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GemPharmatech

Mcm9 Cas9-CKO Strategy

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Reviewer: Miaomiao Cui

Design Date: 2021-5-26

Project Overview

Project Name	***Mcm9***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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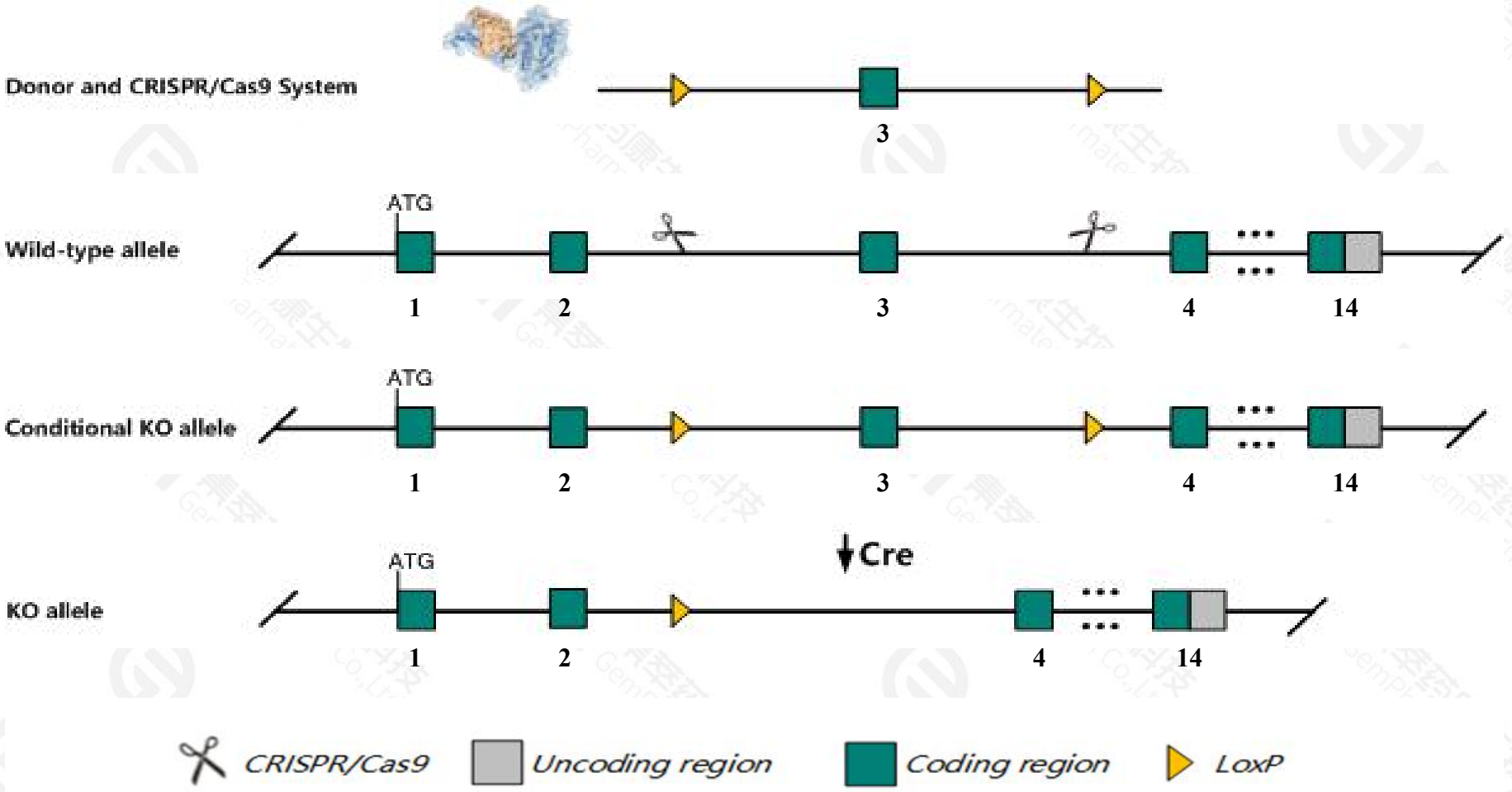
This model will use CRISPR/Cas9 technology to edit the *Mcm9* gene. The schematic diagram is as follows:



Technical routes

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➢ The *Mcm9* gene has 8 transcripts. According to the structure of *Mcm9* gene, exon3 of *Mcm9-201*(ENSMUST00000075540.8) transcript is recommended as the knockout region.The region contains 319bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Mcm9* 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 gene trap alleles display germ cell loss with reduced fertility or infertility and increased tumor incidence, particulary of hepatocellular carcinomas.

➢ Transcript *Mcm9*-203&205&206 may not be affected.

➢ The *Mcm9* gene is located on the Chr10. 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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Mcm9 minichromosome maintenance 9 homologous recombination repair factor [Mus musculus (house mouse)]

Gene ID: 71567, updated on 17-Feb-2021

Summary

Official Symbol	Mcm9 provided by MGI
Official Full Name	minichromosome maintenance 9 homologous recombination repair factor provided byMGI
Primary source	MGI:MGI:1918817
See related	Ensembl:ENSMUSG00000058298
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	9030408O17Rik, Gm235, Mcmdc1
Expression	Ubiquitous expression in CNS E11.5 (RPKM 3.4), liver E14 (RPKM 2.7) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

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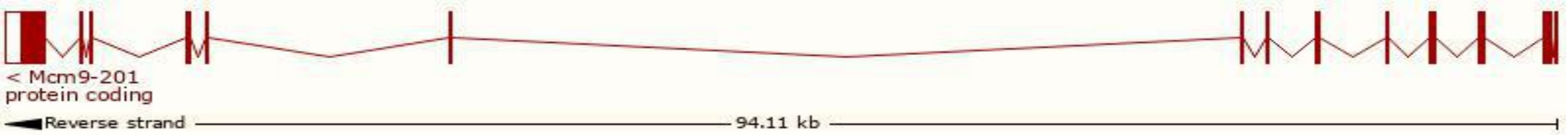
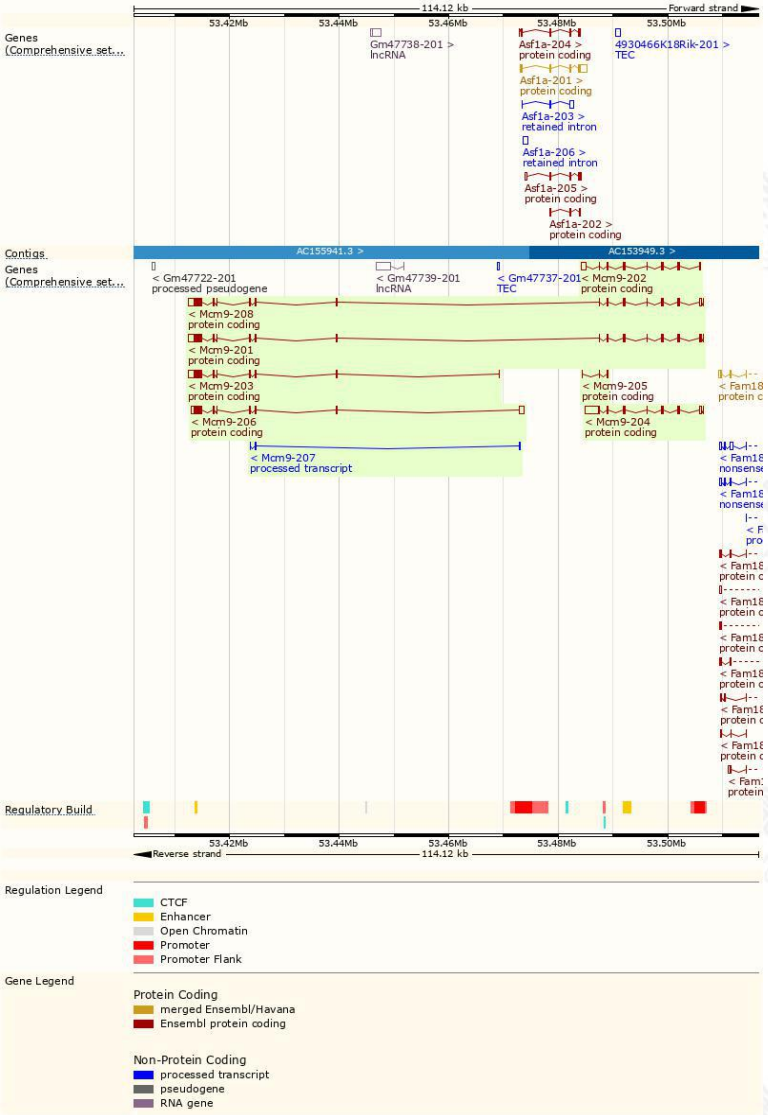
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mcm9-201	ENSMUST00000075540.8	4868	1290aa	Protein coding	CCDS23846		TSL:2 , GENCODE basic , APPRIS P2 ,
Mcm9-208	ENSMUST00000237608.2	4882	1134aa	Protein coding	-		GENCODE basic , APPRIS ALT2 ,
Mcm9-204	ENSMUST00000219838.3	4132	386aa	Protein coding	-		TSL:5 , GENCODE basic ,
Mcm9-206	ENSMUST00000220007.2	3513	552aa	Protein coding	-		TSL:1 , GENCODE basic ,
Mcm9-203	ENSMUST00000219547.2	3168	552aa	Protein coding	-		TSL:1 , GENCODE basic ,
Mcm9-202	ENSMUST00000218549.3	2421	414aa	Protein coding	-		TSL:1 , GENCODE basic ,
Mcm9-205	ENSMUST00000219841.3	307	98aa	Protein coding	-		CDS 5' incomplete , TSL:5 ,
Mcm9-207	ENSMUST00000220260.2	464	No protein	Processed transcript	-		TSL:5 ,

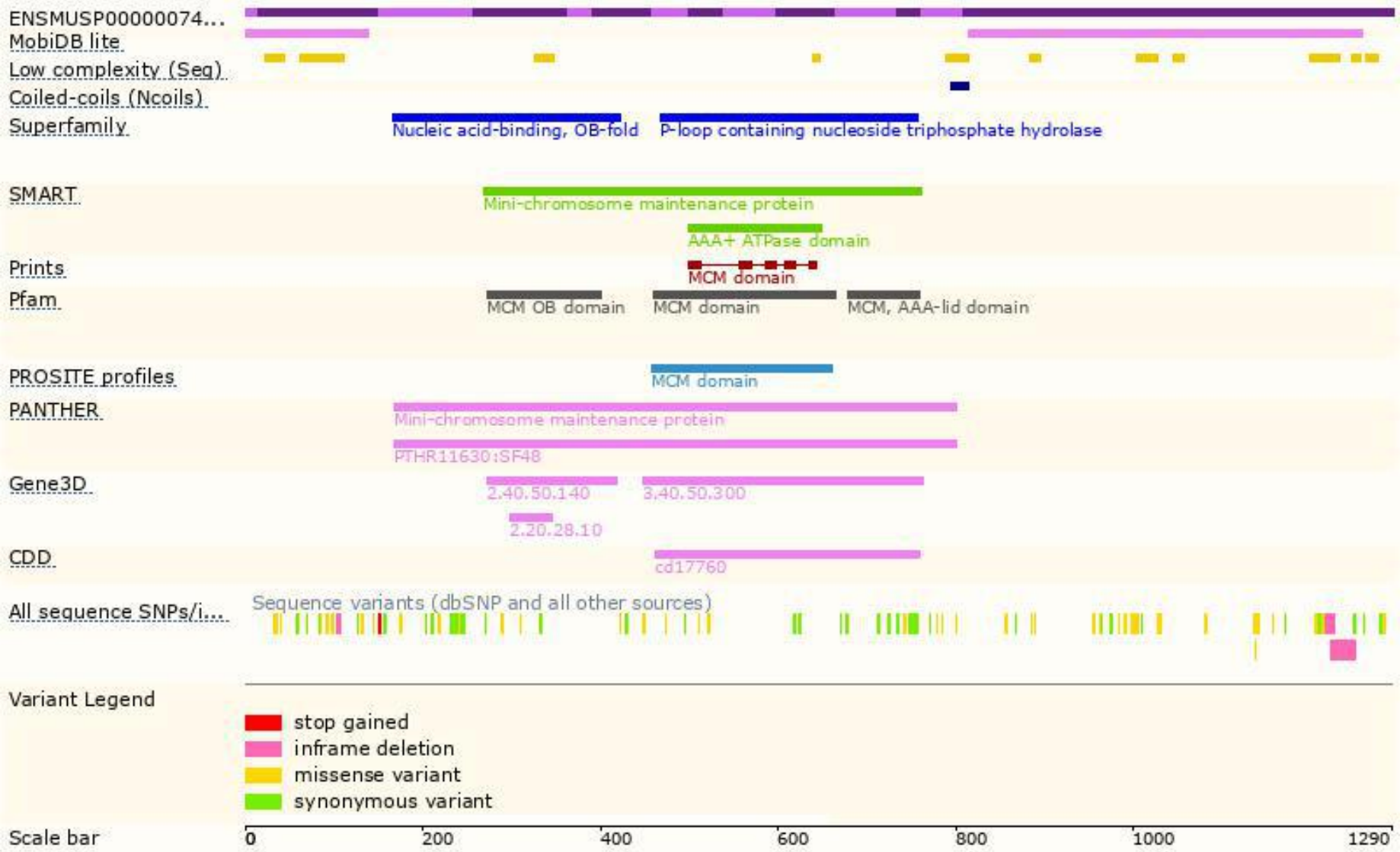
The strategy is based on the design of *Mcm9-201* transcript,the transcription is shown below:



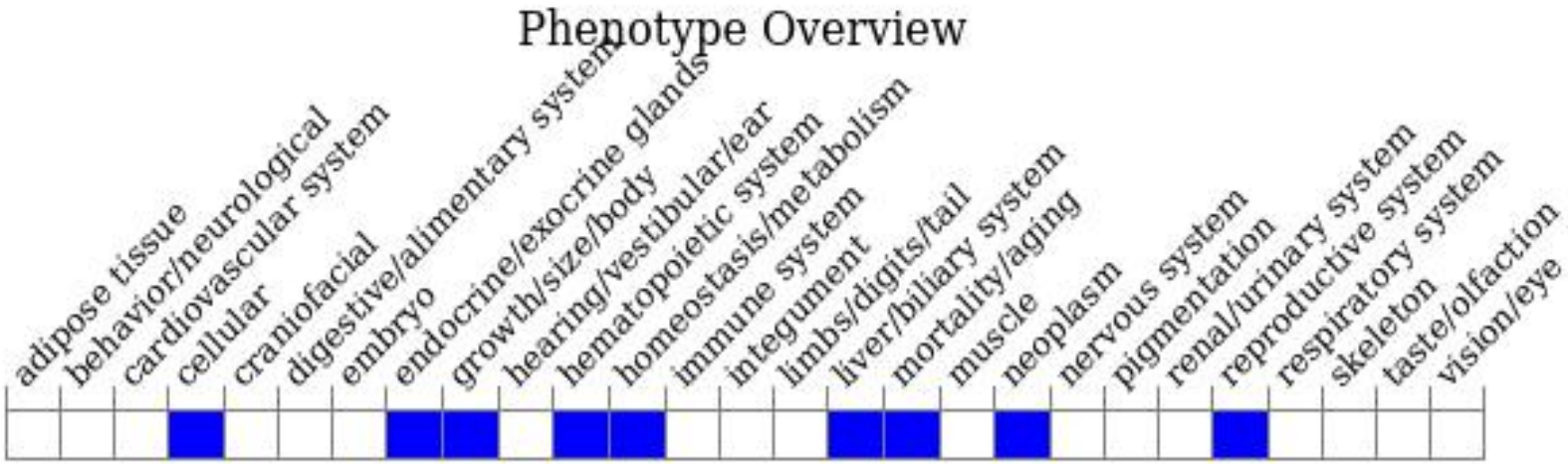
Genomic location distribution



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 gene trap alleles display germ cell loss with reduced fertility or infertility and increased tumor incidence, particulary of hepatocellular carcinomas.

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