

Wfdc8 Cas9-CKO Strategy

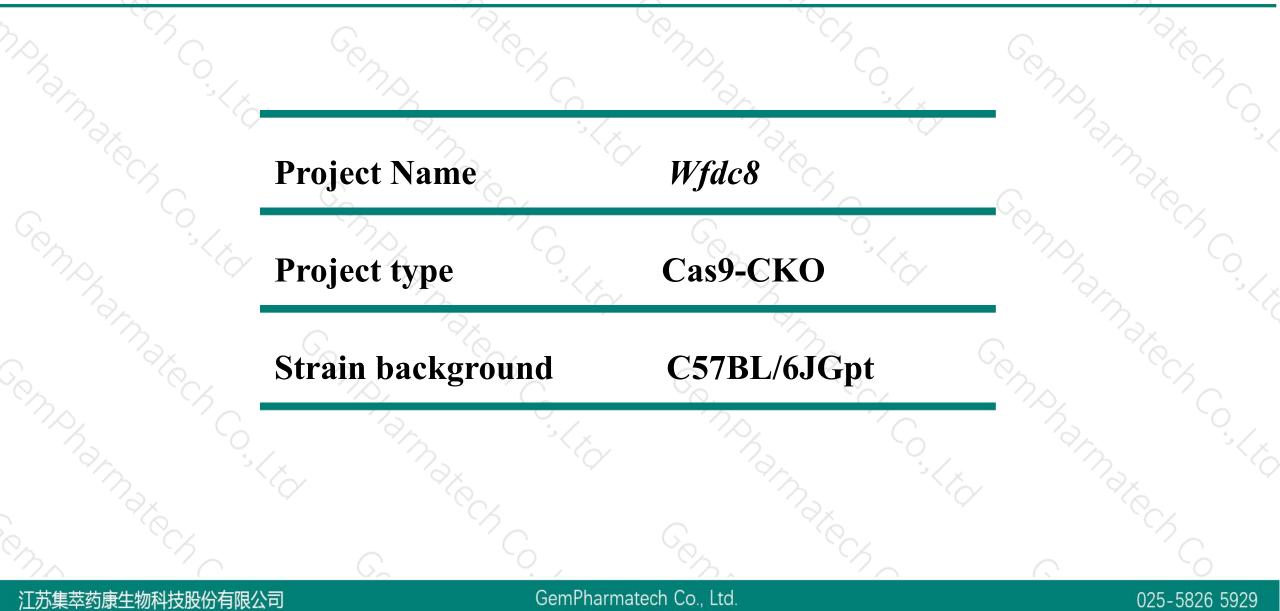
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Reviewer: Daohua Xu

Design Date: 2020-11-18

Project Overview



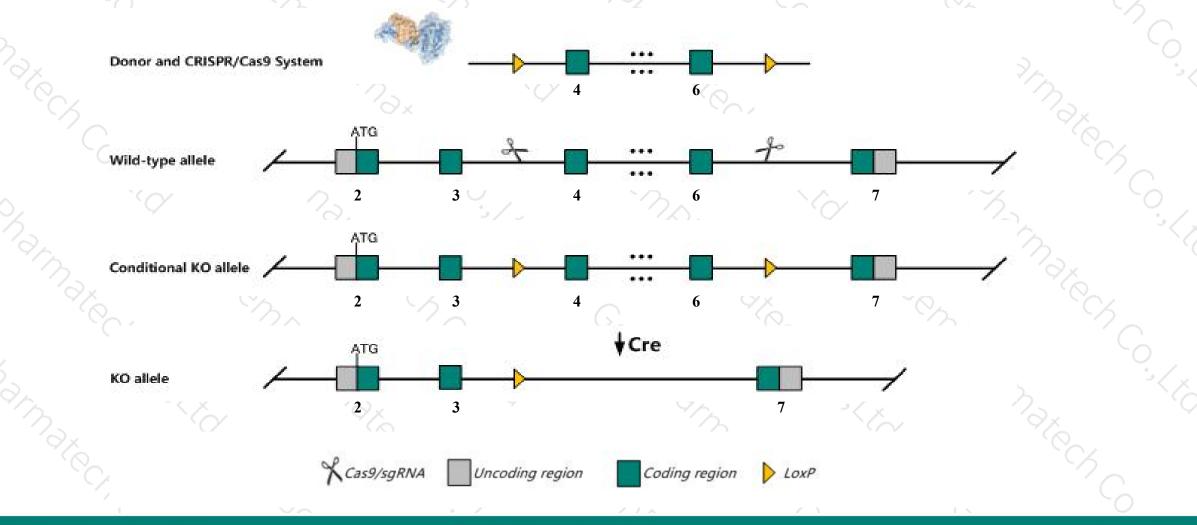


Conditional Knockout strategy



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This model will use CRISPR/Cas9 technology to edit the *Wfdc8* gene. The schematic diagram is as follows:



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➤ The Wfdc8 gene has 3 transcripts. According to the structure of Wfdc8 gene, exon4-exon6 of Wfdc8-203(ENSMUST00000109339.7) 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 *Wfdc8* 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.



> The *Wfdc8* 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.

> Gm25512 gene will be deleted.

> 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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Wfdc8 WAP four-disulfide core domain 8 [Mus musculus (house mouse)]

Gene ID: 277343, updated on 13-Mar-2020

Summary

Official Symbol	Wfdc8 provided by MGI
Official Full Name	WAP four-disulfide core domain 8 provided by MGI
Primary source	MGI:MGI:2685552
See related	Ensembl:ENSMUSG0000070533
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	Gm706
Expression	Restricted expression toward genital fat pad adult (RPKM 66.5)See more
Orthologs	human all



Transcript information (Ensembl)

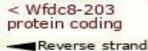


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The gene has 3 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Wfdc8-203	ENSMUST00000109339.7	3227	<u>426aa</u>	Protein coding	CCDS71191	A2A5H1	TSL:1 GENCODE basic APPRIS ALT2
Wfdc8-202	ENSMUST00000109338.1	1794	<u>273aa</u>	Protein coding	CCD538324	Q4KUS1	TSL:1 GENCODE basic APPRIS P3
Wfdc8-201	ENSMUST00000094351.10	1530	<u>264aa</u>	Protein coding	CCDS71192	Q4KXB5	TSL:1 GENCODE basic APPRIS ALT2

The strategy is based on the design of *Wfdc8-203* transcript, the transcription is shown below:

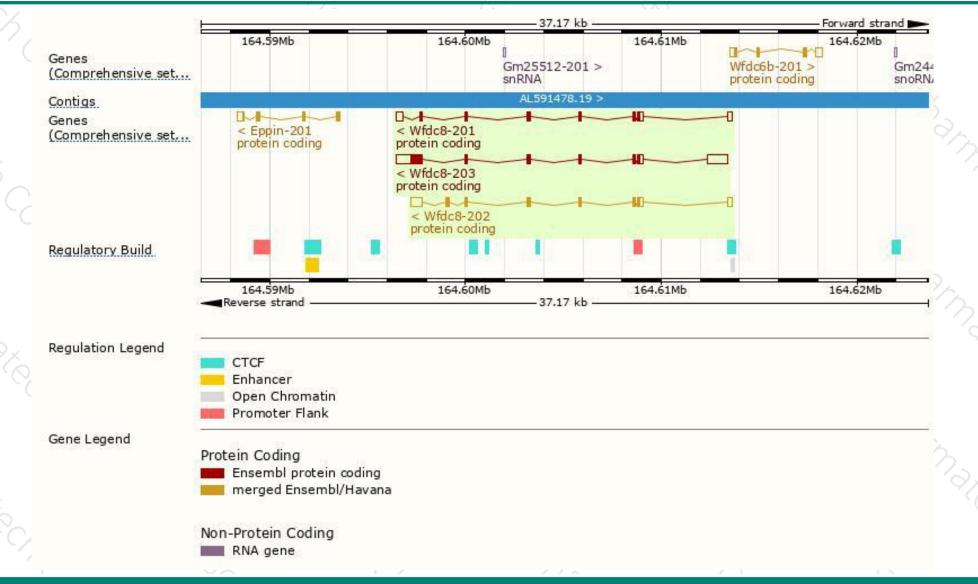


- 16.92 kb -

Genomic location distribution



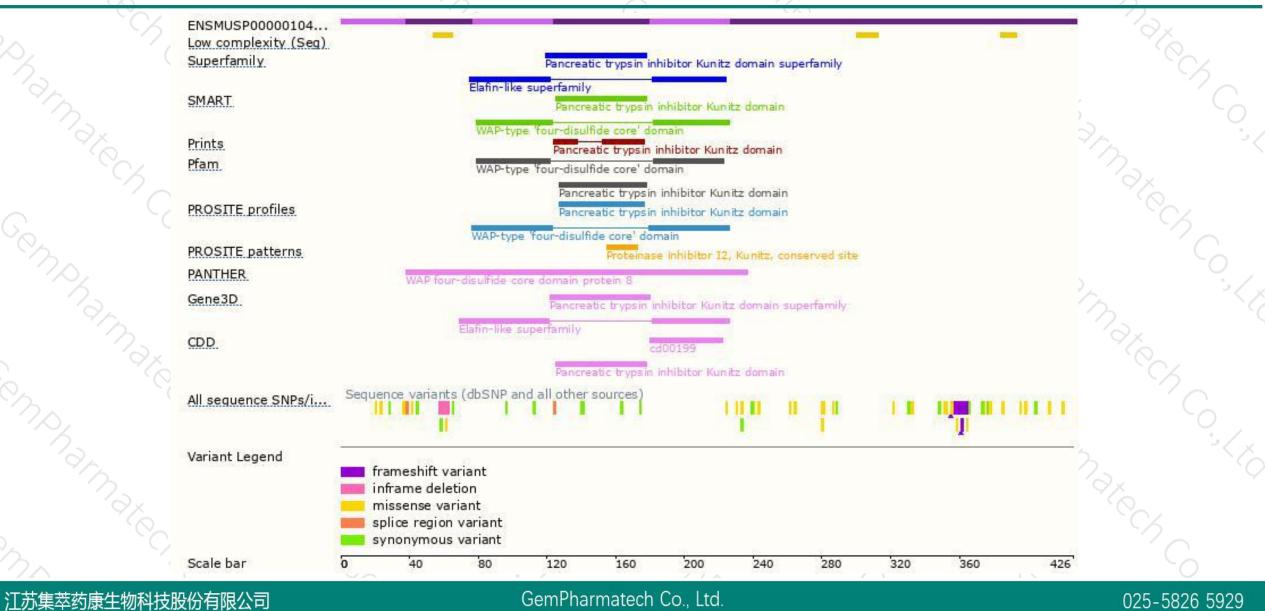
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Protein domain







If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



