Rbbp8 Cas9-CKO *Strategy*

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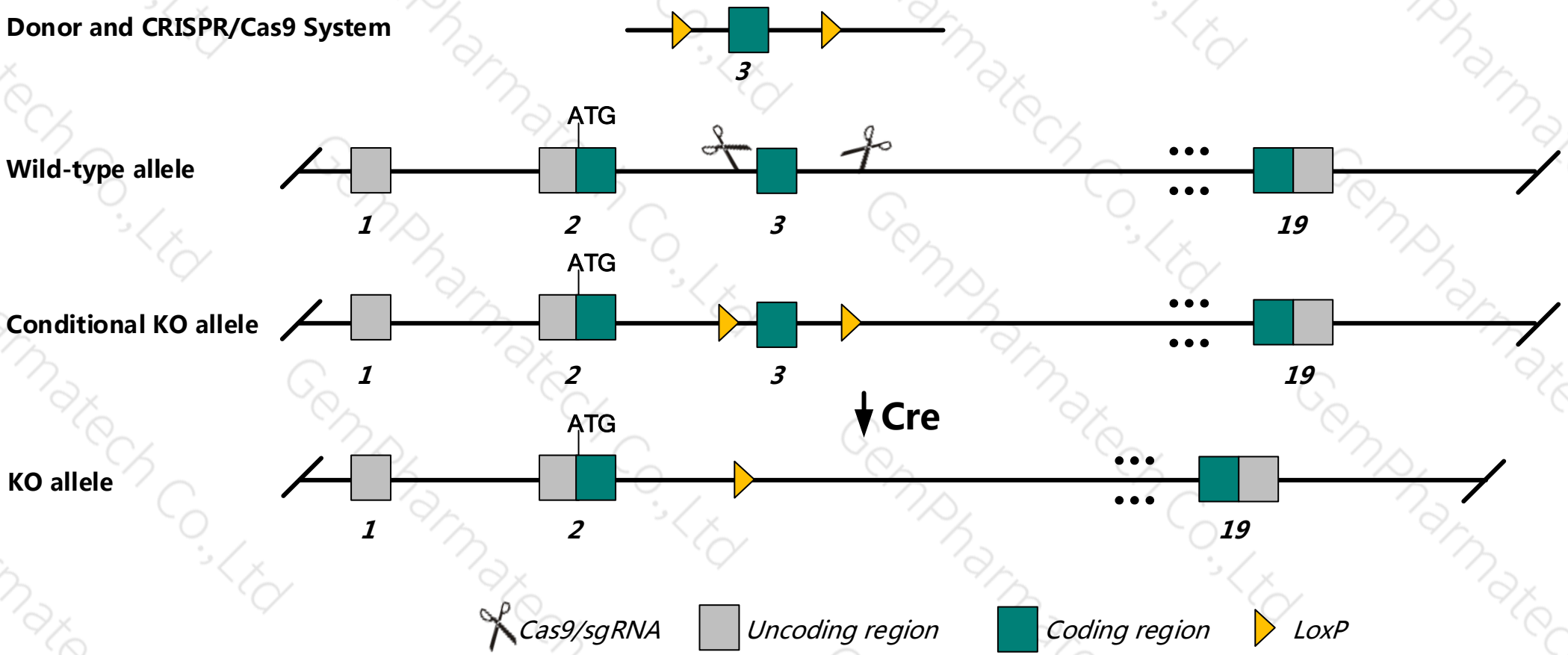
Project Overview

Project Name	***Rbbp8***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rbbp8* gene. The schematic diagram is as follows:



Technical routes

- The *Rbbp8* gene has 11 transcripts.According to the structure of *Rbbp8* gene, exon3 of *Rbbp8*-202 transcript is recommended as the knockout region.The region contains 43bp coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Rbbp8* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues or cell types.

Notice

- According to the existing MGI data，Embryos homozygous for a knock-out allele die at E4.0 as blastocysts fail to enter S phase and arrest at G1, leading to elevated cell death. Heterozygous mutant mice display a shortened lifespan due to formation of multiple tumors, mostly large lymphomasof both B and T cells.

- The *Rbbp8* gene is located on the Chr18. 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information （NCBI）

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Rbbp8 retinoblastoma binding protein 8, endonuclease [*Mus musculus* (house mouse)]

Gene ID: 225182, updated on 31-Jan-2019

Summary

Official Symbol	Rbbp8 provided by MGI
Official Full Name	retinoblastoma binding protein 8, endonuclease provided by MGI
Primary source	MGI:MGI:2442995
See related	Ensembl:ENSMUSG00000041238
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	RIM; CtIP; SAE2; RBBP-8; 9930104E21Rik
Expression	Broad expression in CNS E11.5 (RPKM 6.2), placenta adult (RPKM 5.1) and 23 other tissues See more
Orthologs	human all

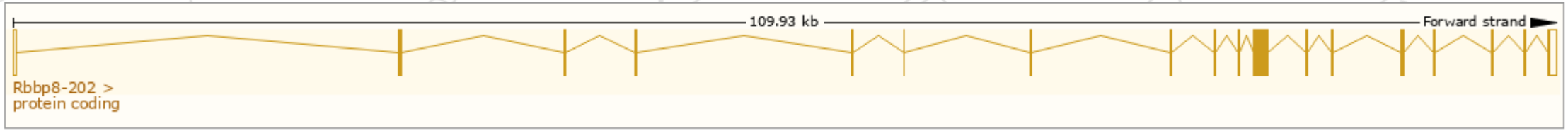
Transcript information（ Ensembl ）

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

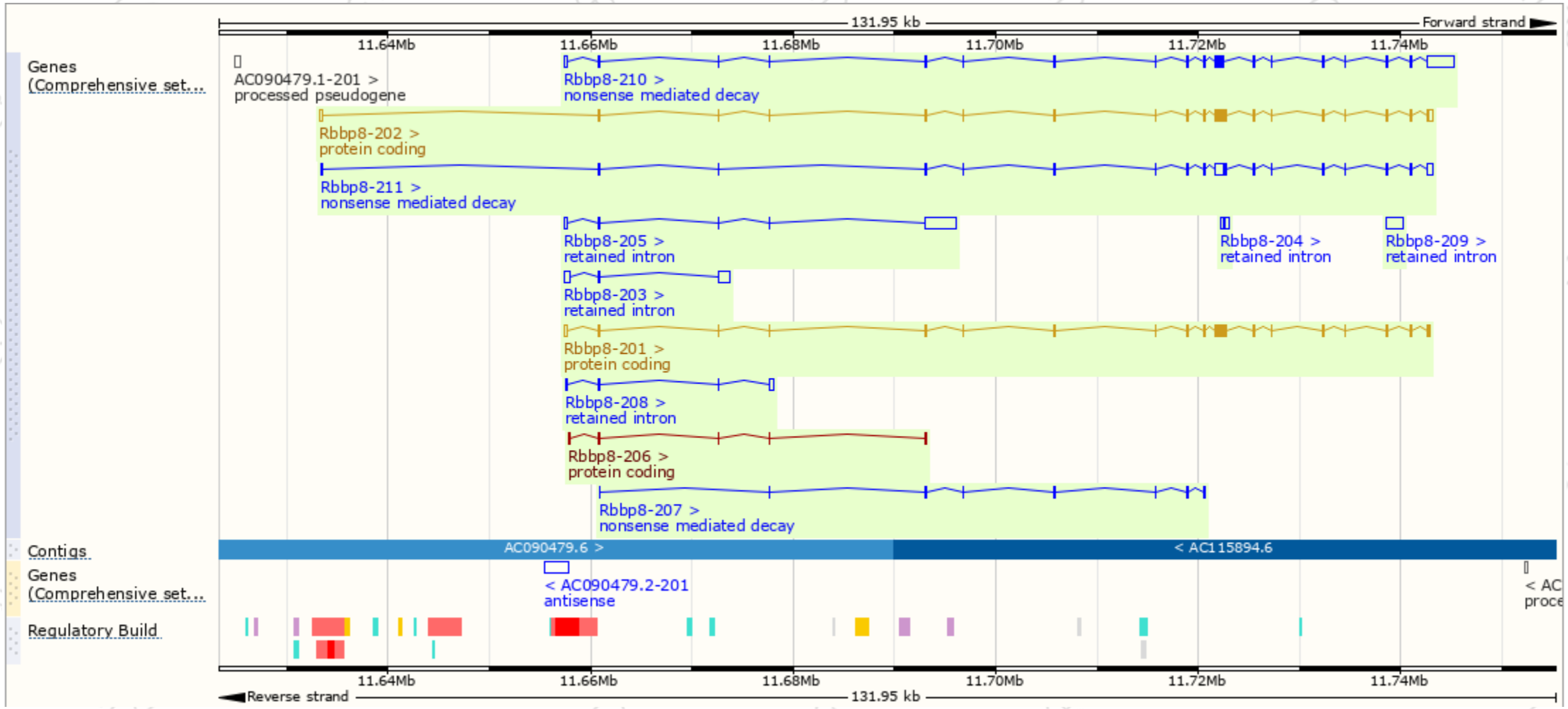
Show/hide columns (1 hidden) Filter

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rbbp8-202	ENSMUST00000115861.8	3523	893aa	Protein coding	CCDS37738	Q80YR6	TSL:1 GENCODE basic APPRIS P1
Rbbp8-201	ENSMUST00000047322.7	3209	893aa	Protein coding	CCDS37738	Q80YR6	TSL:1 GENCODE basic APPRIS P1
Rbbp8-206	ENSMUST00000234499.1	586	118aa	Protein coding	-	-	CDS 3' incomplete
Rbbp8-210	ENSMUST00000234984.1	5471	612aa	Nonsense mediated decay	-	-	-
Rbbp8-211	ENSMUST00000235039.1	3360	247aa	Nonsense mediated decay	-	-	-
Rbbp8-207	ENSMUST00000234616.1	781	26aa	Nonsense mediated decay	-	-	CDS 5' incomplete
Rbbp8-205	ENSMUST00000234184.1	3714	No protein	Retained intron	-	-	-
Rbbp8-203	ENSMUST00000234074.1	1904	No protein	Retained intron	-	-	-
Rbbp8-209	ENSMUST00000234766.1	1736	No protein	Retained intron	-	-	-
Rbbp8-208	ENSMUST00000234744.1	857	No protein	Retained intron	-	-	-
Rbbp8-204	ENSMUST00000234161.1	679	No protein	Retained intron	-	-	-

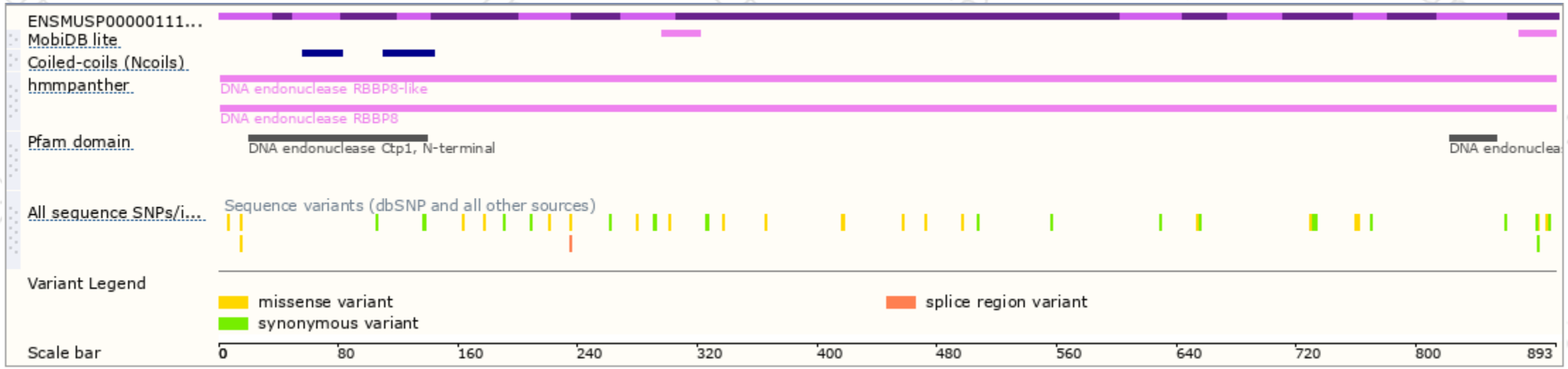
The strategy is based on the design of *Rbbp8*-202 transcript,The transcription is shown below



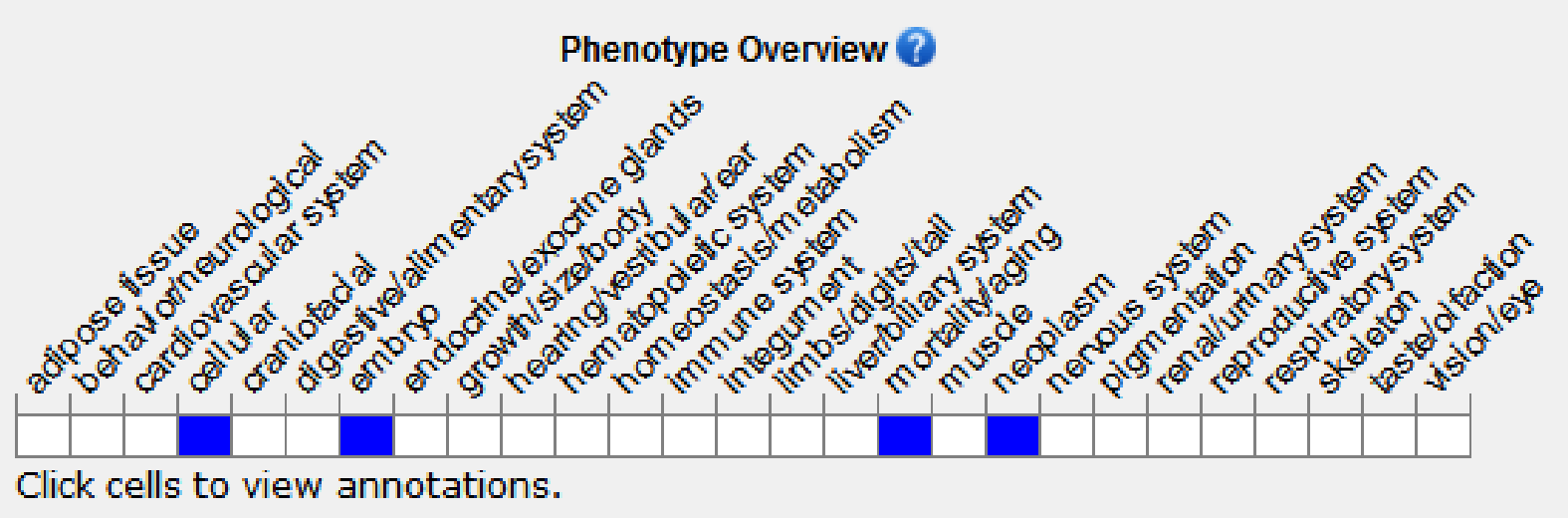
Genomic location distribution



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,Embryos homozygous for a knock-out allele die at E4.0 as blastocysts fail to enter S phase and arrest at G1, leading to elevated cell death. Heterozygous mutant mice display a shortened lifespan due to formation of multiple tumors, mostly large lymphomasof both B and T cells.

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

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