

# Cdk6 Cas9-CKO Strategy

Designer: Jinling Wang

**Design Date:** 2019-7-17

## **Project Overview**



**Project Name** 

Cdk6

**Project type** 

Cas9-CKO

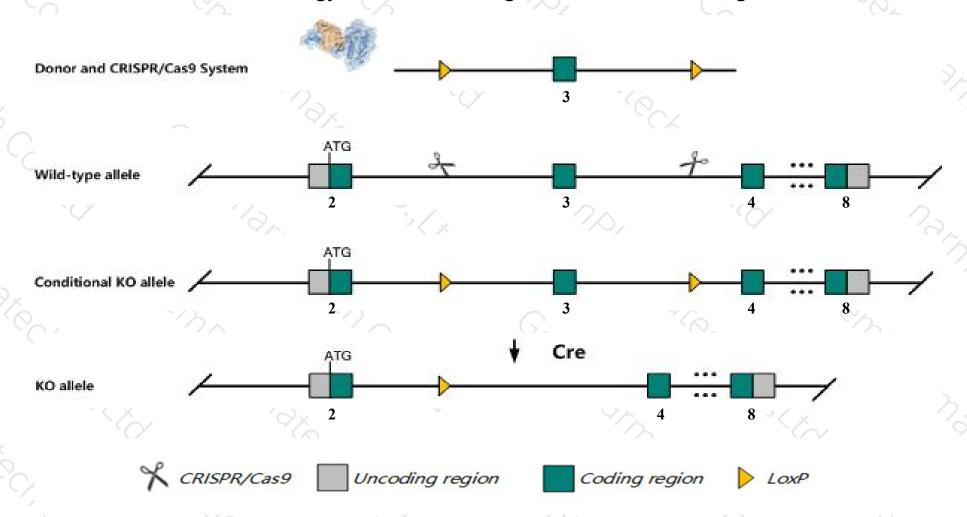
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Cdk6* gene. The schematic diagram is as follows:



### Technical routes



- ➤ The *Cdk6* gene has 6 transcripts. According to the structure of *Cdk6* gene, exon3 of *Cdk6-202*(ENSMUST00000165117.7) transcript is recommended as the knockout region. The region contains 136bp coding sequence.

  Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Cdk6* 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, Mice homozygous for disruptions in this gene display hematopoietic abnormalities affecting spleen and thymus size. Female body weight and fertility are also reduced.
- The *Cdk6* gene is located on the Chr5. 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)



#### Cdk6 cyclin-dependent kinase 6 [Mus musculus (house mouse)]

Gene ID: 12571, updated on 31-Jan-2019

#### Summary

☆ ?

Official Symbol Cdk6 provided by MGI

Official Full Name cyclin-dependent kinase 6 provided by MGI

Primary source MGI:MGI:1277162

See related Ensembl: ENSMUSG00000040274

Gene type protein coding
RefSeq status REVIEWED
Organism Mus musculus

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;

Muroidea; Muridae; Murinae; Mus; Mus

Also known as Al504062, Crk2

Summary This gene encodes a member of the cyclin dependent kinase family of proteins that play important roles in the progression and regulation of

the cell cycle. The encoded protein binds to a D-type cyclin to form an active kinase complex to regulate progression through the G1 phase of the cell cycle. Mice lacking the encoded protein exhibit thymic and splenic hypoplasia, and hematopoietic defects such as reduced number of megakaryocytes and erythrocytes. A pseudogene of this gene has been defined on chromosome 4. [provided by RefSeq, Aug

2015]

Expression Ubiquitous expression in thymus adult (RPKM 7.0), lung adult (RPKM 3.6) and 26 other tissuesSee more

Orthologs <u>human</u> all

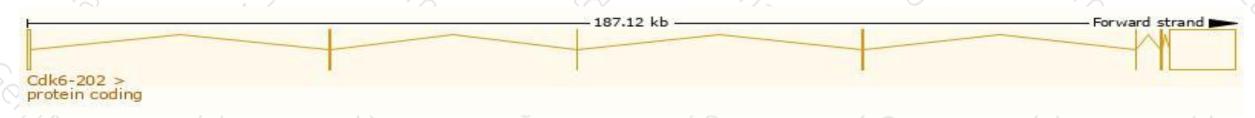
## Transcript information (Ensembl)



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

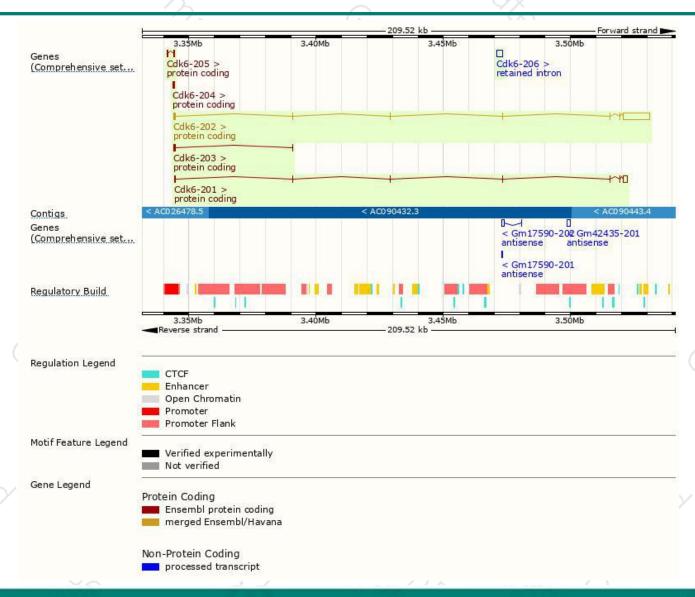
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cdk6-202	ENSMUST00000165117.7	11525	326aa	Protein coding	CCDS19062	Q0VBK8 Q64261	TSL:1 GENCODE basic APPRIS P1
Cdk6-201	ENSMUST00000042410.4	2470	326aa	Protein coding	CCDS19062	Q0VBK8 Q64261	TSL:1 GENCODE basic APPRIS P1
Cdk6-203	ENSMUST00000197385.1	697	82aa	Protein coding	49	A0A0G2JGA8	CDS 3' incomplete TSL:2
Cdk6-205	ENSMUST00000199156.1	425	<u>29aa</u>	Protein coding	29	A0A0G2JGH2	CDS 3' incomplete TSL:5
Cdk6-204	ENSMUST00000197607.1	376	<u>23aa</u>	Protein coding	- Tá	A0A0G2JEJ6	CDS 3' incomplete TSL:3
Cdk6-206	ENSMUST00000199396.1	2232	No protein	Retained intron	-	i <del>-</del>	TSL:NA

The strategy is based on the design of *Cdk6-202* transcript, The transcription is shown below



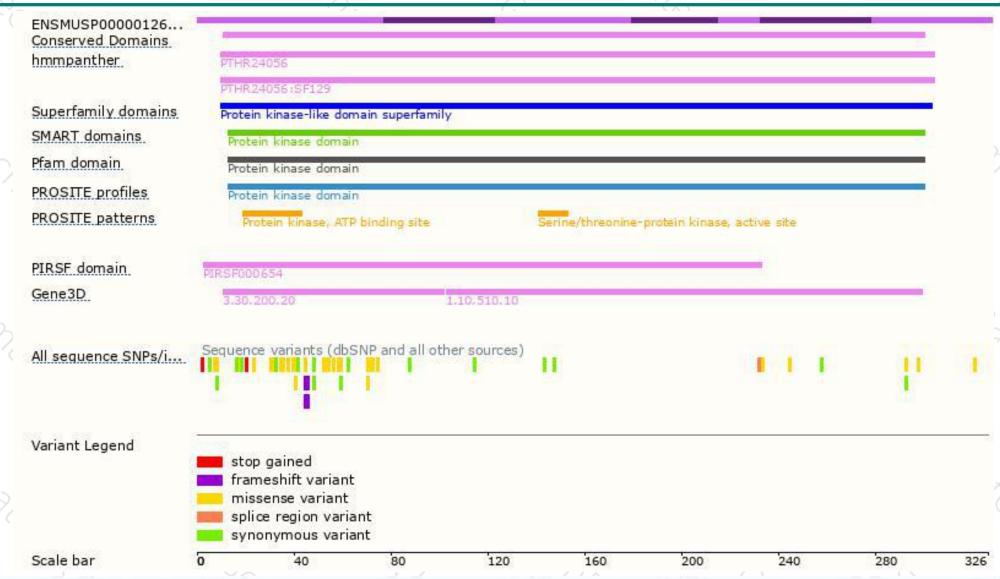
### Genomic location distribution





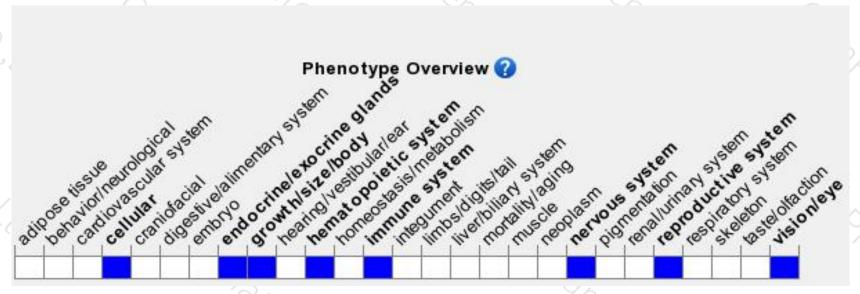
## Protein domain





## 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, Mice homozygous for disruptions in this gene display hematopoietic abnormalities affecting spleen and thymus size. Female body weight and fertility are also reduced.



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





