

# Marchf9 Cas9-CKO Strategy

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Reviewer: Huimin Su

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## **Project Overview**



Project Name Marchf9

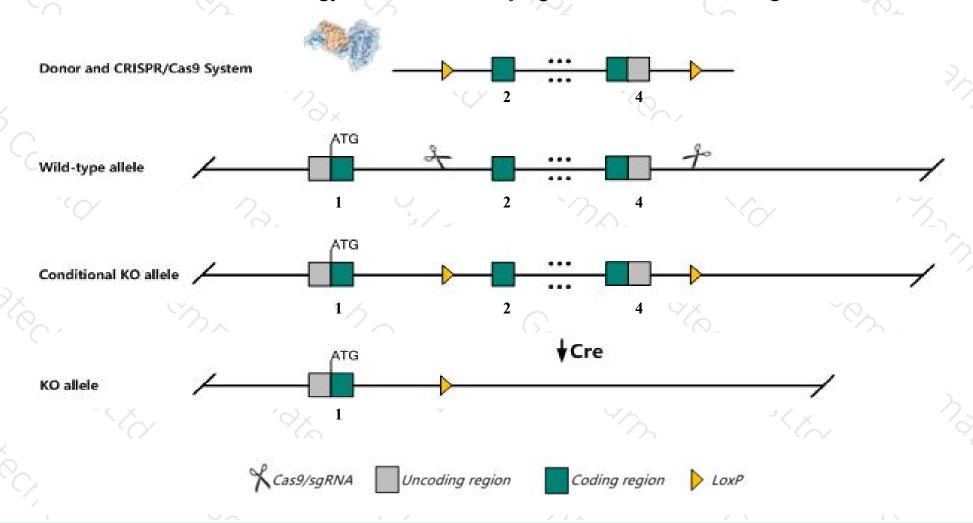
Project type Cas9-CKO

Strain background C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Marchf9* gene. The schematic diagram is as follows:



### Technical routes



- ➤ The *Marchf9* gene has 1 transcript. According to the structure of *Marchf9* gene, exon2-exon4 of *March9-201* (ENSMUST00000040307.5) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Marchf9* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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**



- > The *Marchf9* 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)



#### Marchf9 membrane associated ring-CH-type finger 9 [Mus musculus (house mouse)]

Gene ID: 216438, updated on 13-Mar-2020

#### Summary

☆ ?

Official Symbol Marchf9 provided by MGI

Official Full Name membrane associated ring-CH-type finger 9 provided byMGI

Primary source MGI:MGI:2446144

See related Ensembl:ENSMUSG00000040502

Gene type protein coding
RefSeq status PROVISIONAL
Organism Mus musculus

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

Muroidea; Muridae; Murinae; Mus; Mus

Also known as Al608492, BC019560, March9

Expression Broad expression in whole brain E14.5 (RPKM 17.3), CNS E18 (RPKM 15.5) and 24 other tissuesSee more

Orthologs <u>human</u> all

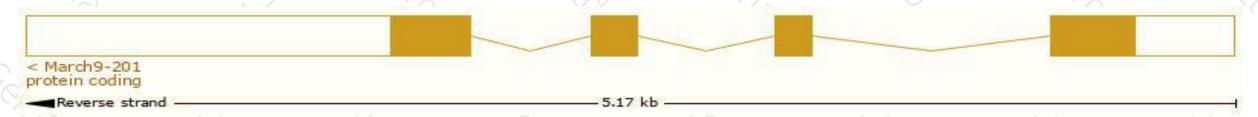
## Transcript information (Ensembl)



The gene has 1 transcript, and the transcript is shown below:

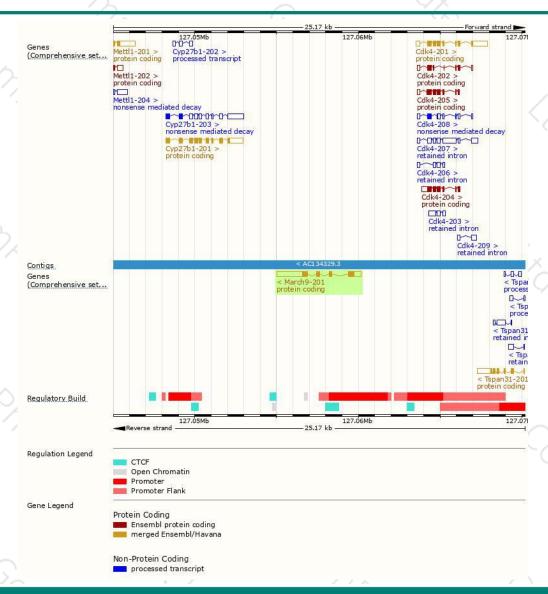
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	l
March9-201	ENSMUST00000040307.5	3037	348aa	Protein coding	CCDS24225	Q3TZ87	TSL:1 GENCODE basic APPRIS P1	ľ

The strategy is based on the design of *March9-201* transcript, the transcription is shown below:



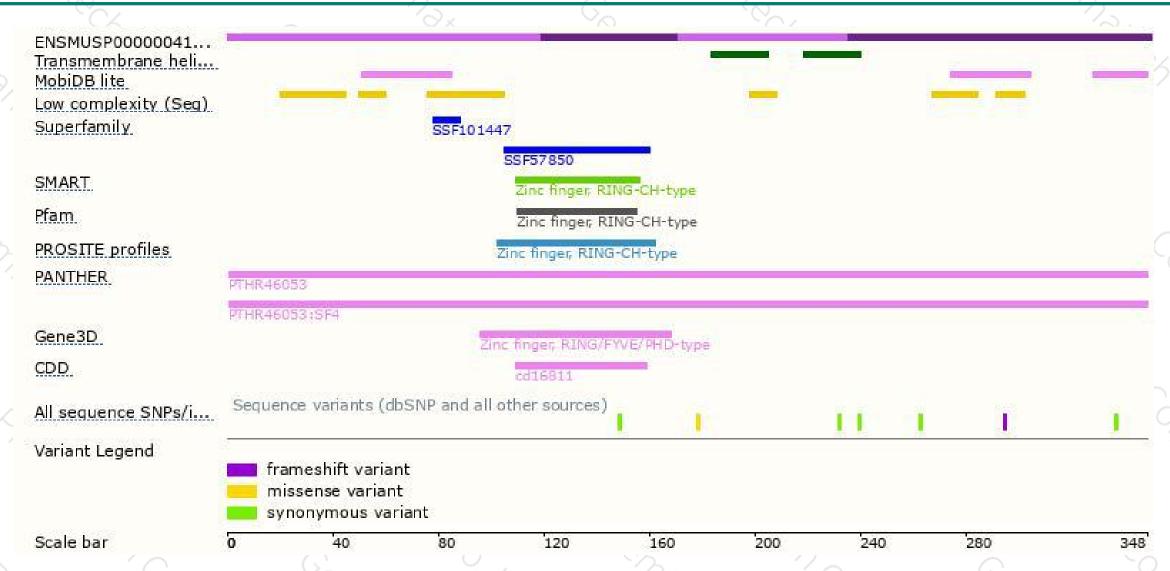
### Genomic location distribution





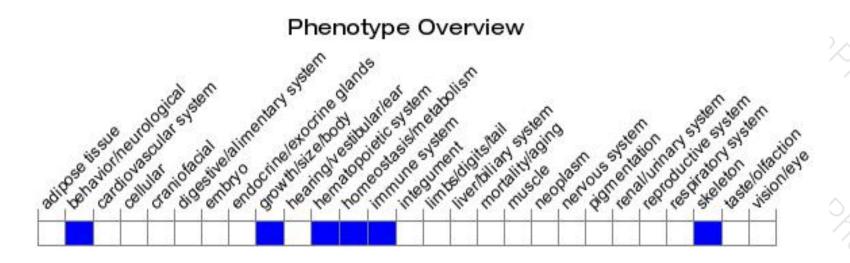
### Protein domain





## Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).



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

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