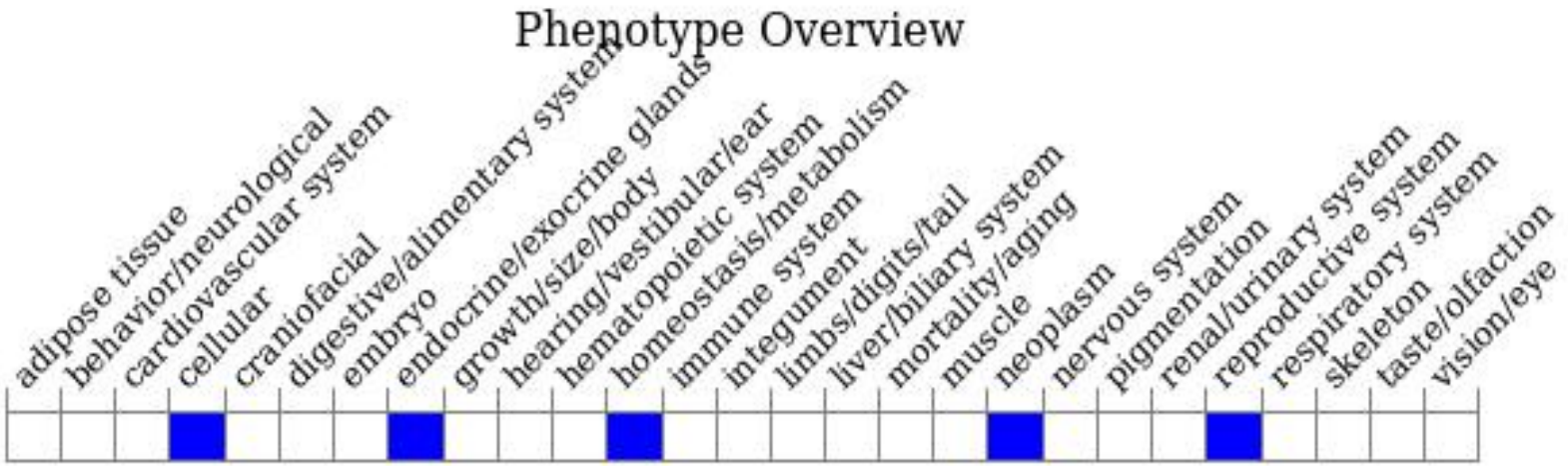
Genomic location distribution



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 a knock-out allele exhibit female and male infertility associated with impaired ovarian development and arrested male meiosis, and impaired sensitivity to homologous recombination double-strand break repair.

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