March5 Cas9-CKO Strategy

Designer: JiaYu

Reviewer: Xiaojing Li

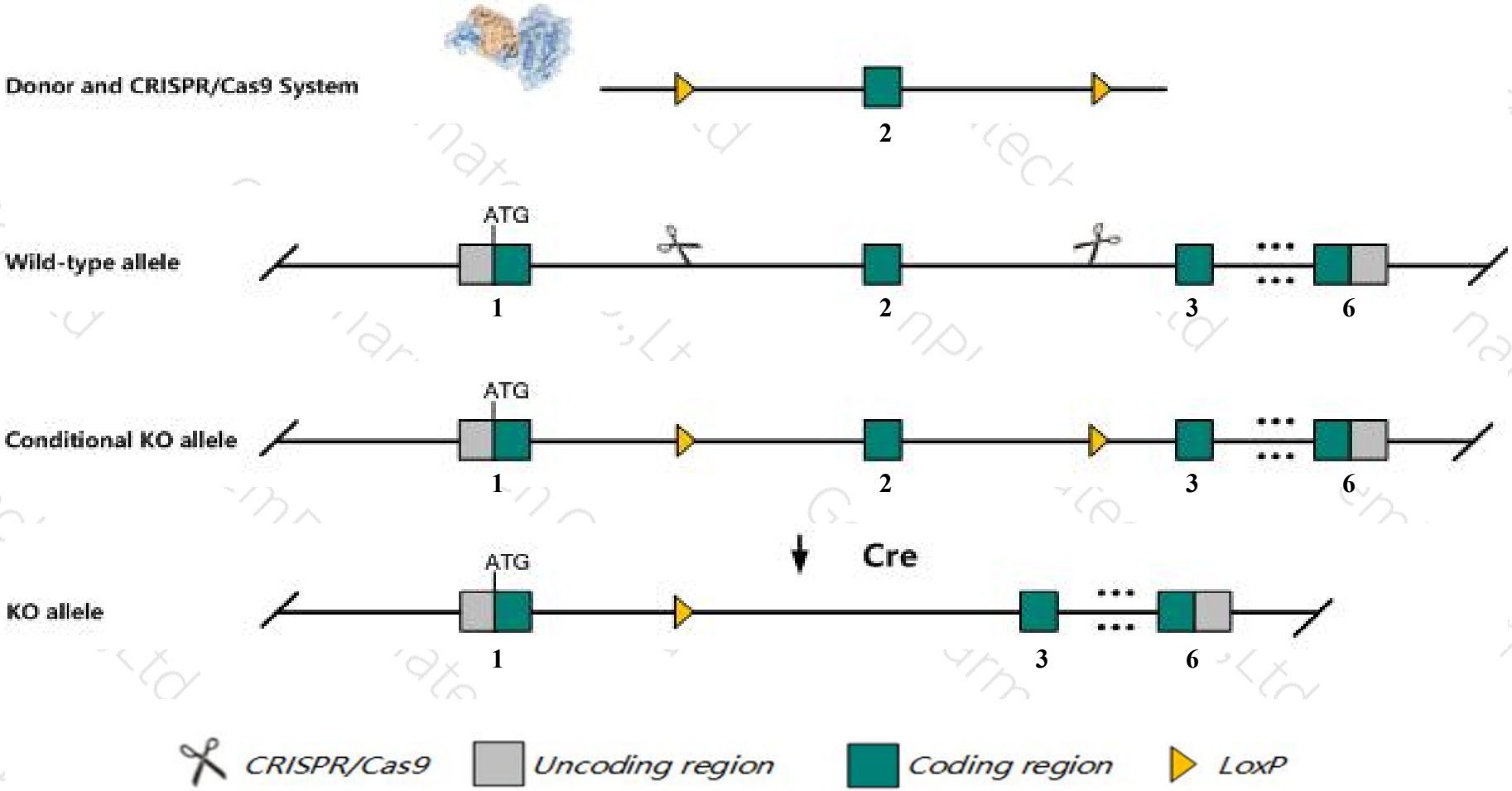
Design Date: 2020-2-25

Project Overview

Project Name	***March5***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *March5* gene. The schematic diagram is as follows:



Technical routes

- The *March5* gene has 4 transcripts. According to the structure of *March5* gene, exon2 of *March5-201* (ENSMUST00000024078.14) transcript is recommended as the knockout region.The region contains 203bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *March5* 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,Homozygous mutant knockout mice exhibit embryonic lethality. Heterozygous mutant mice exhibit defective immune responses to RNA viruses.

➢ The flox region is about 3 kb away from the 5th end of the Cpeb3 gene, which may affect the regulation of this gene.

➢Transcript 203 CDS 5' incomplete the influences is unknown.

➢ The *March5* gene is located on the Chr19. 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）

集萃药康 GemPharmatech

March5 membrane-associated ring finger (C3HC4) 5 [Mus musculus (house mouse)]

Gene ID: 69104, updated on 31-Jan-2019

Summary

Official Symbol	March5 provided by MGI
Official Full Name	membrane-associated ring finger (C3HC4) 5 provided by MGI
Primary source	MGI:MGI:1915207
See related	Ensembl:ENSMUSG00000023307
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	1810015H18Rik, 2310008I22Rik, 2700055A20Rik, 5730499H23Rik, E130202O05Rik, MARCH-V, MITOL, Marchv, Rnf153
Expression	Ubiquitous expression in liver E14 (RPKM 15.2), CNS E14 (RPKM 13.7) and 28 other tissues See more
Orthologs	human all

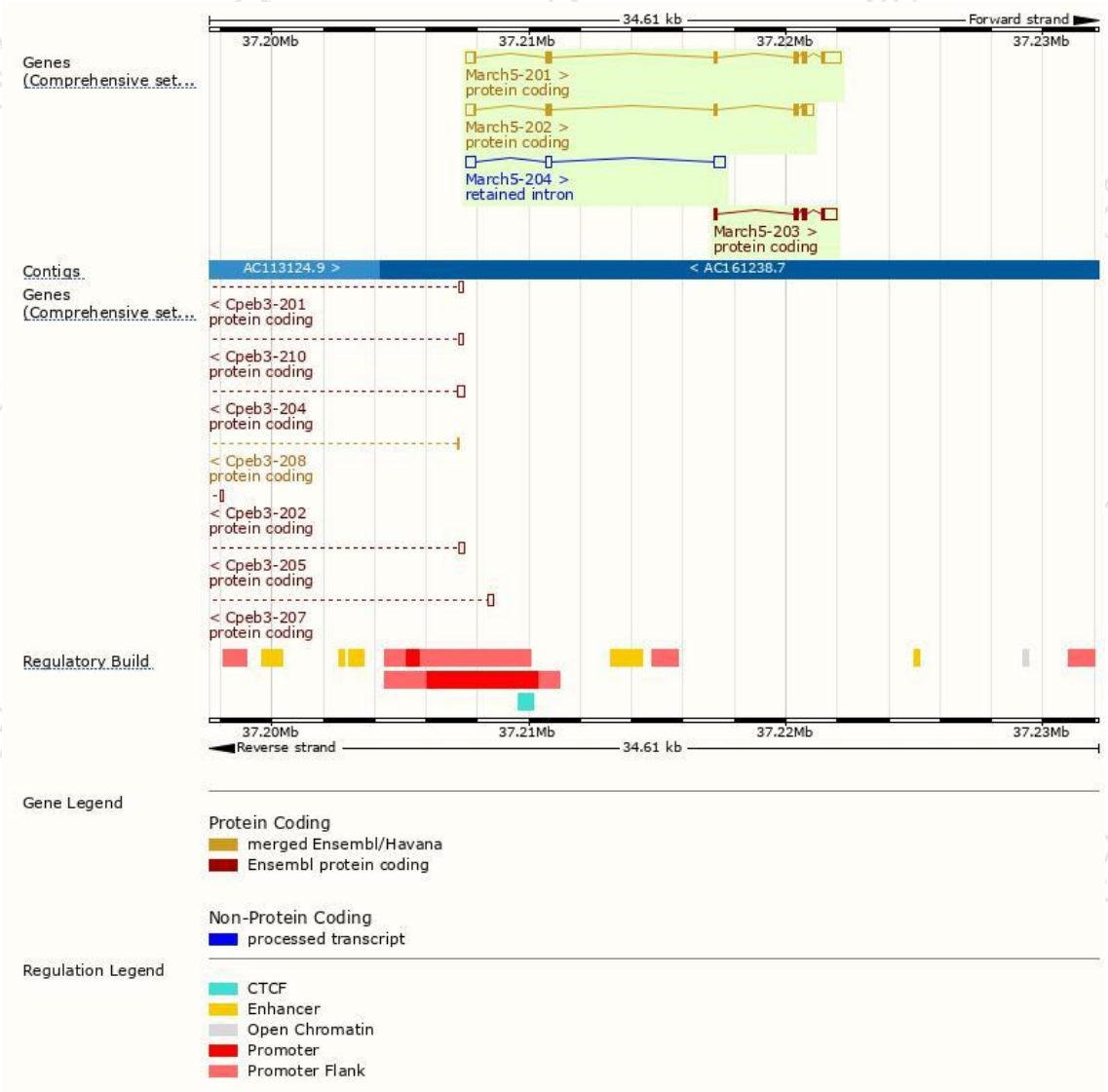
Transcript information（Ensembl）

The gene has 4 transcripts,all transcripts are shown below:

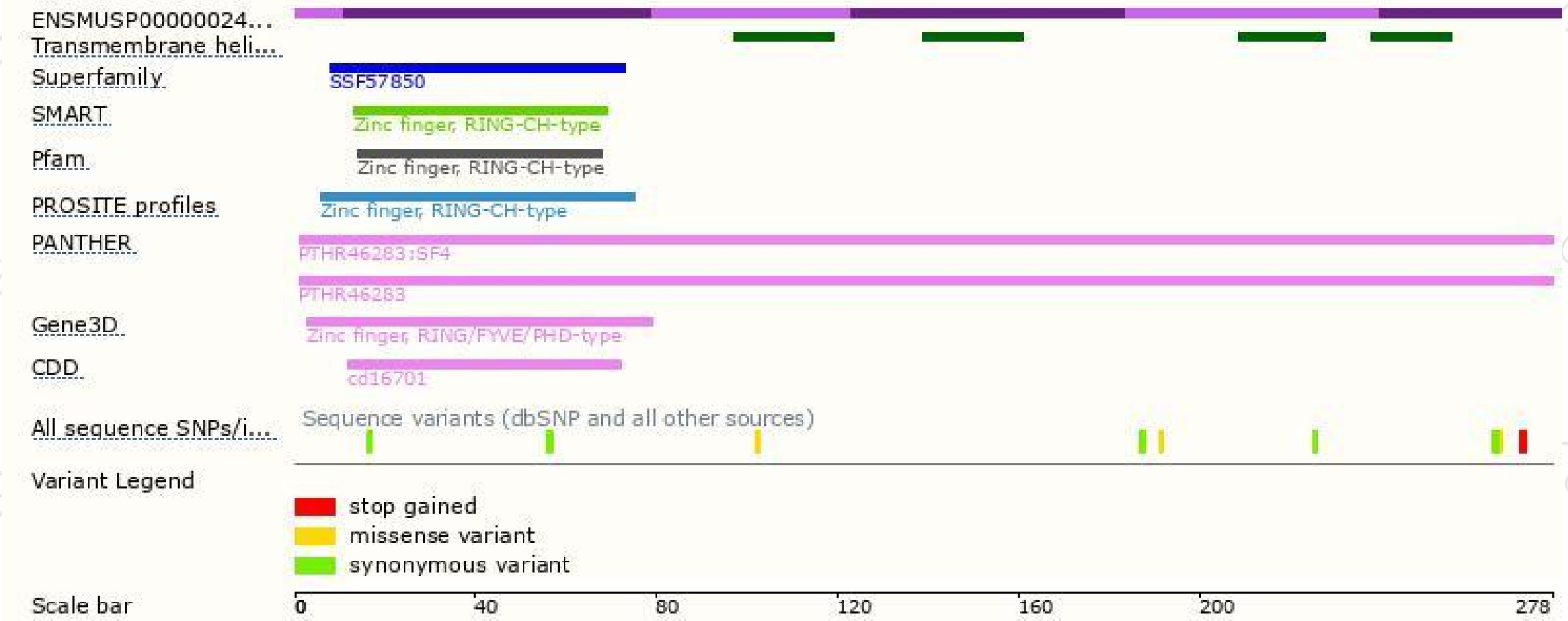
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
March5-201	ENSMUST00000024078.14	1809	278aa	Protein coding	CCDS29776	A2RTC8 Q3KNM2	TSL:1 GENCODE basic APPRIS P1
March5-202	ENSMUST00000112391.7	1293	244aa	Protein coding	CCDS50427	A0A0R4J1H0	TSL:1 GENCODE basic
March5-203	ENSMUST00000128530.1	1033	187aa	Protein coding	-	F6W8T3	CDS 5' incomplete TSL:3
March5-204	ENSMUST00000148105.1	991	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *March5-201* transcript,The transcription is shown below

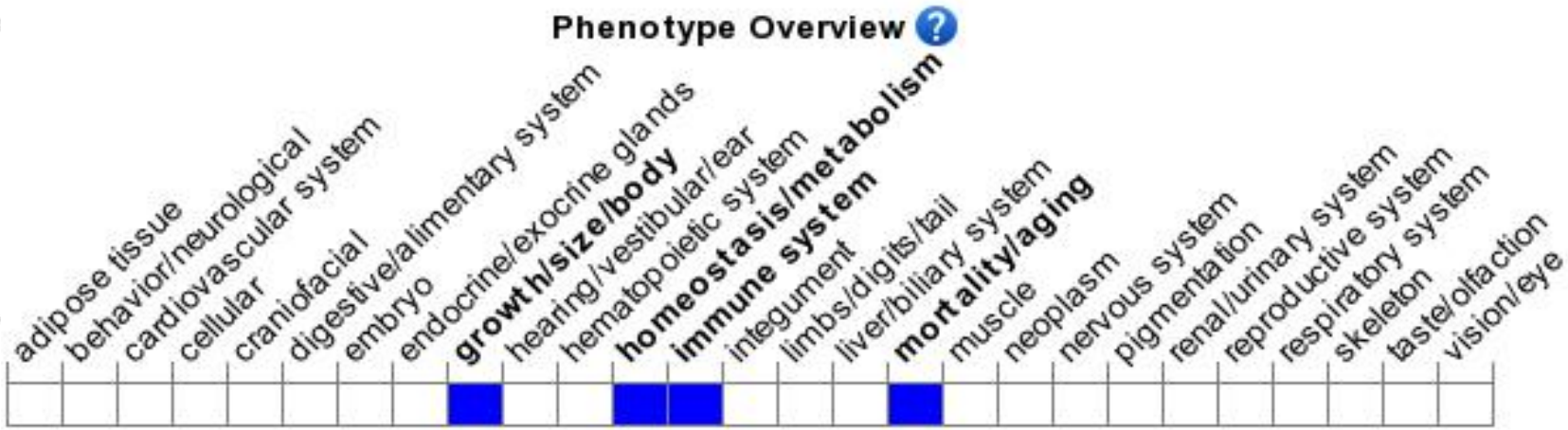
Genomic location distribution



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,Homozygous mutant knockout mice exhibit embryonic lethality. Heterozygous mutant mice exhibit defective immune responses to RNA viruses.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd