

Dapk3 Cas9-CKO Strategy

Designer: Longyun Hu

Reviewer: Rui Xiong

Design Date: 2021-4-6

Project Overview

Project Name

Dapk3

Project type

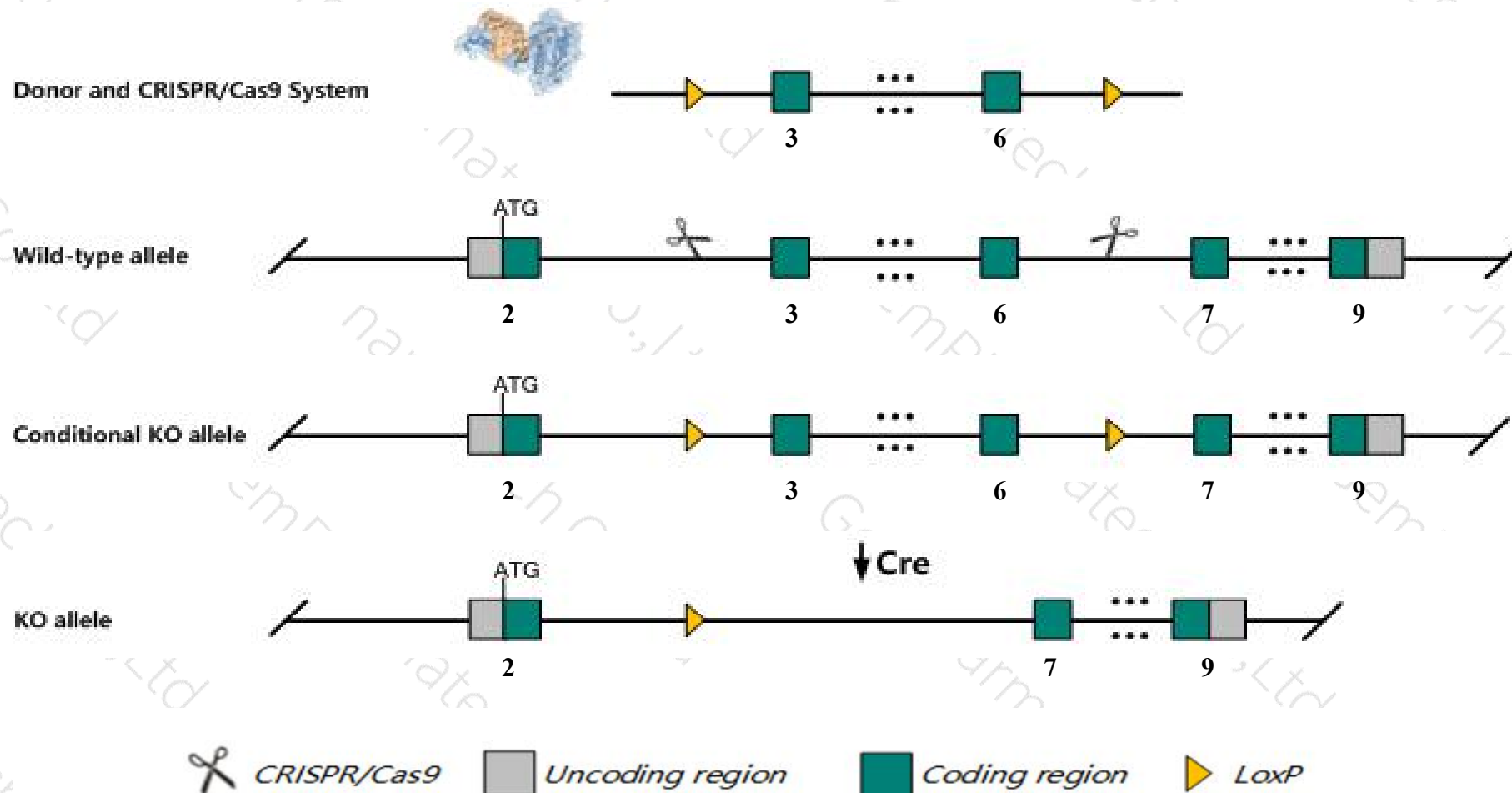
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Dapk3* gene. The schematic diagram is as follows:



Technical routes

- The *Dapk3* gene has 11 transcripts. According to the structure of *Dapk3* gene, exon3-exon6 of *Dapk3*-202(ENSMUST00000178422.8) transcript is recommended as the knockout region. The region contains 567bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Dapk3* 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.

- According to the existing MGI data, mice homozygous for a gene-trapped allele exhibit embryonic lethality after blastocyst implantation.
- The KO region deletes most of the coding sequence, but does not result in frameshift.
- The *Dapk3* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)

Dapk3 death-associated protein kinase 3 [Mus musculus (house mouse)]

Gene ID: 13144, updated on 13-Mar-2020

Summary

Official Symbol Dapk3 provided by [MGI](#)

Official Full Name death-associated protein kinase 3 provided by [MGI](#)

Primary source [MGI:MGI:1203520](#)

See related [Ensembl:ENSMUSG00000034974](#)

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 ZIPK, dlk

Expression Ubiquitous expression in ovary adult (RPKM 67.6), duodenum adult (RPKM 59.4) and 28 other tissues [See more](#)

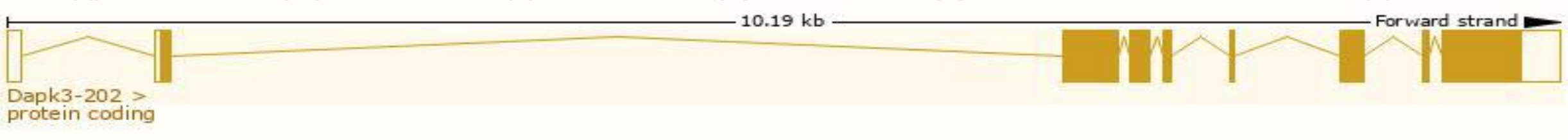
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

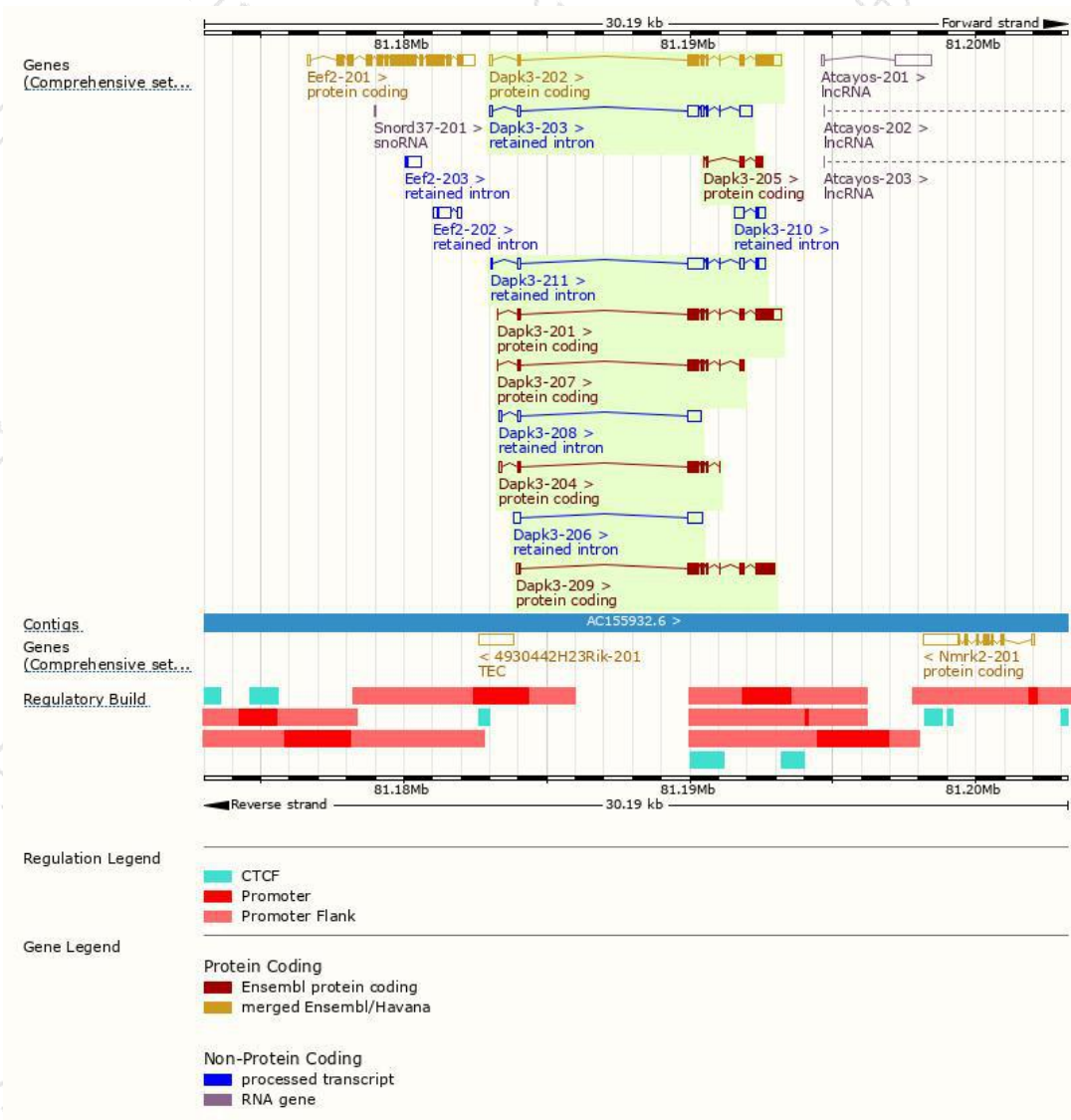
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dapk3-202	ENSMUST00000178422.8	1730	448aa	Protein coding	CCDS24045	Q54784 Q05A21	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
Dapk3-201	ENSMUST00000047665.6	1676	448aa	Protein coding	CCDS24045	Q54784 Q05A21	TSL:5 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
Dapk3-209	ENSMUST00000219850.1	1482	448aa	Protein coding	CCDS24045	Q54784 Q05A21	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
Dapk3-207	ENSMUST00000219133.1	844	256aa	Protein coding	-	A0A1W2P6J6	CDS 3' incomplete TSL:3
Dapk3-204	ENSMUST00000218157.1	729	205aa	Protein coding	-	A0A1W2P6K3	CDS 3' incomplete TSL:3
Dapk3-205	ENSMUST00000218227.1	403	135aa	Protein coding	-	A0A1W2P730	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Dapk3-211	ENSMUST00000220076.1	1204	No protein	Retained intron	-	-	TSL:1
Dapk3-203	ENSMUST00000217936.1	1171	No protein	Retained intron	-	-	TSL:1
Dapk3-206	ENSMUST00000218698.1	734	No protein	Retained intron	-	-	TSL:2
Dapk3-210	ENSMUST00000219944.1	630	No protein	Retained intron	-	-	TSL:2
Dapk3-208	ENSMUST00000219329.1	614	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Dapk3-202* transcript,the transcription is shown below:



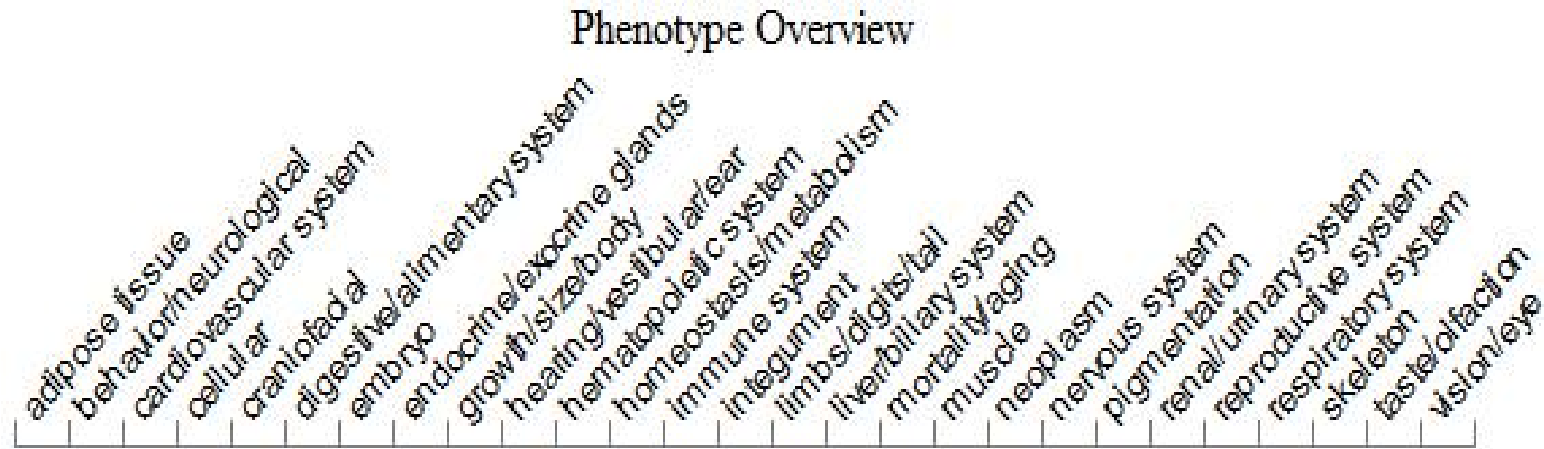
Genomic location distribution



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 a gene-trapped allele exhibit embryonic lethality after blastocyst implantation.

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

Tel: 400-9660890

