

Vim Cas9-CKO Strategy

Designer:

Jinlong Zhao

Reviewer:

Shilei Zhu

Design Date:

2018-9-28

Project Overview



Project Name Vim

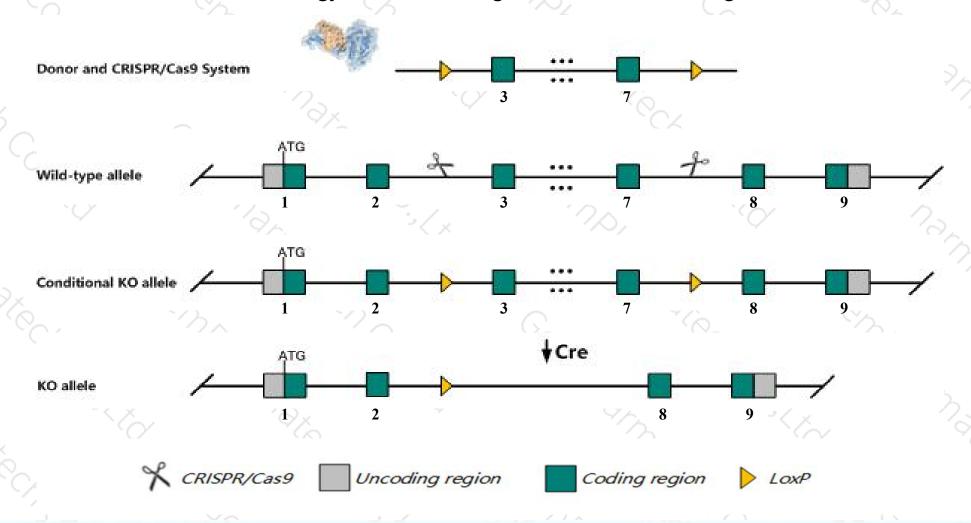
Project type Cas9-CKO

Strain background C57BL/6JGpt

Conditional Knockout strategy



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



Technical routes



- The *Vim* gene has 6 transcripts. According to the structure of *Vim* gene, exon3-exon7 of *Vim-201*(ENSMUST00000028062.7) transcript is recommended as the knockout region. The region contains 649bp coding sequence.

 Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Vim* 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 null mutants exhibit impaired performance in motor coordination tests; cerebellum shows underdeveloped/abnormal Bergman glia and stunted, poorly branched Purkinje cells. Mutants are unable to survive experimental 75% reduction of kidney mass.
- > The *Vim* 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)



Vim vimentin [Mus musculus (house mouse)]

Gene ID: 22352, updated on 9-Apr-2019

Summary

?

Official Symbol Vim provided by MGI

Official Full Name vimentin provided by MGI

Primary source MGI:MGI:98932

See related Ensembl: ENSMUSG00000026728

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

Expression Broad expression in subcutaneous fat pad adult (RPKM 622.6), mammary gland adult (RPKM 524.7) and 24 other tissues See more

Orthologs <u>human</u> all

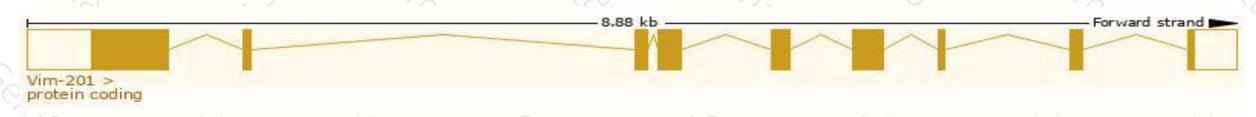
Transcript information (Ensembl)



The gene has 6 transcripts, all transcripts are shown below:

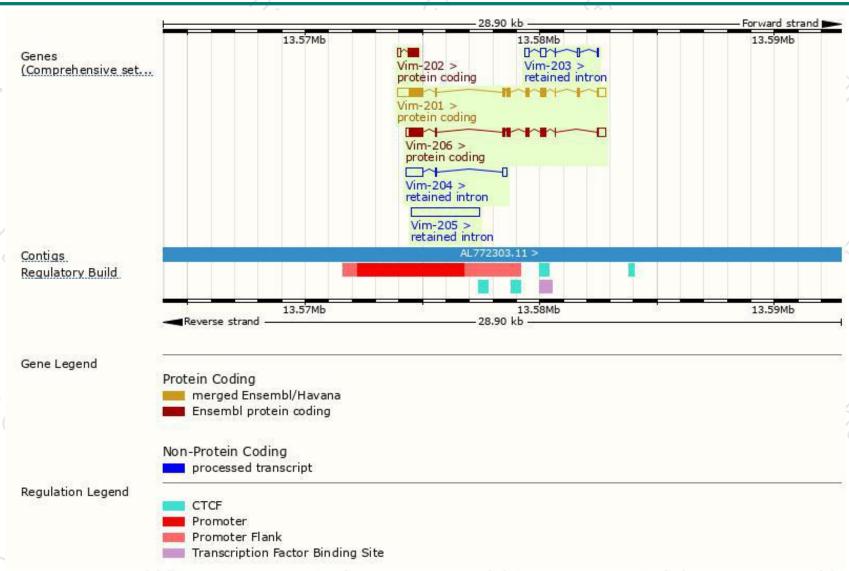
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Vim-201	ENSMUST00000028062.7	2193	466aa	Protein coding	CCDS15696	P20152 Q5FWJ3	TSL:1 GENCODE basic APPRIS P2
Vim-206	ENSMUST00000193675.1	1777	<u>427aa</u>	Protein coding	5.00	A0A0A6YWC8	TSL:5 GENCODE basic APPRIS ALT2
Vim-202	ENSMUST00000141365.2	574	<u>134aa</u>	Protein coding	(s 4 -8	A2AKJ2	CDS 3' incomplete TSL:2
Vim-205	ENSMUST00000191615.1	2926	No protein	Retained intron		-	TSL:NA
Vim-204	ENSMUST00000155605.1	926	No protein	Retained intron	150	5	TSL:1
Vim-203	ENSMUST00000148248.2	535	No protein	Retained intron	6.00		TSL:2

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



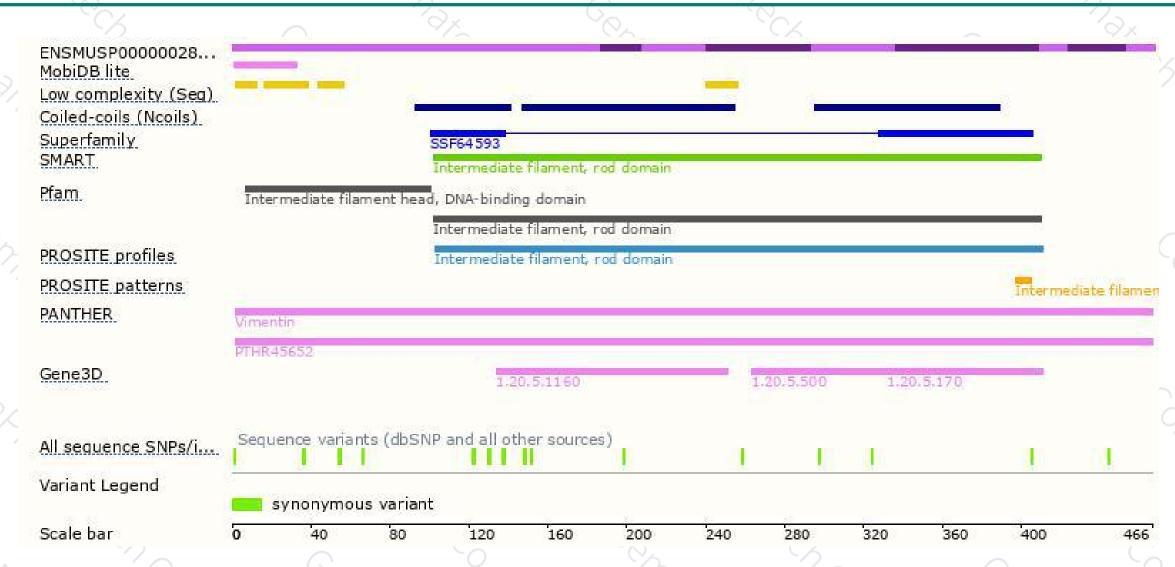
Genomic location distribution





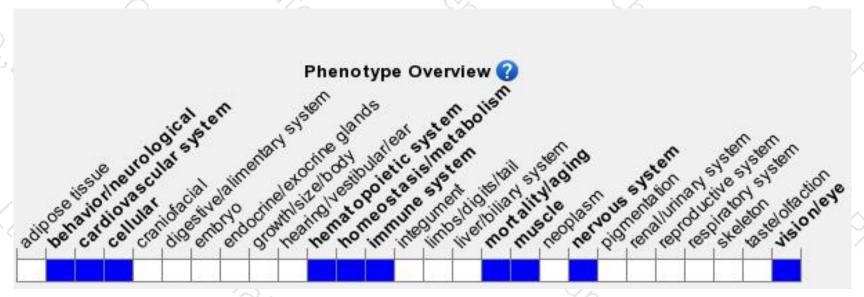
Protein domain





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 null mutants exhibit impaired performance in motor coordination tests; cerebellum shows underdeveloped/abnormal Bergman glia and stunted, poorly branched Purkinje cells. Mutants are unable to survive experimental 75% reduction of kidney mass.



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





