Becn1 Cas9-CKO Strategy

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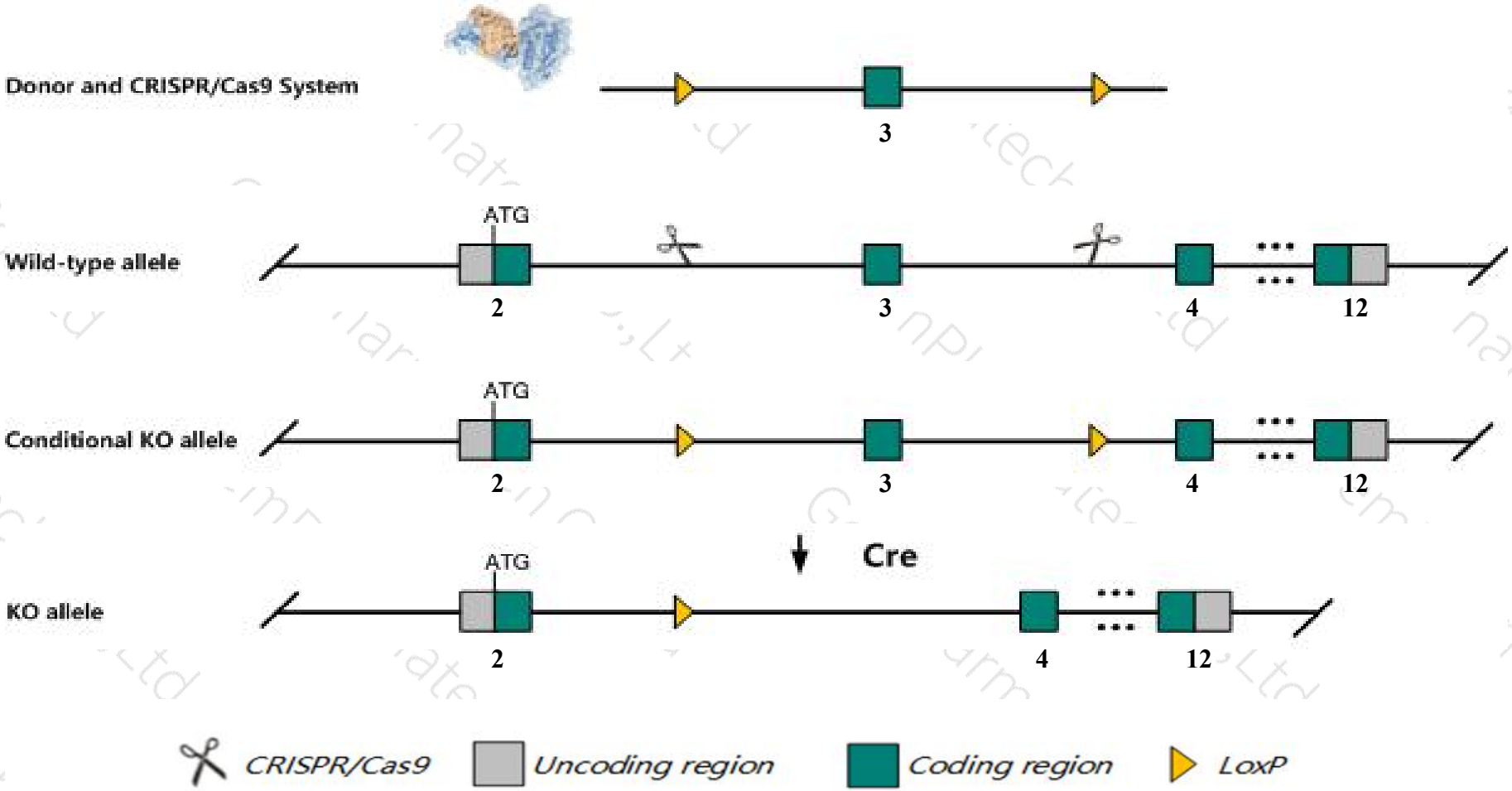
Project Overview

Project Name	***Becn1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Becn1* gene. The schematic diagram is as follows:



Technical routes

- The *Becn1* gene has 17 transcripts. According to the structure of *Becn1* gene, exon3 of *Becn1-205* (ENSMUST00000130916.7) transcript is recommended as the knockout region.The region contains 68bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Becn1* 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 exhibit prenatal lethality.
  Mice heterozygous for this allele exhibit premature death, increased tumor incidence and reduced autophagy.

- The *Becn1* gene is located on the Chr11. 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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Becn1 beclin 1, autophagy related [Mus musculus (house mouse)]

Gene ID: 56208, updated on 2-Apr-2019

Summary

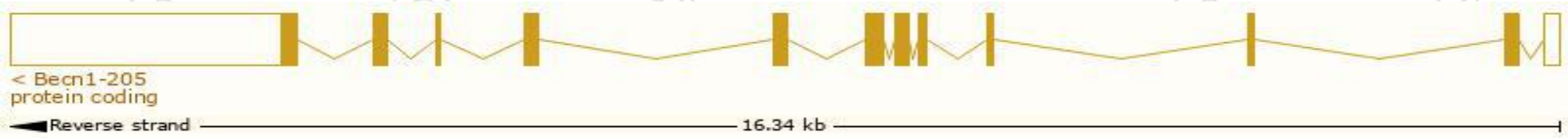
Official Symbol	Becn1 provided by MGI
Official Full Name	beclin 1, autophagy related provided by MGI
Primary source	MGI:MGI:1891828
See related	Ensembl:ENSMUSG00000035086
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Atg6
Expression	Ubiquitous expression in CNS E11.5 (RPKM 39.6), bladder adult (RPKM 35.6) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

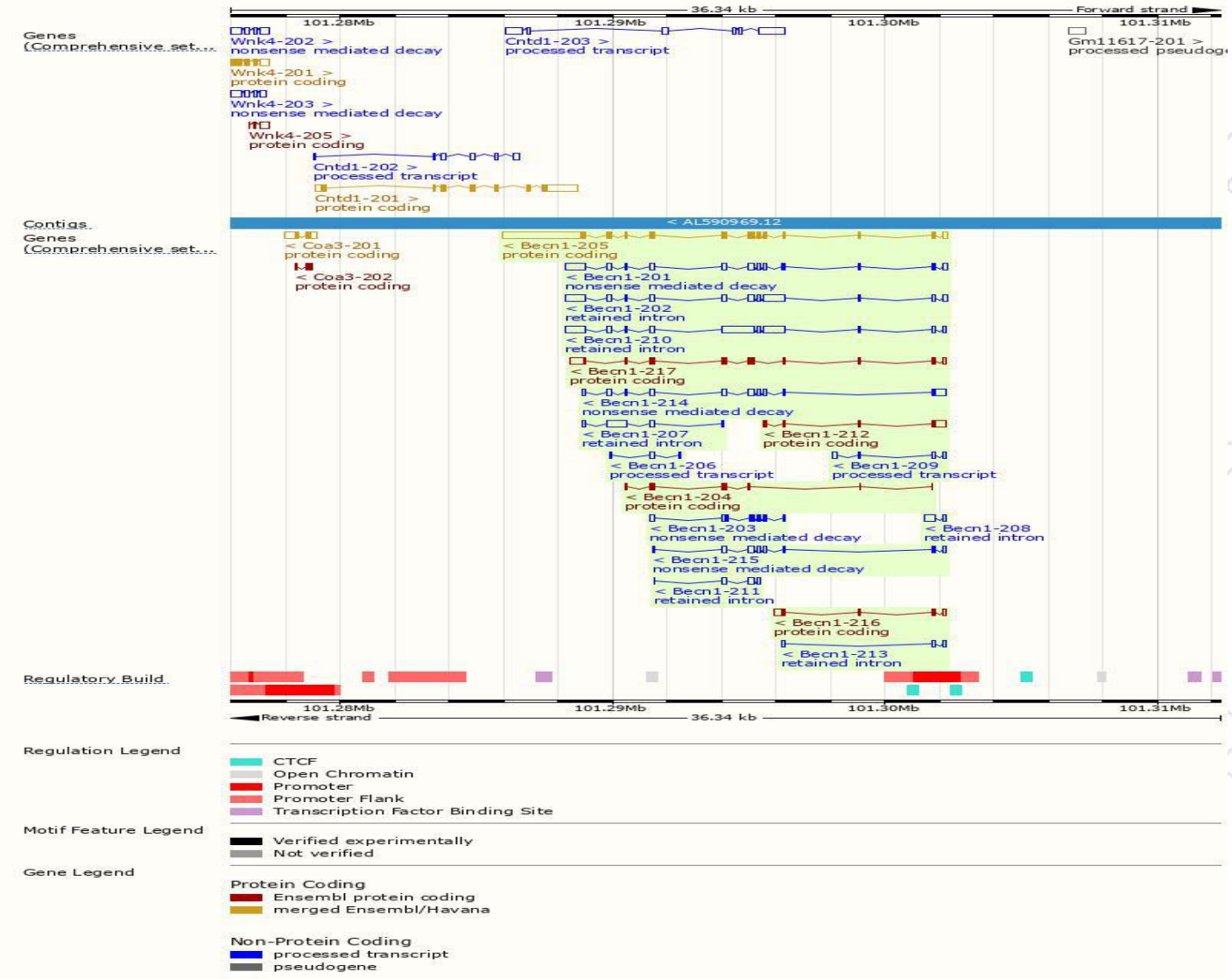
The gene has 17 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Becn1-217	ENSMUST00000172233.7	1493	277aa	Protein coding	-	E9PYD6	TSL:5 GENCODE basic
Becn1-216	ENSMUST00000170502.1	725	105aa	Protein coding	-	E9QAM0	TSL:1 GENCODE basic
Becn1-215	ENSMUST00000167818.7	899	46aa	Nonsense mediated decay	-	E9PX06	TSL:5
Becn1-214	ENSMUST00000167667.7	1599	46aa	Nonsense mediated decay	-	E9PX06	TSL:5
Becn1-213	ENSMUST00000164036.7	329	No protein	Retained intron	-	-	TSL:3
Becn1-212	ENSMUST00000140706.7	684	110aa	Protein coding	-	F7DB15	CDS 3' incomplete TSL:2
Becn1-211	ENSMUST00000140286.1	487	No protein	Retained intron	-	-	TSL:5
Becn1-210	ENSMUST00000139997.8	3476	No protein	Retained intron	-	-	TSL:1
Becn1-209	ENSMUST00000139669.1	463	No protein	lncRNA	-	-	TSL:3
Becn1-208	ENSMUST00000137614.1	506	No protein	Retained intron	-	-	TSL:1
Becn1-207	ENSMUST00000136535.7	1002	No protein	Retained intron	-	-	TSL:5
Becn1-206	ENSMUST00000135805.7	239	No protein	lncRNA	-	-	TSL:5
Becn1-205	ENSMUST00000130916.7	4365	448aa	Protein coding	CCDS25462	O88597	TSL:1 GENCODE basic APPRIS P1
Becn1-204	ENSMUST00000129863.1	375	125aa	Protein coding	-	F7CVL9	CDS 5' and 3' incomplete TSL:5
Becn1-203	ENSMUST00000126195.1	786	168aa	Nonsense mediated decay	-	F7C090	CDS 5' incomplete TSL:3
Becn1-202	ENSMUST00000122817.7	2662	No protein	Retained intron	-	-	TSL:1
Becn1-201	ENSMUST00000041403.11	2123	105aa	Nonsense mediated decay	-	E9QAM0	TSL:1

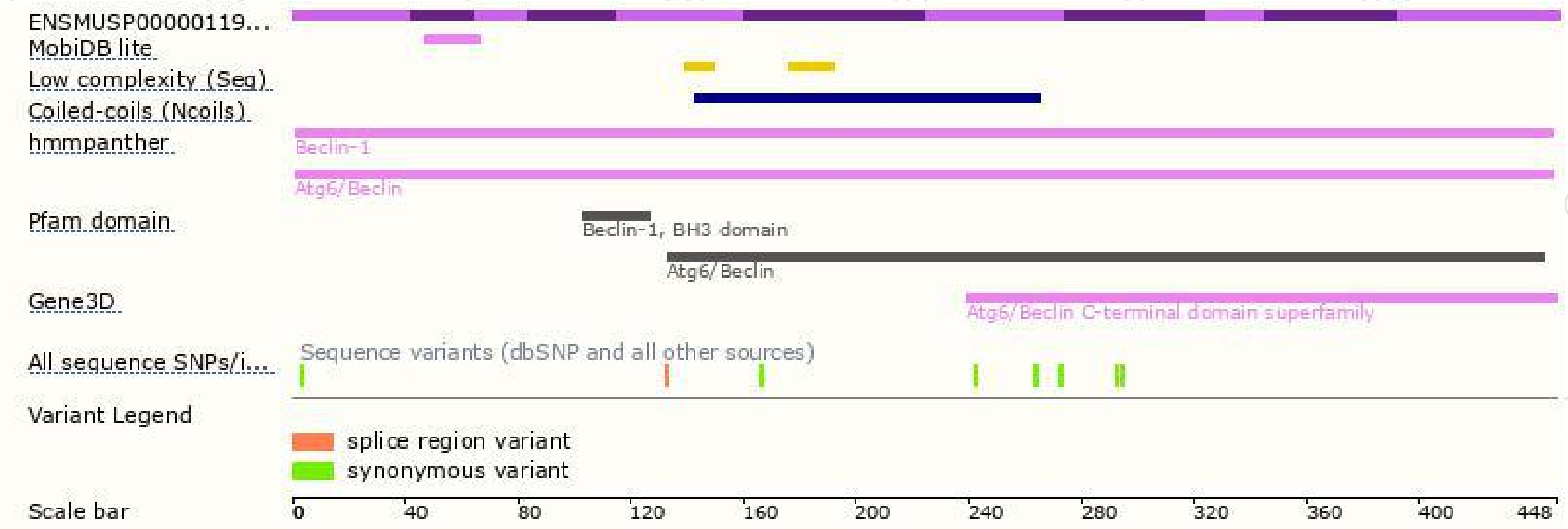
The strategy is based on the design of *Becn1-205* transcript,The transcription is shown below



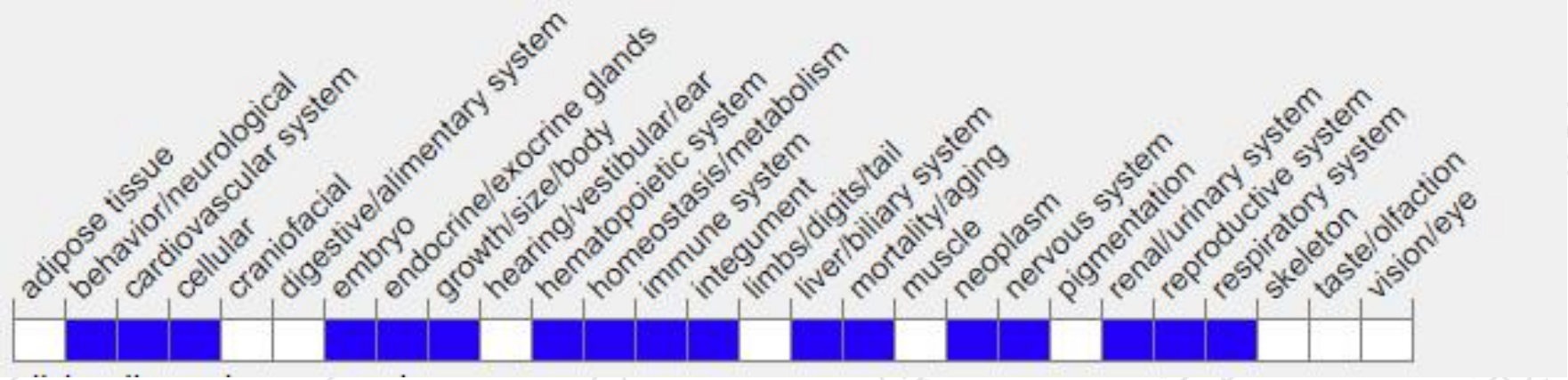
Genomic location distribution



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 exhibit prenatal lethality. Mice heterozygous for this allele exhibit premature death, increased tumor incidence and reduced autophagy.

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

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