Cdk4 Cas9-KO Strategy

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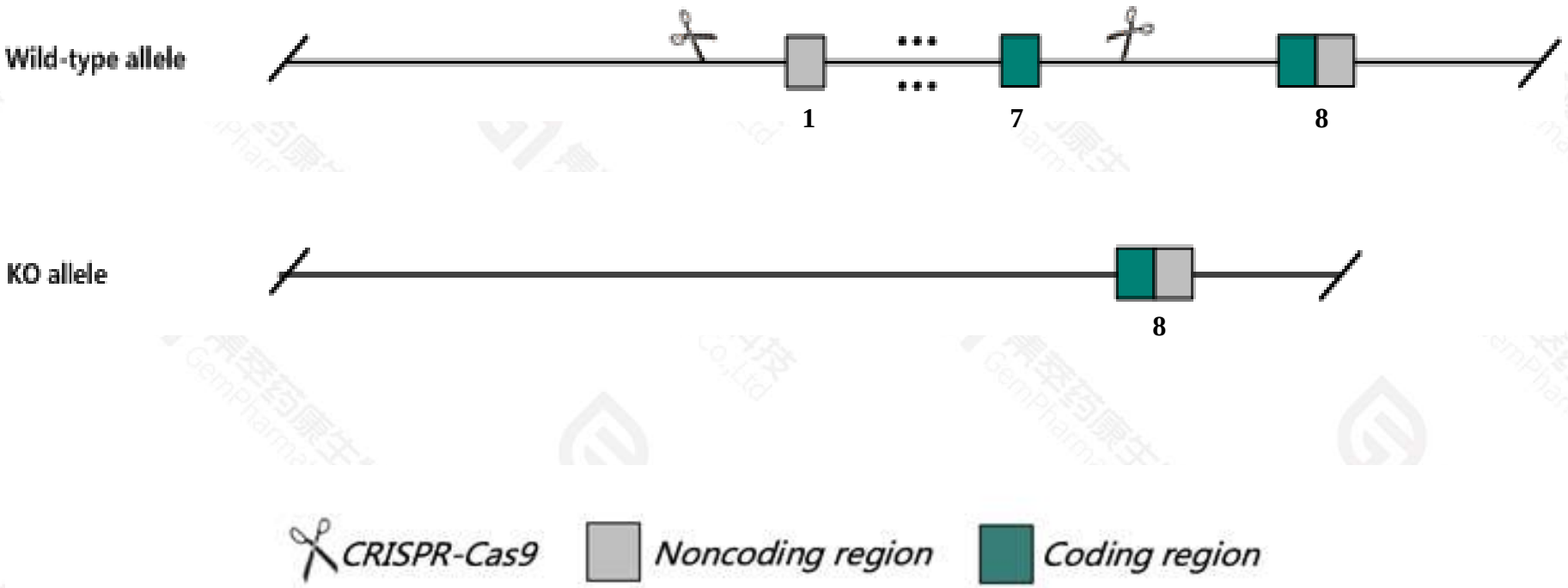
Project Overview

Project Name	***Cdk4***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR-Cas9 technology to edit the *Cdk4* gene. The schematic diagram is as follows:



Technical routes

➢ The *Cdk4* gene has 9 transcripts. According to the structure of *Cdk4* gene, exon1-exon7 of *Cdk4-201*(ENSMUST00000006911.12) 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 *Cdk4* 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.

Notice

➢ According to the existing MGI data,homozygous null mutants have small size, insulin-deficient diabetes, sterility in females; near-sterility in males and impaired prolactin secretion due to hypoplastic pituitary development. Locomotor and endocrine gland defects are seen with some alleles.

➢ The *Cdk4* 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.

➢ The insertion of the first loxp may affect promoter function of the *Marchf9* gene.

➢ 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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Cdk4 cyclin-dependent kinase 4 [Mus musculus (house mouse)]

Gene ID: 12567, updated on 13-Mar-2020

Summary

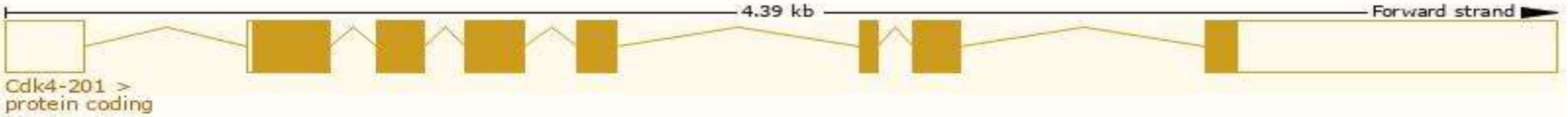
Official Symbol	Cdk4 provided by MGI
Official Full Name	cyclin-dependent kinase 4 provided byMGI
Primary source	MGI:MGI:88357
See related	Ensembl:ENSMUSG00000006728
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Crk3
Expression	Broad expression in CNS E11.5 (RPKM 315.3), limb E14.5 (RPKM 224.3) and 25 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

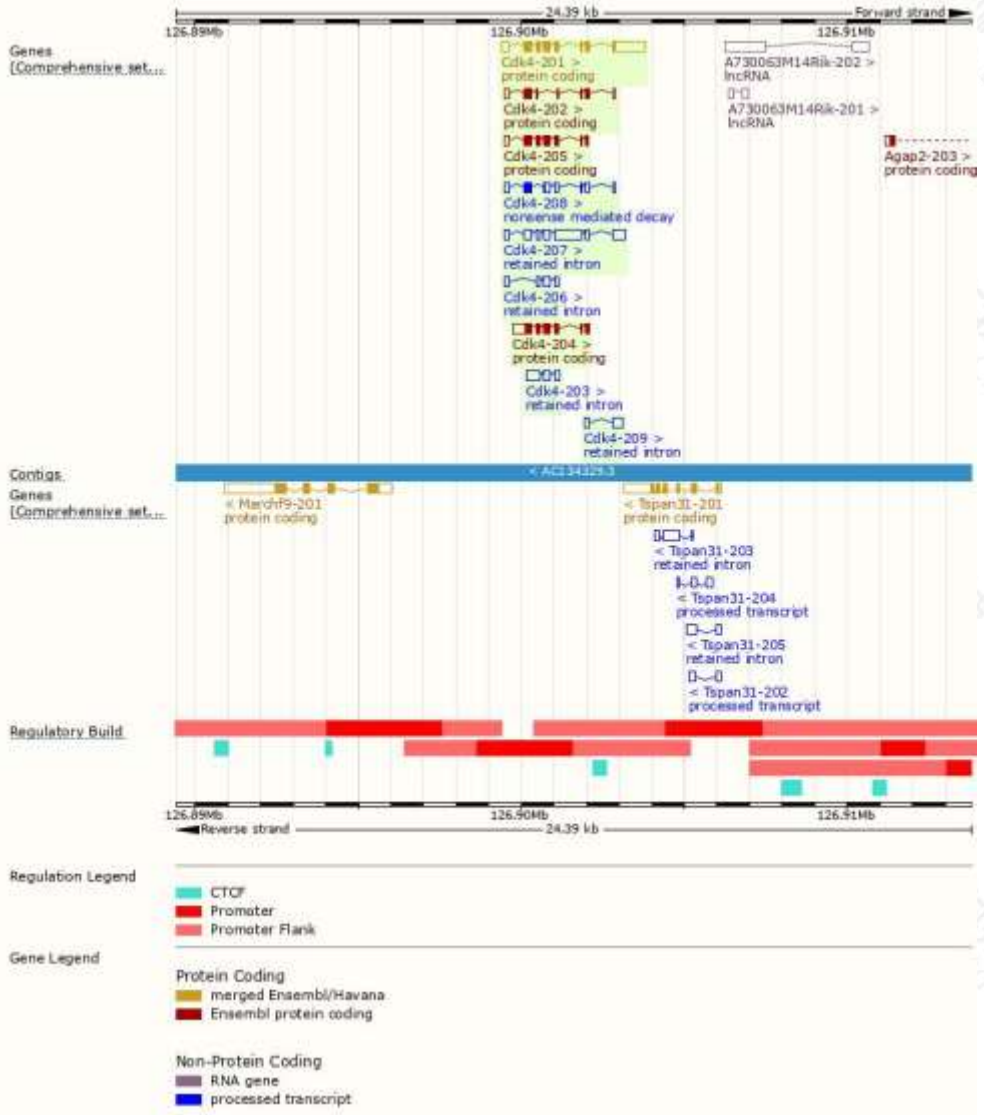
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cdk4-201	ENSMUST00000006911.11	2053	303aa	Protein coding	CCDS24226	P30285 Q545C3	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
Cdk4-204	ENSMUST00000125682.1	1182	271aa	Protein coding	-	E9PZX7	CDS 3' incomplete TSL:2
Cdk4-205	ENSMUST00000133115.7	950	253aa	Protein coding	-	E9Q9E4	CDS 3' incomplete TSL:5
Cdk4-202	ENSMUST00000120226.7	714	158aa	Protein coding	-	D3YWB0	TSL:5 GENCODE basic
Cdk4-208	ENSMUST00000142558.7	924	74aa	Nonsense mediated decay	-	D6RHS5	TSL:5
Cdk4-207	ENSMUST00000140254.7	2038	No protein	Retained intron	-	-	TSL:1
Cdk4-203	ENSMUST00000123456.1	752	No protein	Retained intron	-	-	TSL:2
Cdk4-206	ENSMUST00000135179.7	574	No protein	Retained intron	-	-	TSL:2
Cdk4-209	ENSMUST00000145670.1	468	No protein	Retained intron	-	-	TSL:1

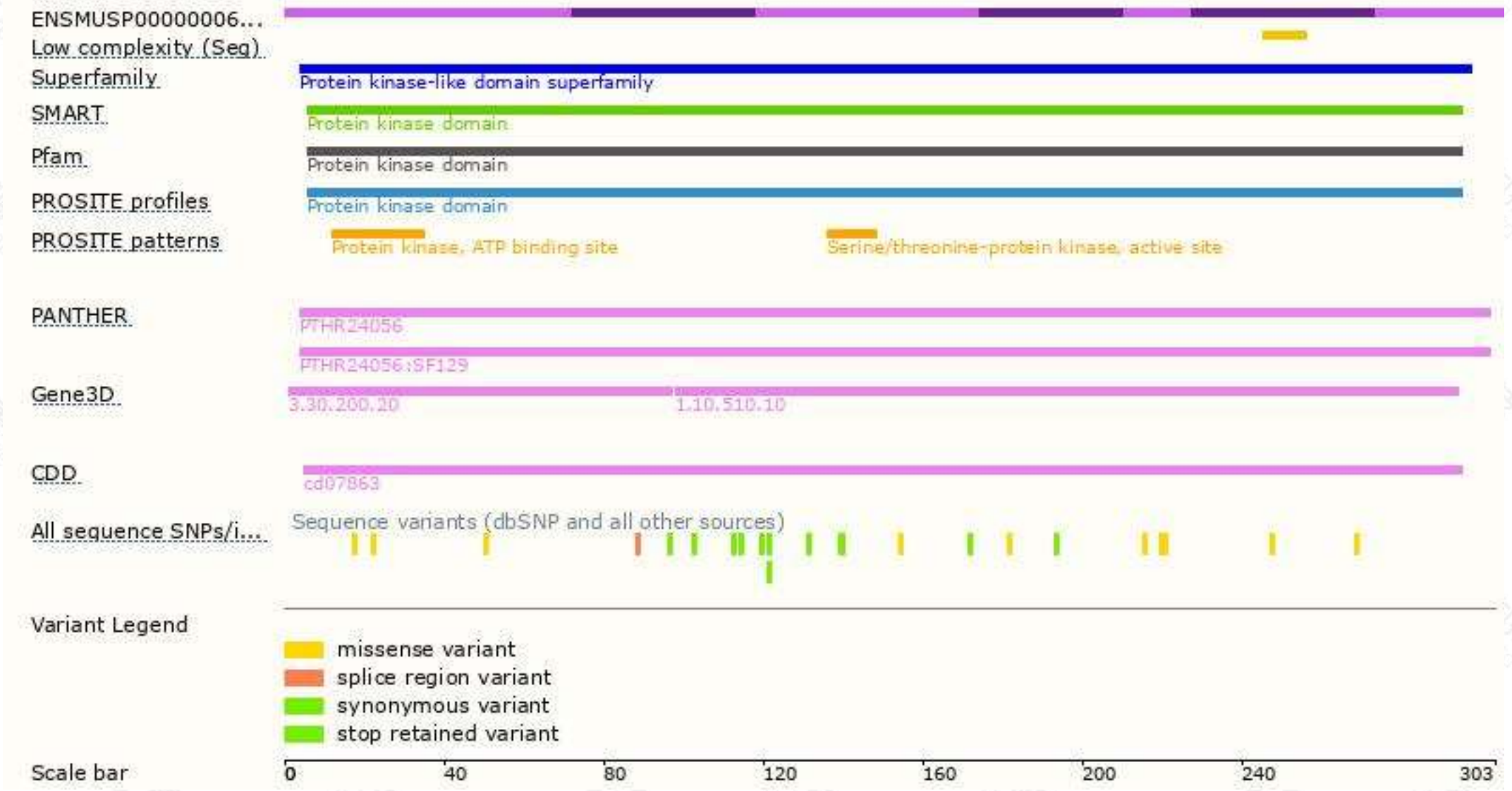
The strategy is based on the design of *Cdk4-201* transcript,the transcription is shown below:



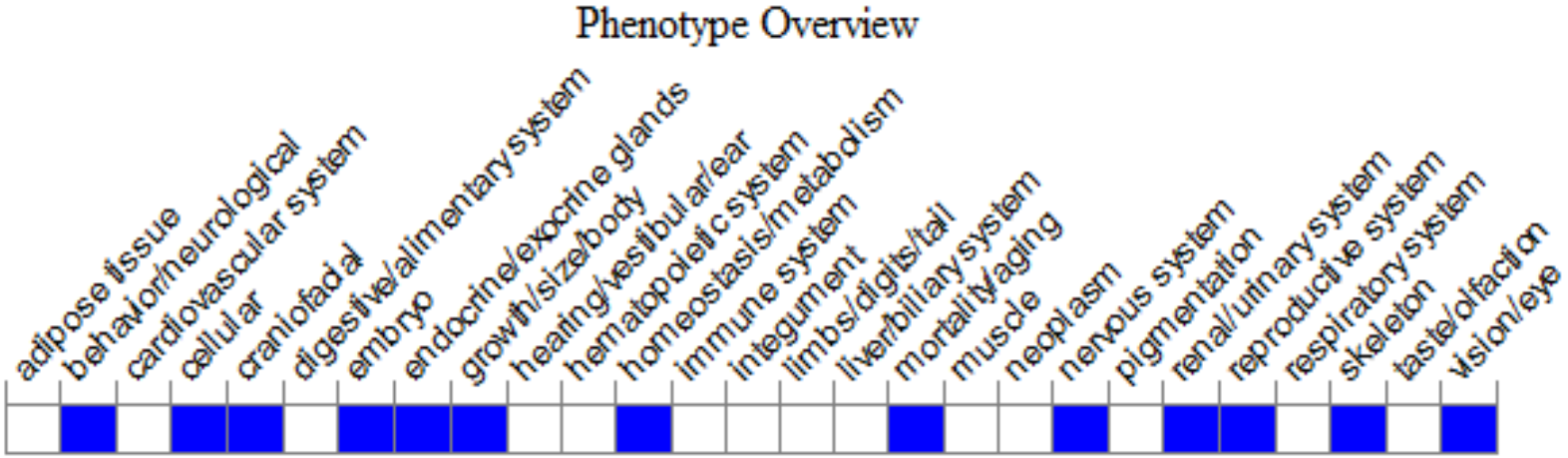
Genomic location distribution



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/).

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If you have any questions, you are welcome to inquire.
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