

Cdk2 Cas9-KO Strategy

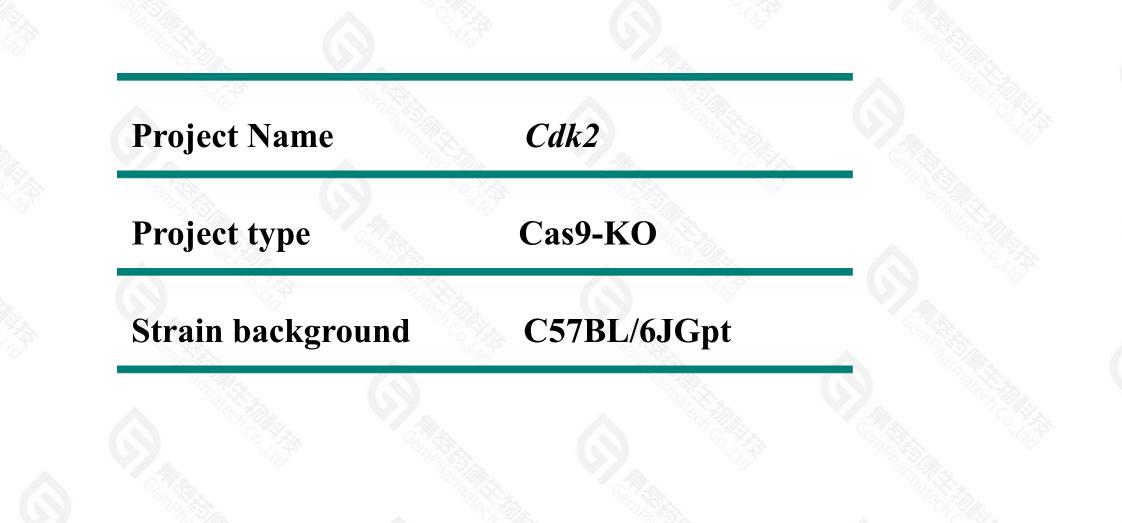
Designer: Huan Wang

Reviewer: Lingyan Wu

Design Date: 2021-7-27

Project Overview





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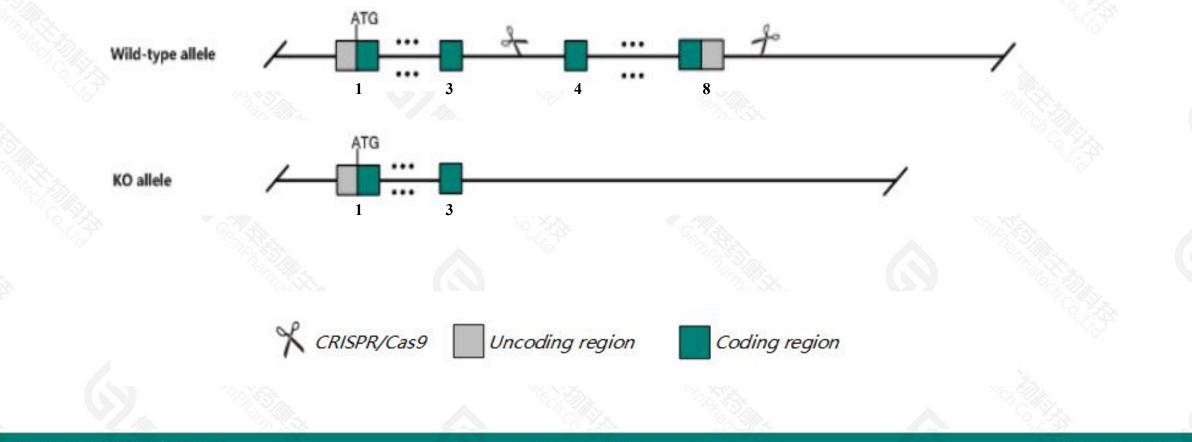
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Knockout strategy



400-9660890

This model will use CRISPR/Cas9 technology to edit the *Cdk2* gene. The schematic diagram is as follows:



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> The *Cdk2* gene has 7 transcripts. According to the structure of *Cdk2* gene, exon4-exon8 of *Cdk2*-202(ENSMUST00000026416.15) 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 Cdk2 gene. The brief process is as follows: CRISPR/Cas9 system 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.



- According to the existing MGI data, reproductive system abnormalities are observed in mice homozygous for disruptions in this gene. Gametogenesis fails in both males and females, leading to atrophy of the testes and ovaries. Both sexes are sterile.
 The KO region is about 1.1kb and 1kb from *Rab5b* and *Pmel* gene. Knockout the region may affect the function of *Rab5b* and *Pmel* gene.
- > Some amino acids remain at the N terminal, and part of the protein's function may be preserved.
- > The *Cdk2* gene is located on the Chr10. 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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Cdk2 cyclin-dependent kinase 2 [Mus musculus (house mouse)]

Gene ID: 12566, updated on 13-Mar-2020

Summary

 Official Symbol
 Cdk2 provided by MGI

 Official Full Name
 cyclin-dependent kinase 2 provided by MGI

 Primary source
 MGI:MGI:104772

 See related
 Ensembl:ENSMUSG00000025358

 Gene type
 protein coding

 RefSeq status
 VALIDATED

 Organism
 Mus musculus

 Lineage
 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muriade; Murinae; Mus; Mus

 Also known as
 A630093N05Rik

 Expression
 Broad expression in limb E14.5 (RPKM 35.9), thymus adult (RPKM 24.5) and 25 other tissues<u>See more</u>

 Orthologs
 human all

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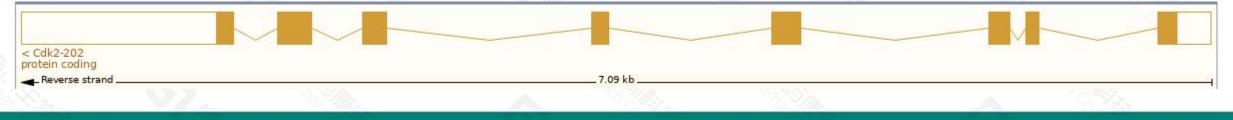
Transcript information (Ensembl)



The gene has 7 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cdk2-202	ENSMUST00000026416.14	2406	<u>346aa</u>	Protein coding	CCDS24289	P97377 Q3UGB9	TSL:1 GENCODE basic
Cdk2-201	ENSMUST0000026415.8	2240	<u>298aa</u>	Protein coding	CCDS24288	P97377 Q3U6X7	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Cdk2-207	ENSMUST00000220407.1	549	No protein	Processed transcript	-		TSL:2
Cdk2-203	ENSMUST00000219047.1	3264	No protein	Retained intron	-	1	TSL:NA
Cdk2-204	ENSMUST00000219099.1	2971	No protein	Retained intron		1.00	TSL:1
Cdk2-205	ENSMUST00000219601.1	2673	No protein	Retained intron			TSL:1
Cdk2-206	ENSMUST00000219983.1	1708	No protein	Retained intron		-	TSL:1

The strategy is based on the design of *Cdk2-202* transcript, the transcription is shown below:

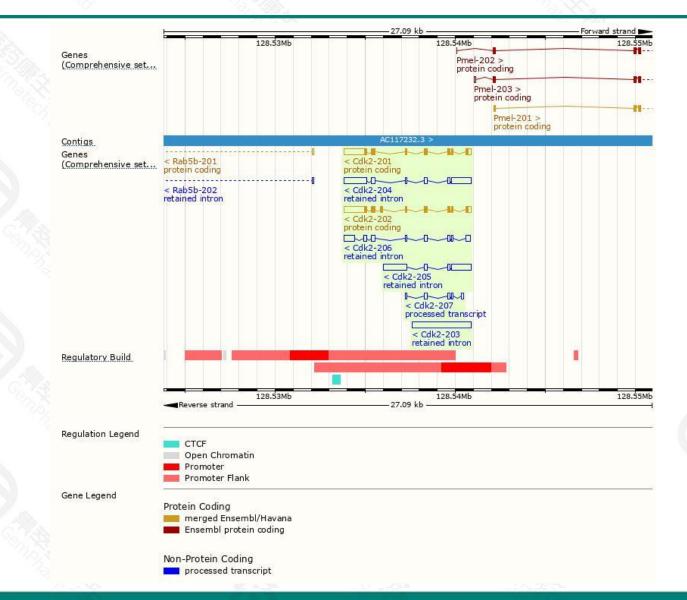


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Genomic location distribution





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Protein domain



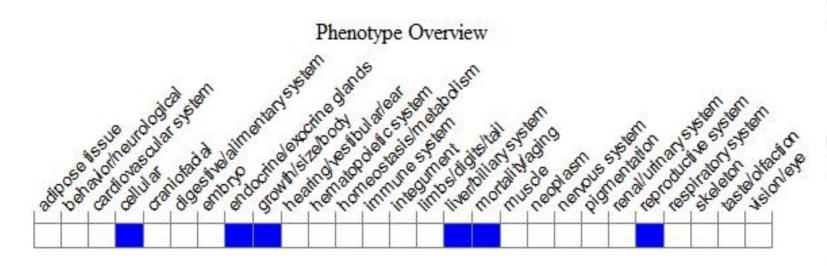
ENSMUSP00000026 Superfamily	Protein kinase-lik	ke domain superfami	ily			a la A La	2 117						
MART	Protein kinase domain												
<u>Yam</u>	Protein kinase d	domain											
ROSITE profiles	Protein kinase d	domain											
PROSITE patterns	Protein kinas	e, ATP binding site	Serine/thr	eonine-proteir	kinase, active	site							
PANTHER	PTHR24056:SF3	71						-					
Gene3D	PTHR24056							-					
	3,30,200,20	1.10	.510.10					20					
<u>DD</u>	cd07860												
All sequence SNPs/i	Sequence varia	ants (dbSNP and a	ll other sources)	Ú.		7.400	110	н					
/ariant Legend	8			74	1.74		T						
	missense	variant ous variant											
Scale bar	0 4		120	160	200	240	280	34					

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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, reproductive system abnormalities are observed in mice homozygous for disruptions in this gene. Gametogenesis fails in both males and females, leading to atrophy of the testes and ovaries. Both sexes are sterile.

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



