Pif1 Cas9-CKO Strategy

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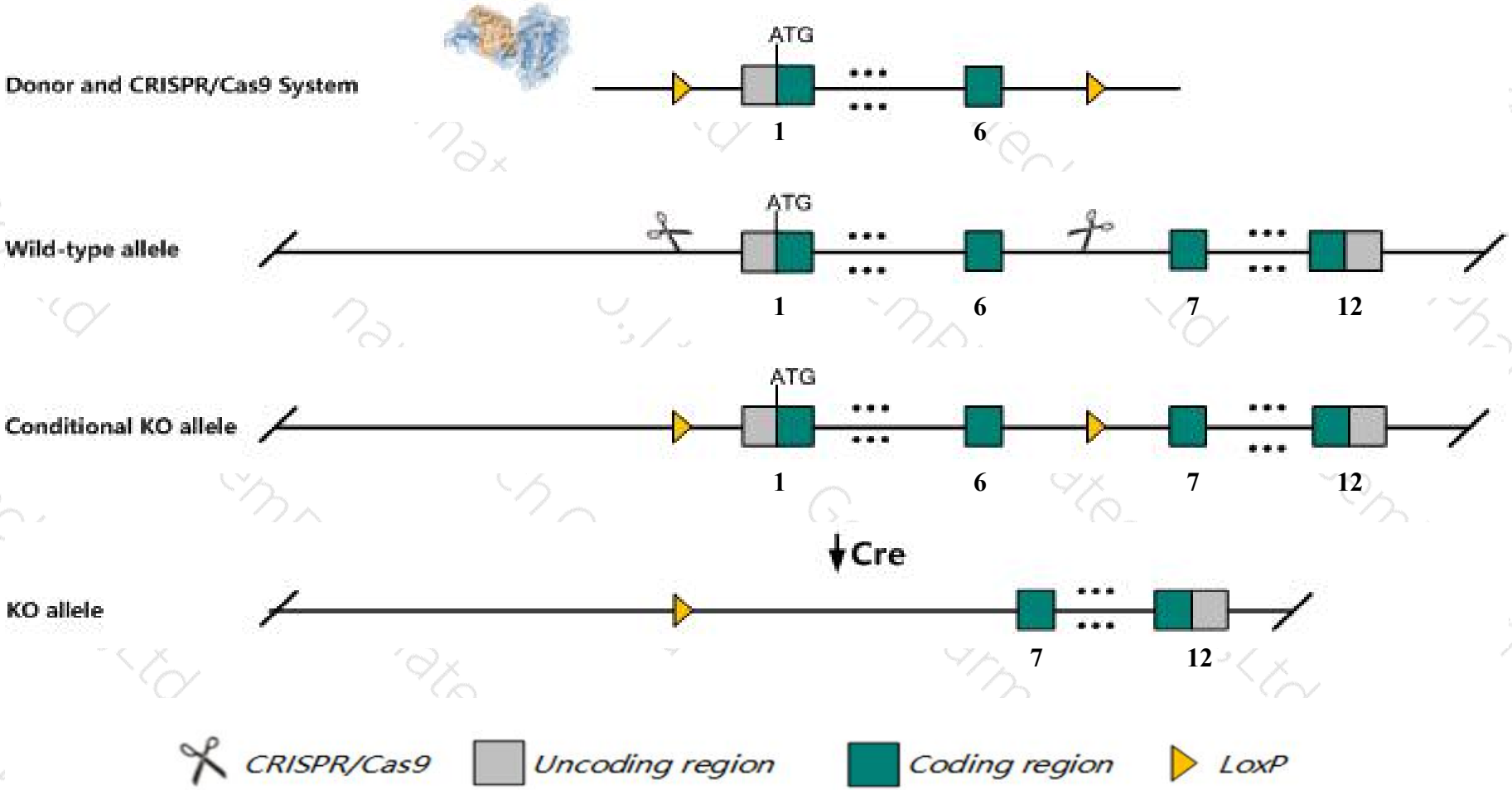
Project Overview

Project Name	***Pif1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pif1* gene. The schematic diagram is as follows:



Technical routes

- The *Pif1* gene has 8 transcripts. According to the structure of *Pif1* gene, exon1-exon6 of *Pif1-201* (ENSMUST00000047099.11) transcript is recommended as the knockout region.The region contains start codon ATG. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Pif1* 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 a knock-out allele are viable and overtly normal and show no evidence of increased sensitivity to DNA damage, genetic instability, reproducible telomere length alteration or other cellular abnormalities.

➢ The *Pif1* gene is located on the Chr9. 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）

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Pif1 PIF1 5'-to-3' DNA helicase [Mus musculus (house mouse)]

Gene ID: 208084, updated on 13-Mar-2020

Summary

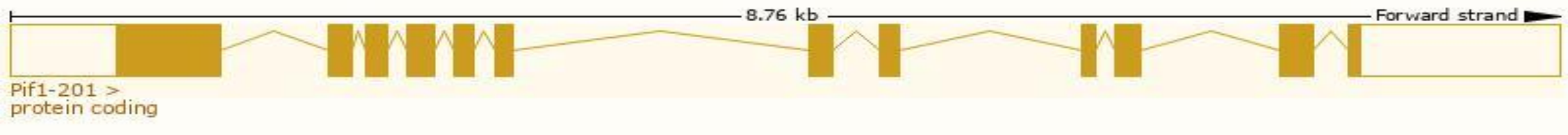
Official Symbol	Pif1 provided by MGI
Official Full Name	PIF1 5'-to-3' DNA helicase provided by MGI
Primary source	MGI:MGI:2143057
See related	Ensembl:ENSMUSG00000041064
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4631410M14, AI449441
Expression	Broad expression in CNS E11.5 (RPKM 6.1), thymus adult (RPKM 5.9) and 15 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

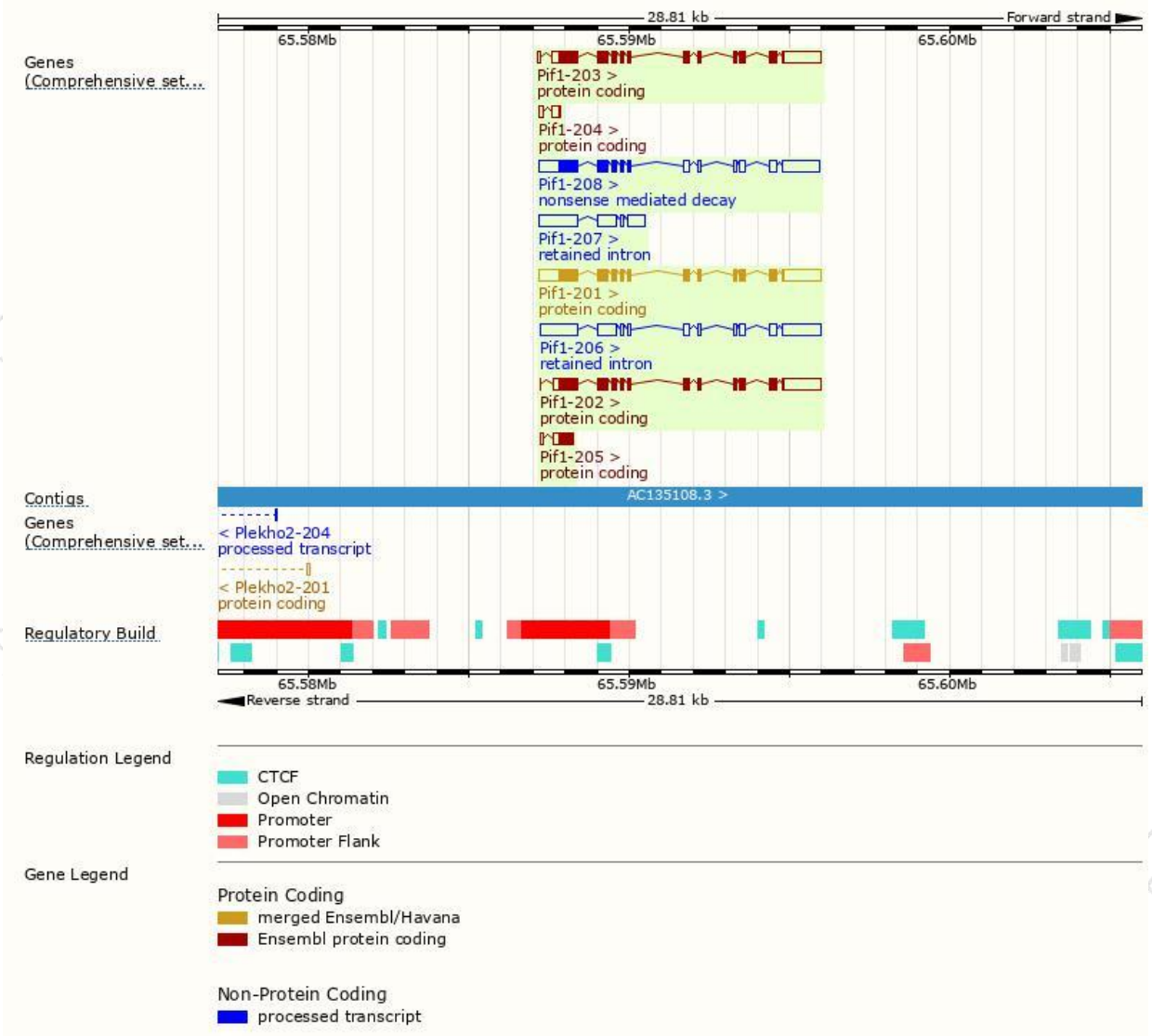
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pif1-201	ENSMUST00000047099.11	3685	650aa	Protein coding	CCDS23295	Q80SX8	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
Pif1-203	ENSMUST00000134538.7	3395	650aa	Protein coding	CCDS23295	Q80SX8	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
Pif1-202	ENSMUST00000131483.1	3309	650aa	Protein coding	CCDS23295	Q80SX8	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
Pif1-205	ENSMUST00000141046.1	742	157aa	Protein coding	-	D3YZ87	CDS 3' incomplete TSL:2
Pif1-204	ENSMUST00000136205.1	371	9aa	Protein coding	-	A0A1C7ZMZ4	CDS 3' incomplete TSL:3
Pif1-208	ENSMUST00000154970.7	3725	411aa	Nonsense mediated decay	-	D6RFC6	TSL:1
Pif1-206	ENSMUST00000152529.1	3897	No protein	Retained intron	-	-	TSL:2
Pif1-207	ENSMUST00000152885.7	2450	No protein	Retained intron	-	-	TSL:2

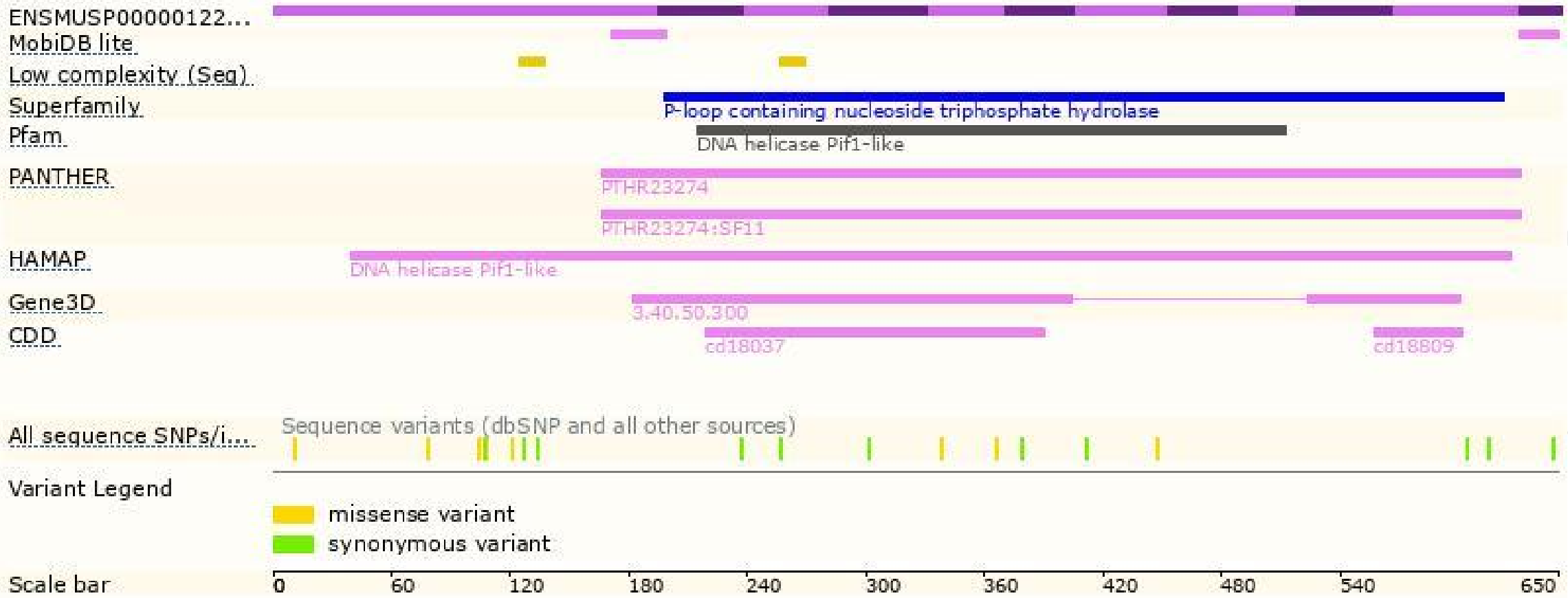
The strategy is based on the design of *Pif1-201* transcript,The transcription is shown below



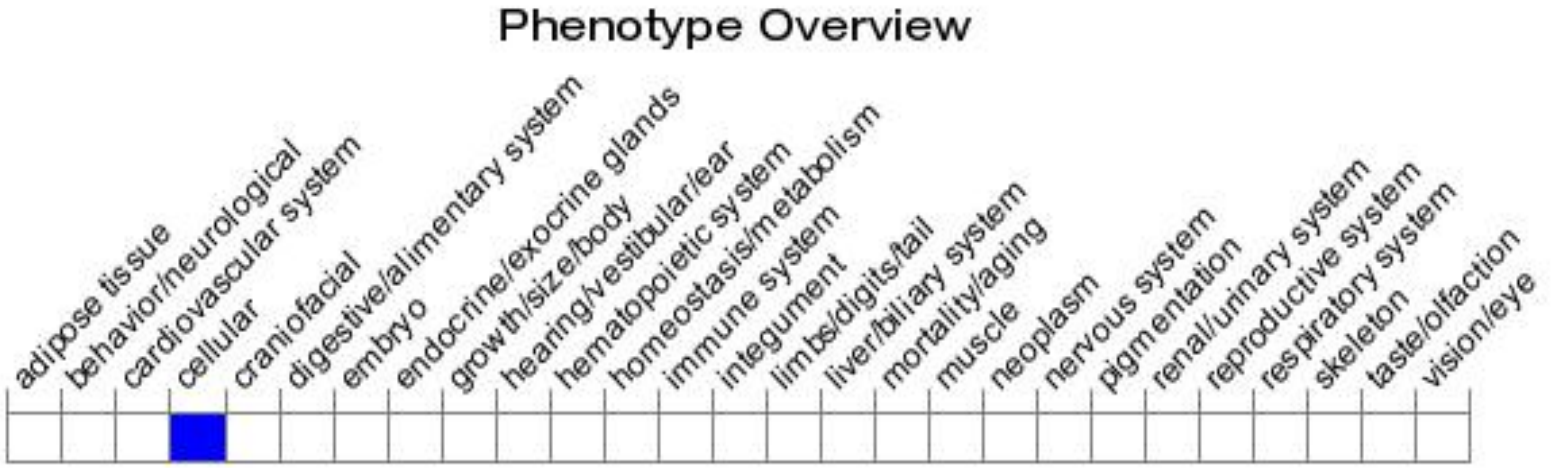
Genomic location distribution



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/).

According to the existing MGI data,Mice homozygous for a knock-out allele are viable and overtly normal and show no evidence of increased sensitivity to DNA damage, genetic instability, reproducible telomere length alteration or other cellular abnormalities.

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

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