

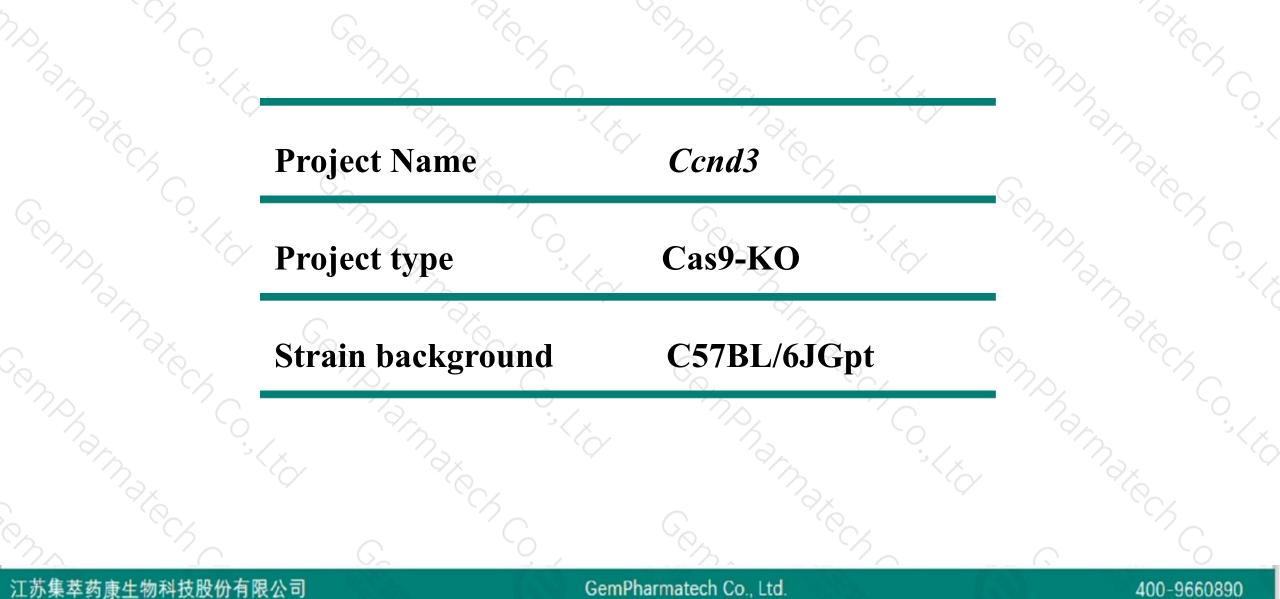
Ccnd3 Cas9-KO Strategy

Designer: Reviewer: Design Date: Ruirui Zhang Huimin Su

2020-1-7

Project Overview

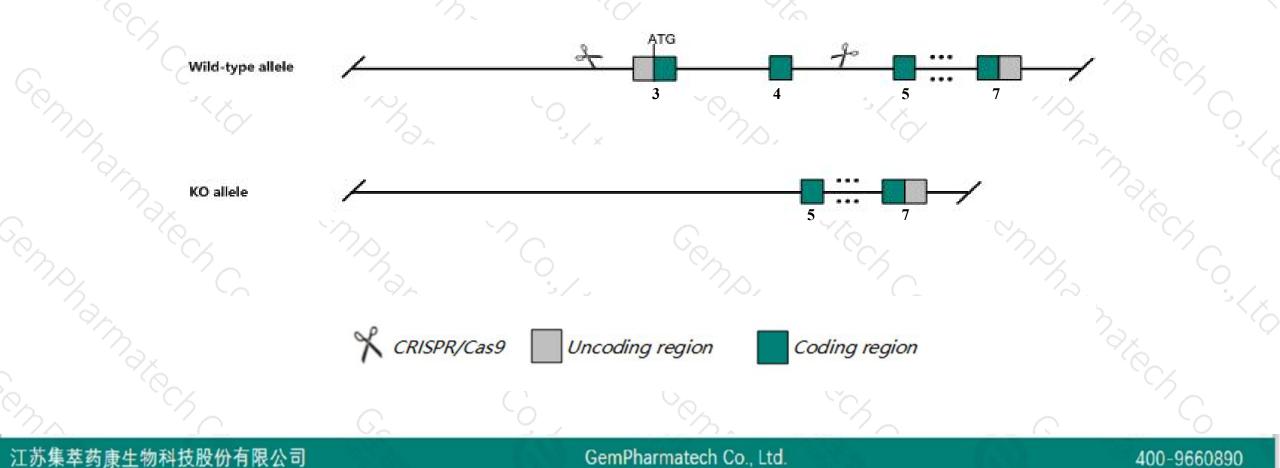




Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Ccnd3 gene. The schematic diagram is as follows:





The Ccnd3 gene has 21 transcripts. According to the structure of Ccnd3 gene, exon3-exon4 of Ccnd3-202 (ENSMUST00000171031.7) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.

> In this project we use CRISPR/Cas9 technology to modify Ccnd3 gene. The brief process is as follows: CRISPR/Cas9 system



- According to the existing MGI data, mice homozygous for a knock-out allele exhibit severe thymus hypoplasia, abnormal thymocyte development, and impaired expansion of immature T lymphocytes.
- ➤ Transcripts *Ccnd3*-209,*Ccnd3*-219, *Ccnd3*-221 may not be affected.
- The Ccnd3 gene is located on the Chr17. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)



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Ccnd3 cyclin D3 [Mus musculus (house mouse)]

Gene ID: 12445, updated on 5-Nov-2019

Summary

Official Symbol	Ccnd3 provided by MGI							
Official Full Name	cyclin D3 provided by MGI							
Primary source	MGI:MGI:88315							
See related	Ensembl:ENSMUSG0000034165							
Gene type	protein coding							
RefSeq status	VALIDATED							
Organism	Mus musculus							
Lineage	Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae							
	Murinae; Mus; Mus							
Also known as	C78795; AA682053; AL024085; AW146355; 9230106B05Rik							
Expression	ion Broad expression in thymus adult (RPKM 402.8), adrenal adult (RPKM 319.1) and 18 other tissues See more							
Orthologs	human all							
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Transcript information (Ensembl)



The gene has 21 transcripts, all transcripts are shown below:

Name 🖕	Transcript ID 💧	bp 🍦	Protein 🖕	Biotype 🝦	CCDS 🖕	UniProt 💧	Flags 🍦
Ccnd3-202	ENSMUST00000171031.7	2113	<u>292aa</u>	Protein coding	CCDS28850	P30282@ Q3TSW4@	TSL:5 GENCODE basic APPRIS P1
Ccnd3-215	ENSMUST00000183044.7	2064	<u>292aa</u>	Protein coding	CCDS28850	P30282@ Q3TSW4@	TSL:1 GENCODE basic APPRIS P1
Ccnd3-218	ENSMUST00000183177.7	2060	<u>292aa</u>	Protein coding	CCDS28850	<u>P30282</u> & <u>Q3TSW4</u> &	TSL:5 GENCODE basic APPRIS P1
Ccnd3-201	ENSMUST0000037333.16	1999	<u>292aa</u>	Protein coding	CCDS28850@	P30282@ Q3TSW4@	TSL:1 GENCODE basic APPRIS P1
Ccnd3-205	ENSMUST00000182209.7	1977	<u>292aa</u>	Protein coding	CCDS28850	P30282@ Q3TSW4@	TSL:1 GENCODE basic APPRIS P1
Ccnd3-204	ENSMUST00000182129.7	952	<u>214aa</u>	Protein coding	=	<u>S4R240</u> ര്	CDS 3' incomplete TSL:5
Ccnd3-208	ENSMUST00000182539.7	931	<u>96aa</u>	Protein coding	-	<u>S4R216</u> ச	TSL:2 GENCODE basic
Ccnd3-211	ENSMUST00000182848.7	926	<u>243aa</u>	Protein coding	-	<u>S4R2N5</u>	CDS 3' incomplete TSL:5
Ccnd3-207	ENSMUST00000182506.7	853	<u>250aa</u>	Protein coding	=	<u>S4R1D9</u> 65	CDS 3' incomplete TSL:5
Ccnd3-221	ENSMUST00000183256.7	560	<u>75aa</u>	Protein coding	-	<u>S4R275</u> മ്	CDS 3' incomplete TSL:5
Ccnd3-212	ENSMUST00000182874.2	484	<u>119aa</u>	Protein coding	-	<u>S4R2N1</u>	CDS 3' incomplete TSL:5
Ccnd3-210	ENSMUST00000182846.2	464	<u>125aa</u>	Protein coding	-	<u>S4R1R1</u>	CDS 5' incomplete TSL:3
Ccnd3-217	ENSMUST00000183158.1	385	<u>99aa</u>	Protein coding	=	<u>S4R1C8</u>	CDS 3' incomplete TSL:2
Ccnd3-219	ENSMUST00000183206.1	336	<u>29aa</u>	Protein coding	-	<u>S4R2Q7</u> @	CDS 3' incomplete TSL:3
Ccnd3-220	ENSMUST00000183210.1	299	<u>52aa</u>	Protein coding	-	<u>S4R215</u> 교	CDS 3' incomplete TSL:5
Ccnd3-213	ENSMUST00000182935.7	277	<u>43aa</u>	Protein coding	=	<u>S4R1L8</u> &	CDS 3' incomplete TSL:3
Ccnd3-206	ENSMUST00000182281.1	3858	No protein	Retained intron	-	-	TSL:1
Ccnd3-216	ENSMUST00000183061.1	2670	No protein	Retained intron	-		TSLINA
Ccnd3-203	ENSMUST00000182060.1	754	No protein	Retained intron	-		TSL:1
Ccnd3-214	ENSMUST00000183014.1	552	No protein	Retained intron	-		TSL:3
Ccnd3-209	ENSMUST00000182591.1	283	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Ccnd3-202* transcript, the transcription is shown below:

Ccnd3-202 > protein coding

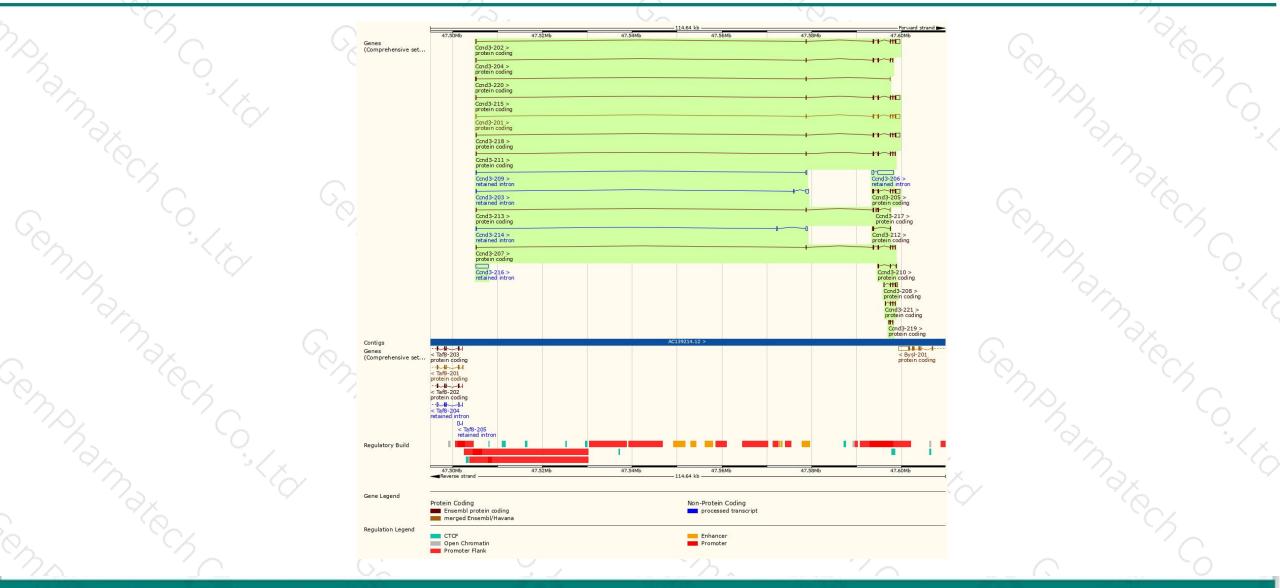
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Genomic location distribution





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Protein domain

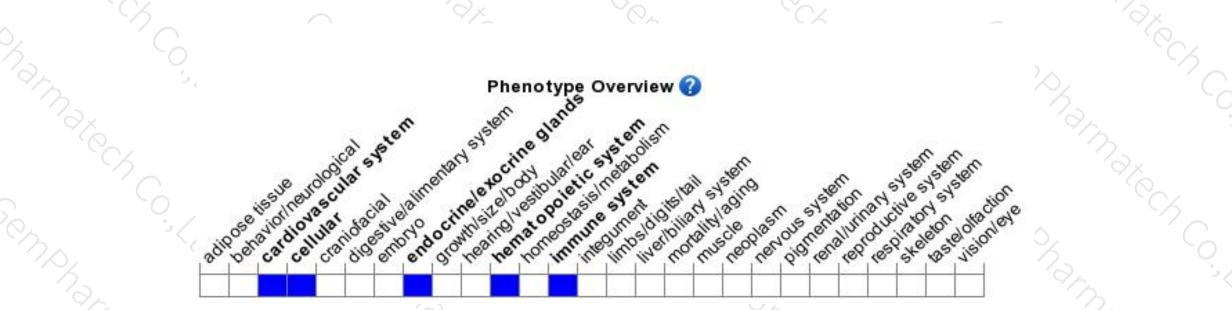


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	Pfam		Oyclin, N-terminal			Cycli	n, C-terminal domain			
	PROSITE patterns PIRSF		Cyclin	Cyclin, N-terminal						
	PANTHER Gene3D CDD	Cyclin Cyclin D	1.10.472.10	Cyclin-like						
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Mouse phenotype 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, Mice homozygous for a knock-out allele exhibit severe thymus hypoplasia, abnormal thymocyte development, and impaired expansion of immature T lymphocytes.



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



