

***Dna2* Cas9-CKO Strategy**

Designer: Xiaojing Li

Reviewer: Jia Yu

Design Date: 2020-4-20

Project Overview

Project Name

Dna2

Project type

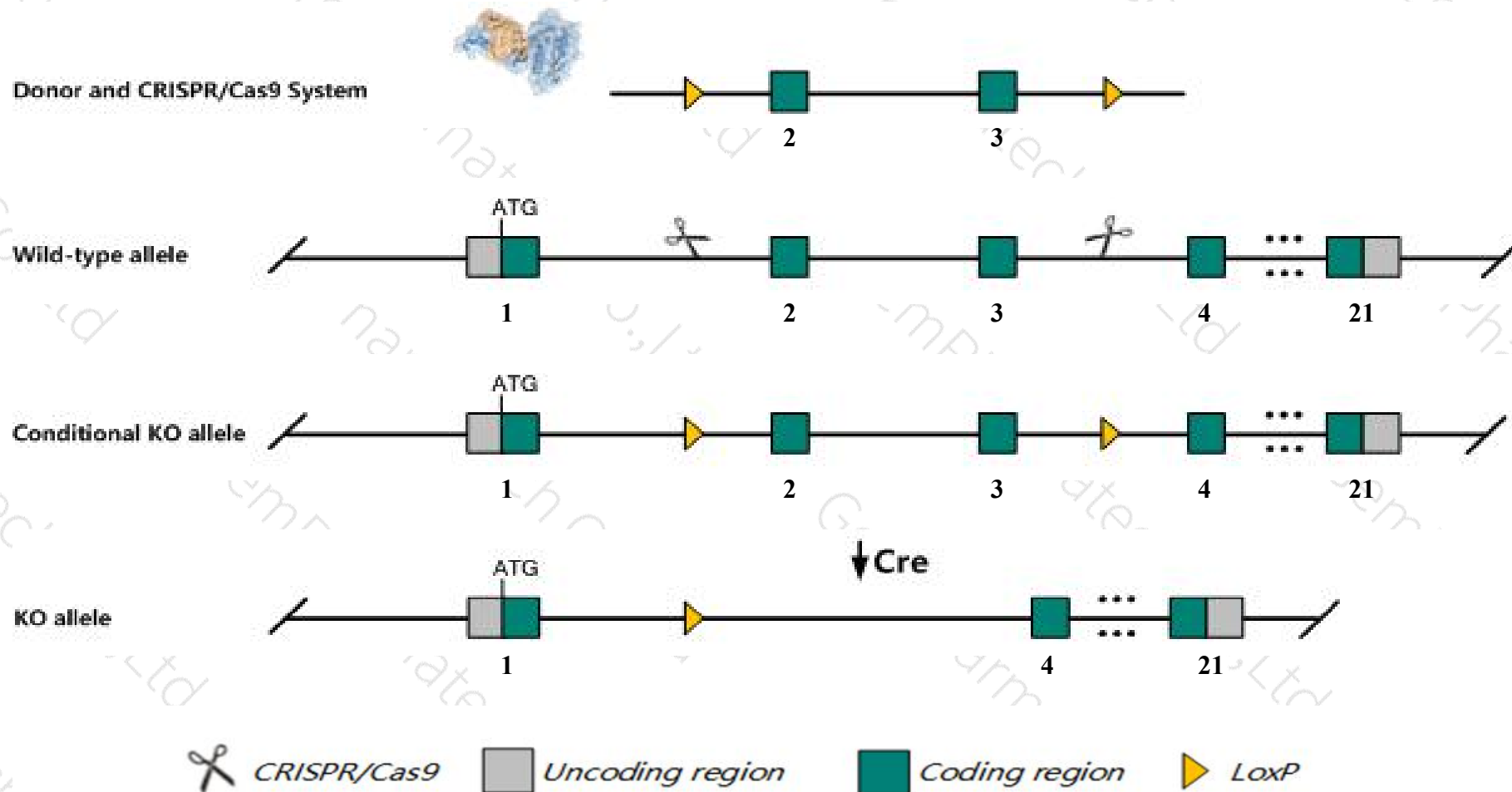
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Dna2* gene. The schematic diagram is as follows:



- The *Dna2* gene has 6 transcripts. According to the structure of *Dna2* gene, exon2-exon3 of *Dna2-203* (ENSMUST00000131422.7) transcript is recommended as the knockout region. The region contains 367bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Dna2* 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 knock-out allele exhibit embryonic lethality before e7.5. mice heterozygous for the allele exhibit shortened telomeres, chromosome segregation errors and increased tumor incidence associated with aneuploidy.
- The *Dna2* 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)

Dna2 DNA replication helicase/nuclease 2 [Mus musculus (house mouse)]

Gene ID: 327762, updated on 13-Mar-2020

Summary



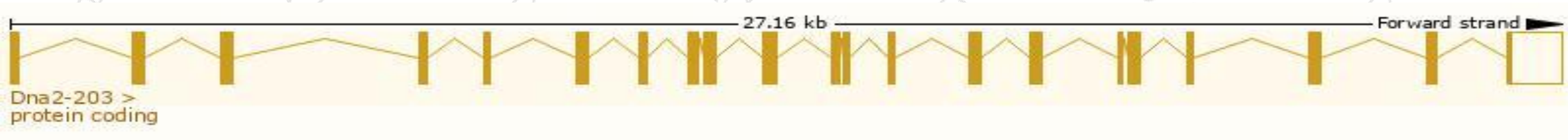
Official Symbol	Dna2 provided by MGI
Official Full Name	DNA replication helicase/nuclease 2 provided by MGI
Primary source	MGI:MGI:2443732
See related	Ensembl:ENSMUSG00000036875
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Dna2l, E130315B21Rik
Expression	Broad expression in liver E14 (RPKM 14.4), liver E14.5 (RPKM 13.2) and 18 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

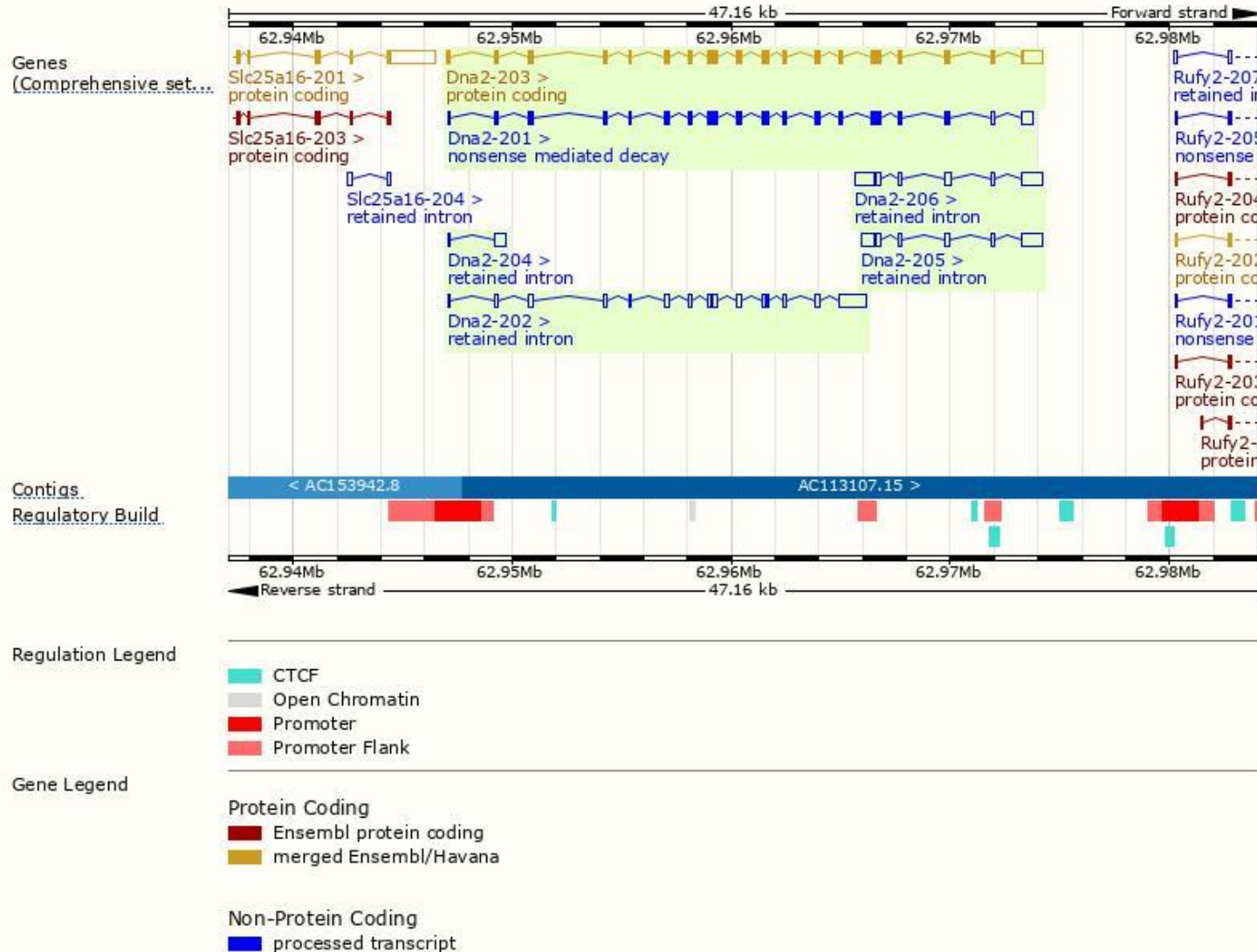
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dna2-203	ENSMUST00000131422.7	4122	1062aa	Protein coding	CCDS35923	Q6ZQJ5	TSL:1 GENCODE basic APPRIS P1
Dna2-201	ENSMUST00000092462.11	3651	966aa	Nonsense mediated decay	-	Q6ZQJ5	TSL:1
Dna2-202	ENSMUST00000129785.1	3430	No protein	Retained intron	-	-	TSL:2
Dna2-206	ENSMUST00000139212.7	2488	No protein	Retained intron	-	-	TSL:1
Dna2-205	ENSMUST00000137378.1	2085	No protein	Retained intron	-	-	TSL:1
Dna2-204	ENSMUST00000131715.1	603	No protein	Retained intron	-	-	TSL:2

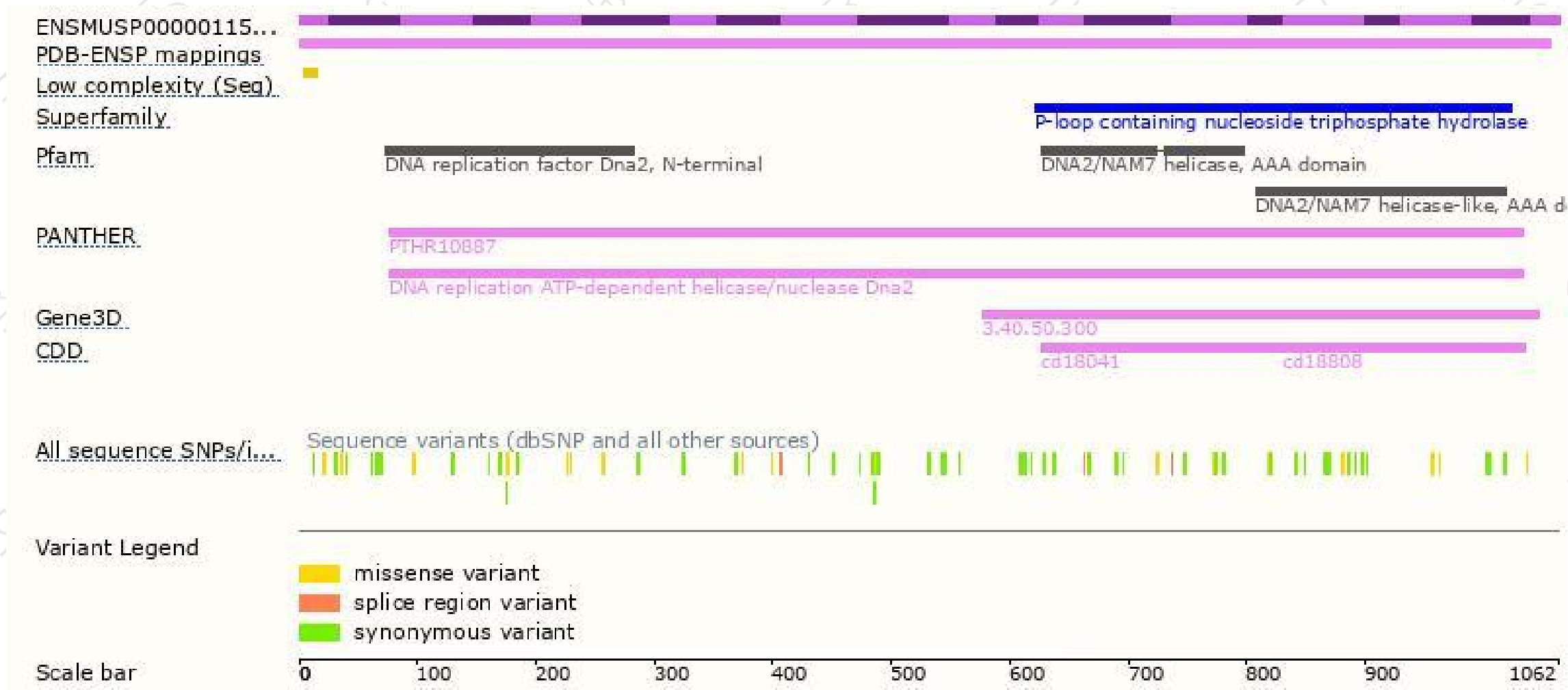
The strategy is based on the design of *Dna2-203* transcript,The transcription is shown below



Genomic location distribution

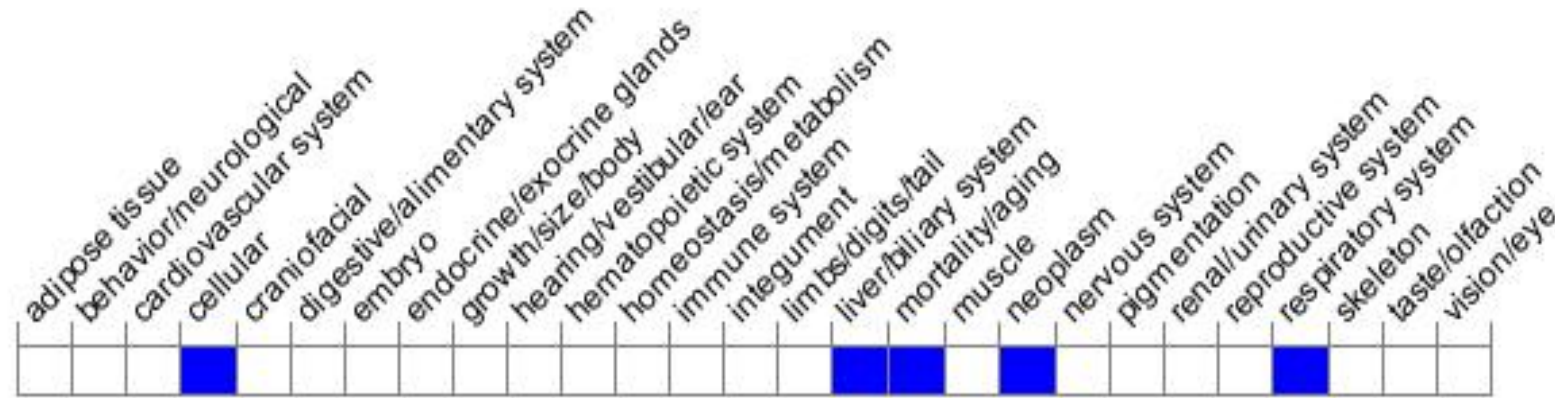


Protein domain



Mouse phenotype description(MGI)

Phenotype Overview



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 embryonic lethality before E7.5. Mice heterozygous for the allele exhibit shortened telomeres, chromosome segregation errors and increased tumor incidence associated with aneuploidy.

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

Tel: 400-9660890

