

Vwc2 Cas9-CKO Strategy

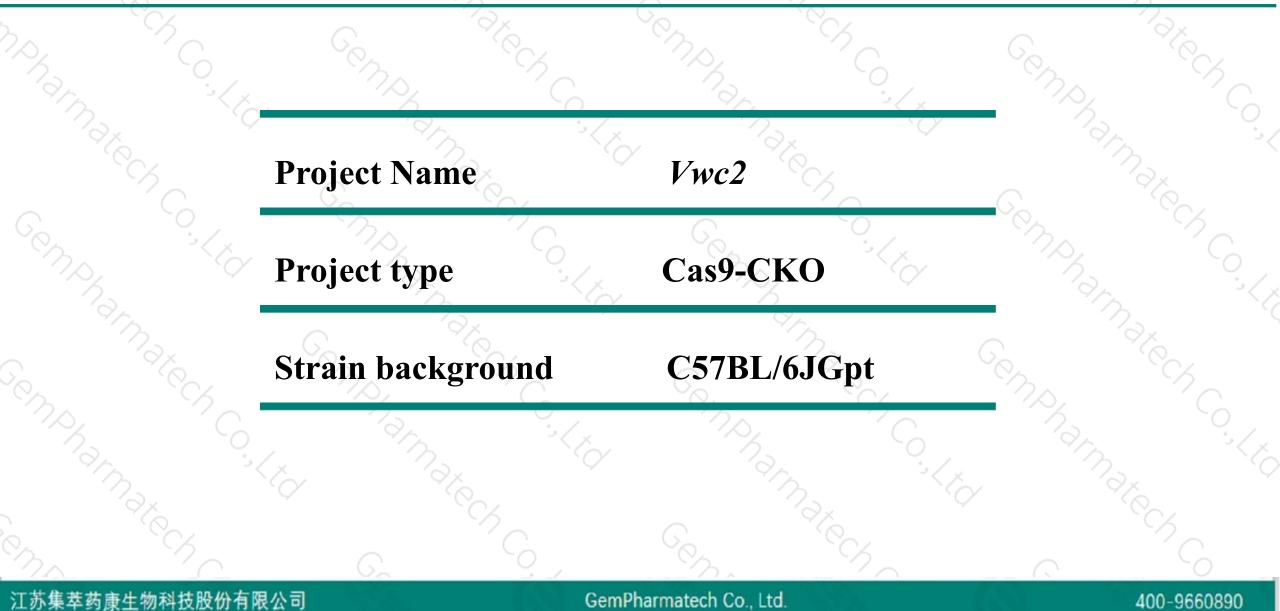
Designer: Reviewer:

Design Date:

Huan Wang Huan Fan 2020-5-12

Project Overview

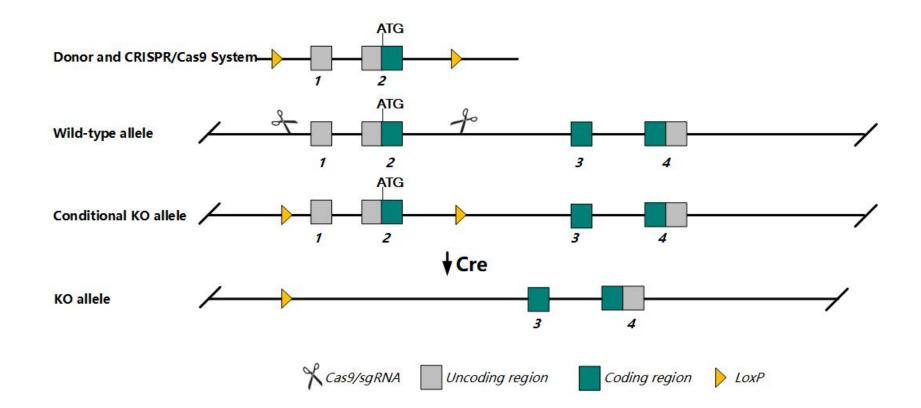




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Vwc2* gene. The schematic diagram is as follows:





The Vwc2 gene has 4 transcripts. According to the structure of Vwc2 gene, exon1-exon2 of Vwc2-202 (ENSMUST00000109681.7) transcript is recommended as the knockout region. The region contains start condon ATG. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Vwc2* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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



- The Vwc2 gene is located on the Chr11. 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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Vwc2 von Willebrand factor C domain containing 2 [Mus musculus (house mouse)]

Gene ID: 319922, updated on 13-Mar-2020

Summary

Official Symbol	Vwc2 provided by MGI
Official Full Name	von Willebrand factor C domain containing 2 provided by MGI
Primary source	MGI:MGI:2442987
See related	Ensembl:ENSMUSG00000050830
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	A930041G11Rik, G11, PSST739, UNQ739, cradin
Expression	Low expression observed in reference datasetSee more
Orthologs	human all

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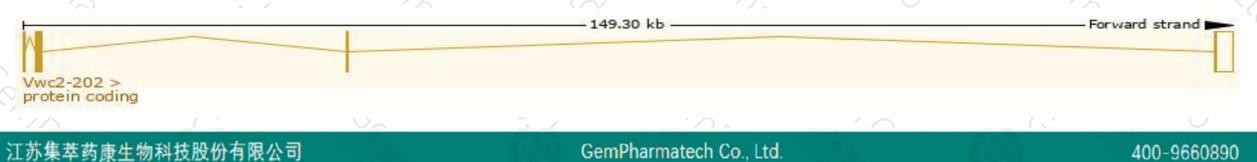
Transcript information (Ensembl)



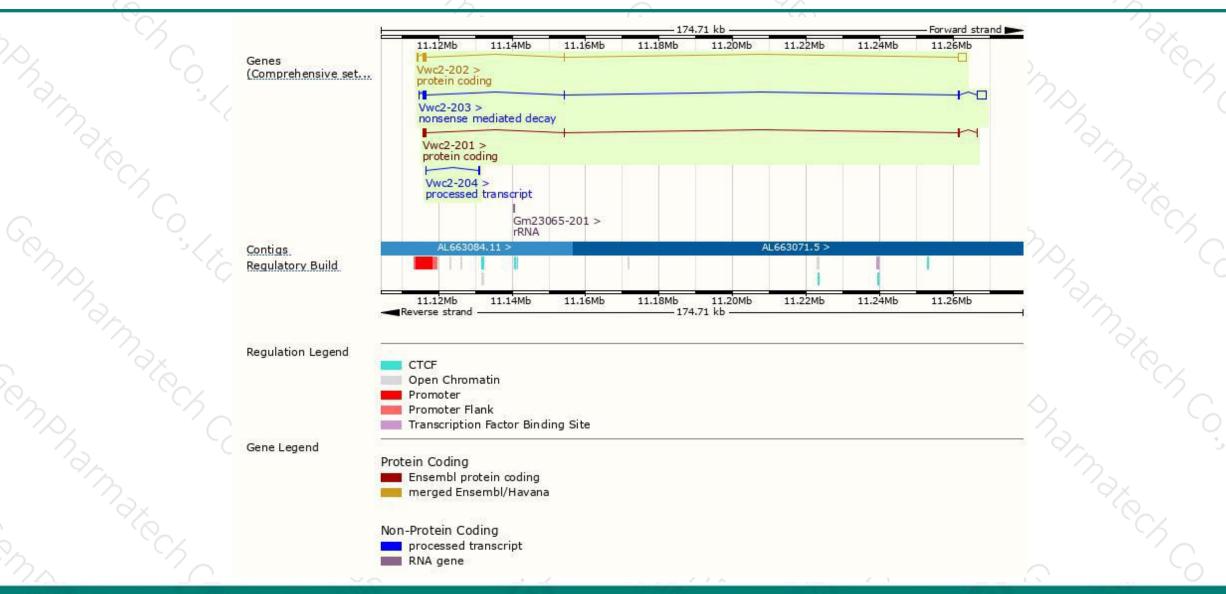
The gene has 4 transcripts, all transcripts are shown below:

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Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Vwc2-202	ENSMUST00000109681.7	3371	<u>324aa</u>	Protein coding	CCDS36111	Q8C8N3	TSL:1 GENCODE basic APPRIS P1
Vwc2-201	ENSMUST0000056344.4	1350	<u>324aa</u>	Protein coding	CCDS36111	Q8C8N3	TSL:1 GENCODE basic APPRIS P1
Vwc2-203	ENSMUST00000129670.7	3712	<u>324aa</u>	Nonsense mediated decay	20	Q8C8N3	TSL:1
Vwc2-204	ENSMUST00000154155.1	397	No protein	Processed transcript	10 B	1020	TSL:3

The strategy is based on the design of *Vwc2-202* transcript, the transcription is shown below



Genomic location distribution



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Protein domain



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All sequence SNPs/i	Sequence vari	ants (dbSNP and all	other sources)	21	10	4	0.010
Variant Legend	synonym	ous variant					
Scale bar	0	40 80	120	160	200	240	280
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If you have any questions, you are welcome to inquire. Tel: 400-9660890



