

# Vcp Cas9-CKO Strategy

Designer: Jinling Wang

**Design Date:** 2019-7-19

## **Project Overview**



**Project Name** 

Project type Cas9-CKO

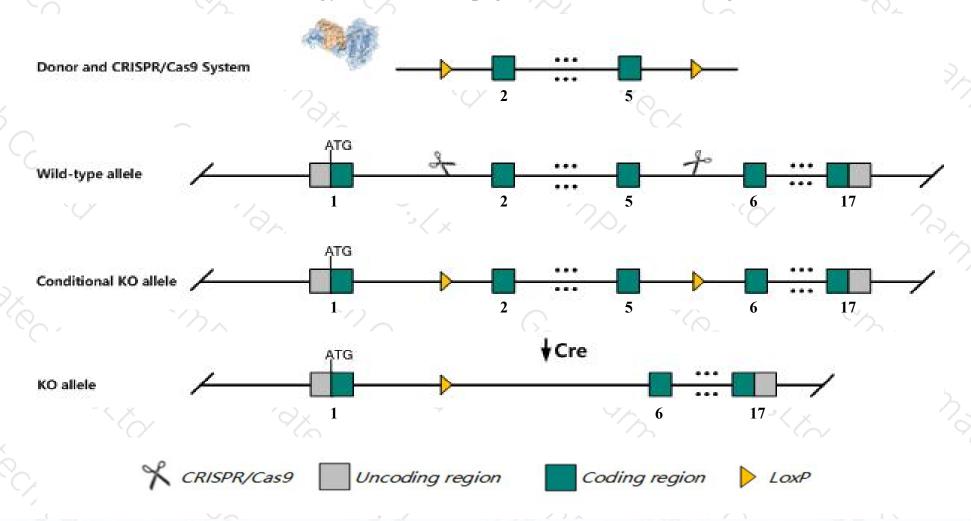
Strain background C57BL/6JGpt

Vcp

## Conditional Knockout strategy



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



### Technical routes



- The *Vcp* gene has 5 transcripts. According to the structure of *Vcp* gene, exon2-exon5 of *Vcp-201*(ENSMUST00000030164.7) transcript is recommended as the knockout region. The region contains 559bp coding sequence.

  Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Vcp* 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 mutation of this gene results in lethality before weaning.

  Mice homozygous for a knock-in allele exhibit progressive muscle weakness, myopathy, decreased bone density, increased osteoclast genesis, and seizures.
- > The *Vcp* gene is located on the Chr4. 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)



#### Vcp valosin containing protein [Mus musculus (house mouse)]

Gene ID: 269523, updated on 7-Apr-2019

#### Summary

☆ ?

Official Symbol Vcp provided by MGI

Official Full Name valosin containing protein provided by MGI

Primary source MGI:MGI:99919

See related Ensembl: ENSMUSG00000028452

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 3110001E05, CDC48, p97, p97/VCP

Expression Ubiquitous expression in placenta adult (RPKM 109.8), adrenal adult (RPKM 109.0) and 28 other tissuesSee more

Orthologs <u>human</u> all

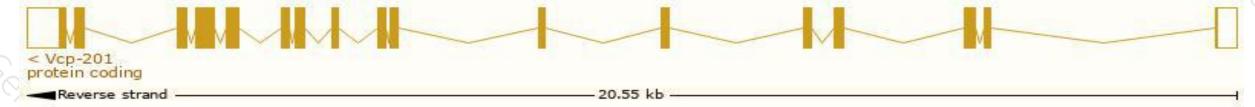
## Transcript information (Ensembl)



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

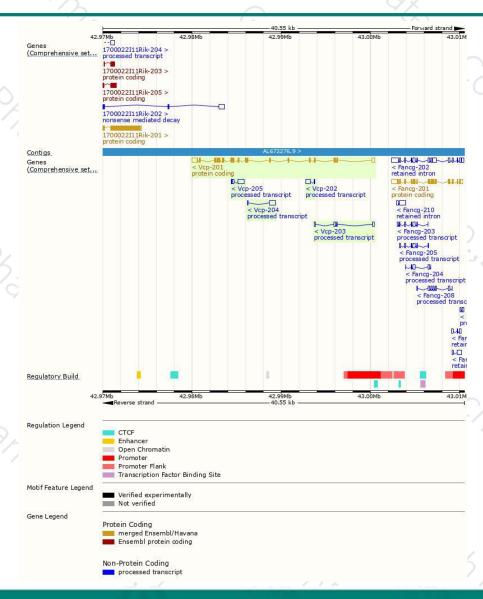
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Vcp-201	ENSMUST00000030164.7	3316	806aa	Protein coding	CCDS18086	Q01853	TSL:1 GENCODE basic APPRIS P1
Vcp-205	ENSMUST00000154541.1	894	No protein	Processed transcript	-	-	TSL:1
Vcp-204	ENSMUST00000154423.1	707	No protein	Processed transcript	9	14	TSL:2
Vcp-202	ENSMUST00000139843.1	618	No protein	Processed transcript	-	150	TSL:1
Vcp-203	ENSMUST00000148182.1	539	No protein	Processed transcript		(2)	TSL:3

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



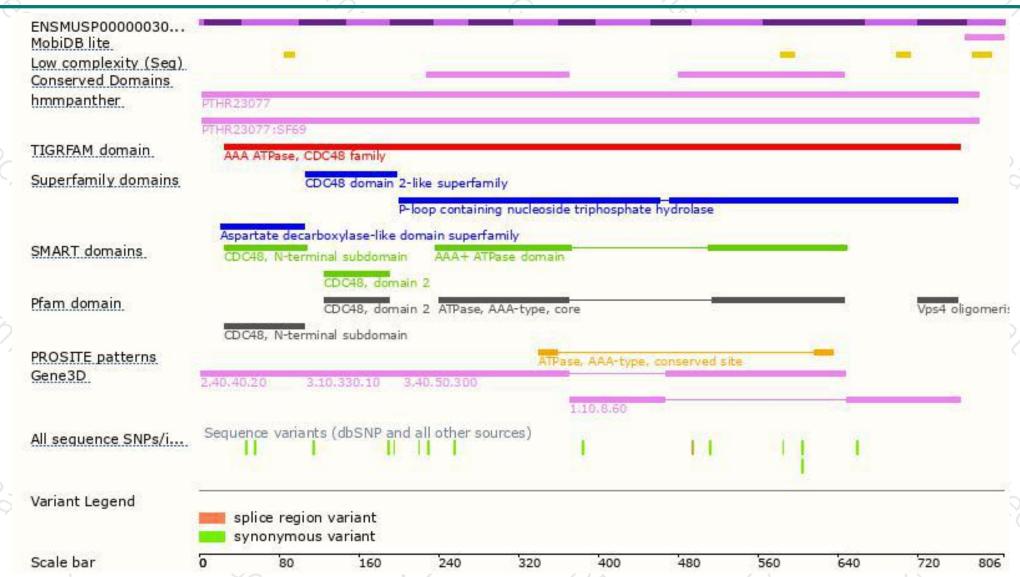
### Genomic location distribution





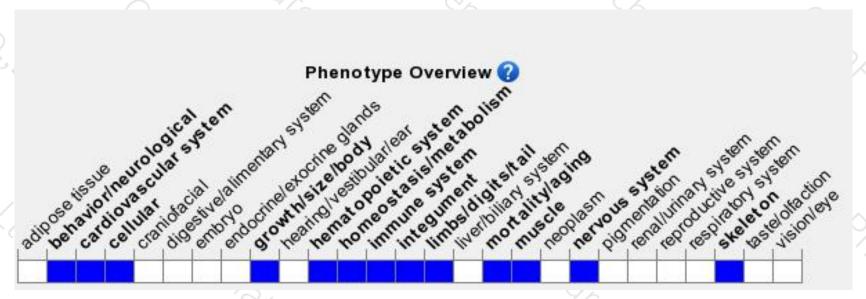
### Protein domain





## 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 mutation of this gene results in lethality before weaning. Mice homozygous for a knock-in allele exhibit progressive muscle weakness, myopathy, decreased bone density, increased osteocla genesis, and seizures.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





