Bglap Cas9-CKO Strategy

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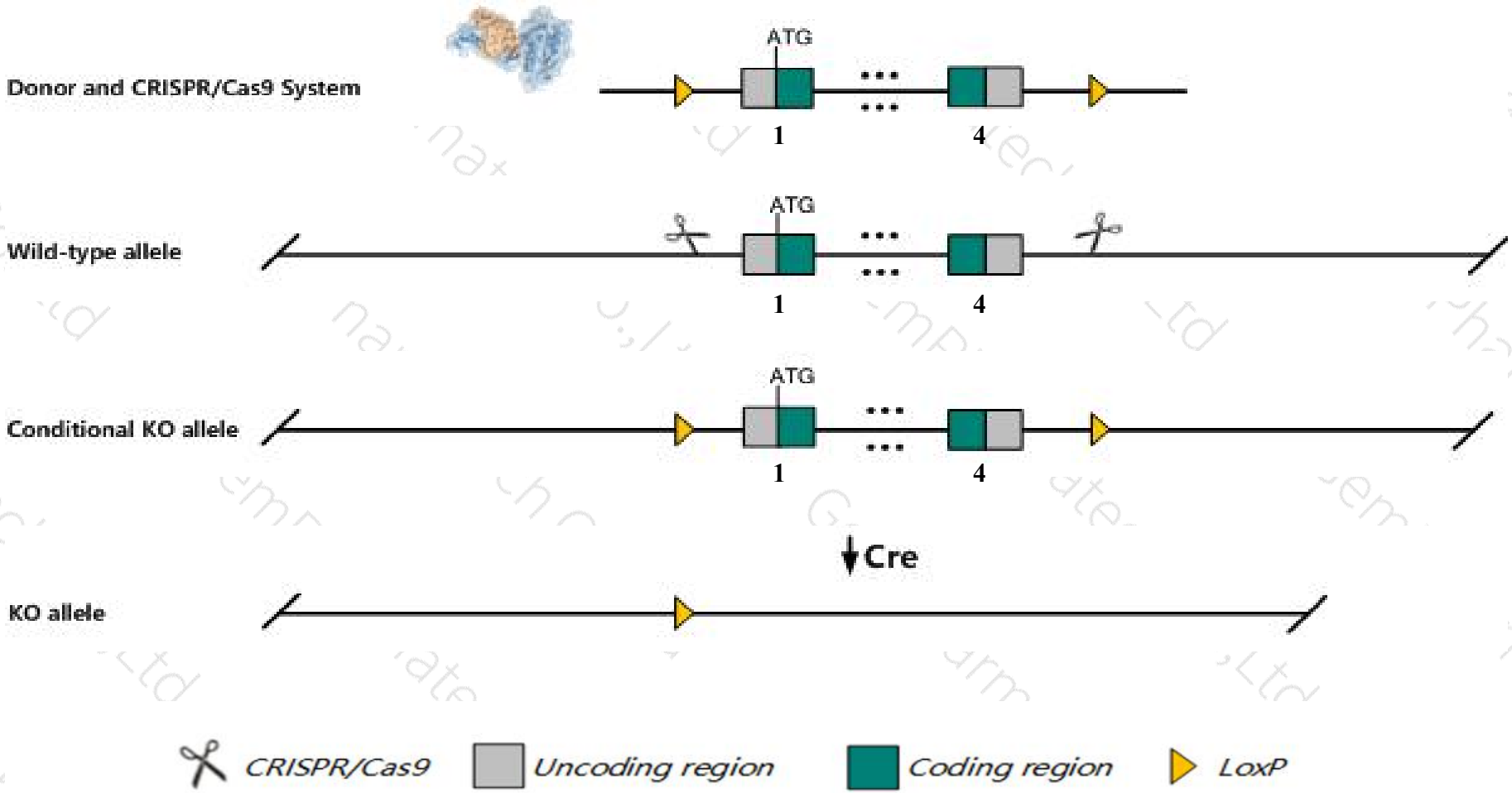
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Bglap* gene has 2 transcripts. According to the structure of *Bglap* gene, exon1-exon4 of *Bglap-201* (ENSMUST00000076048.4) transcript is recommended as the knockout region.The region contains all of the coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Bglap* 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 of both Bglap1 and Bglap2 exhibit decreased circulating insulin, impaired glucose tolerance, increased adipose tissue, increased bone density, and decreased male fertility.

➢The floxed region is near to the C-terminal of *Gm6821* gene and N-terminal of *Bglap2* gene,this strategy may influence the regulatory function of the C-terminal of *Gm6821* gene and N-terminal of *Bglap2* gene.

➢ The *Bglap* gene is located on the Chr3. 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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Bglap bone gamma carboxyglutamate protein [Mus musculus (house mouse)]

Gene ID: 12096, updated on 13-Mar-2020

Summary

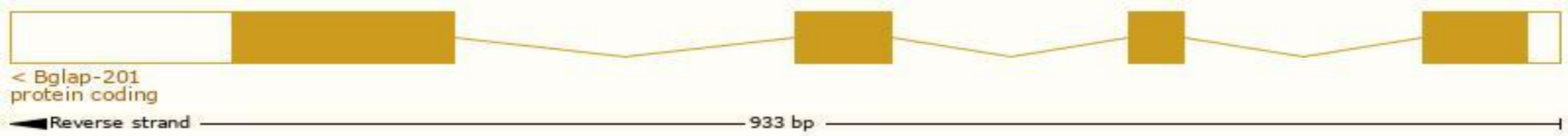
Official Symbol	Bglap provided by MGI
Official Full Name	bone gamma carboxyglutamate protein provided byMGI
Primary source	MGI:MGI:88156
See related	Ensembl:ENSMUSG00000074483
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	BGP, Bglap1, OC, OG1, mOC-A
Summary	This gene encodes one of the most abundant non-collagenous proteins in bone tissue that is localized to the mineralized matrix of bone. The encoded preproprotein undergoes proteolytic processing and post-translational gamma carboxylation to generate a mature, calcium-binding protein. Mice lacking the encoded protein develop abnormalities of bone remodelling. This gene is located adjacent to two other osteocalcin-related genes on chromosome 3. [provided by RefSeq, Oct 2015]
Expression	Biased expression in colon adult (RPKM 5.0), bladder adult (RPKM 3.9) and 14 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

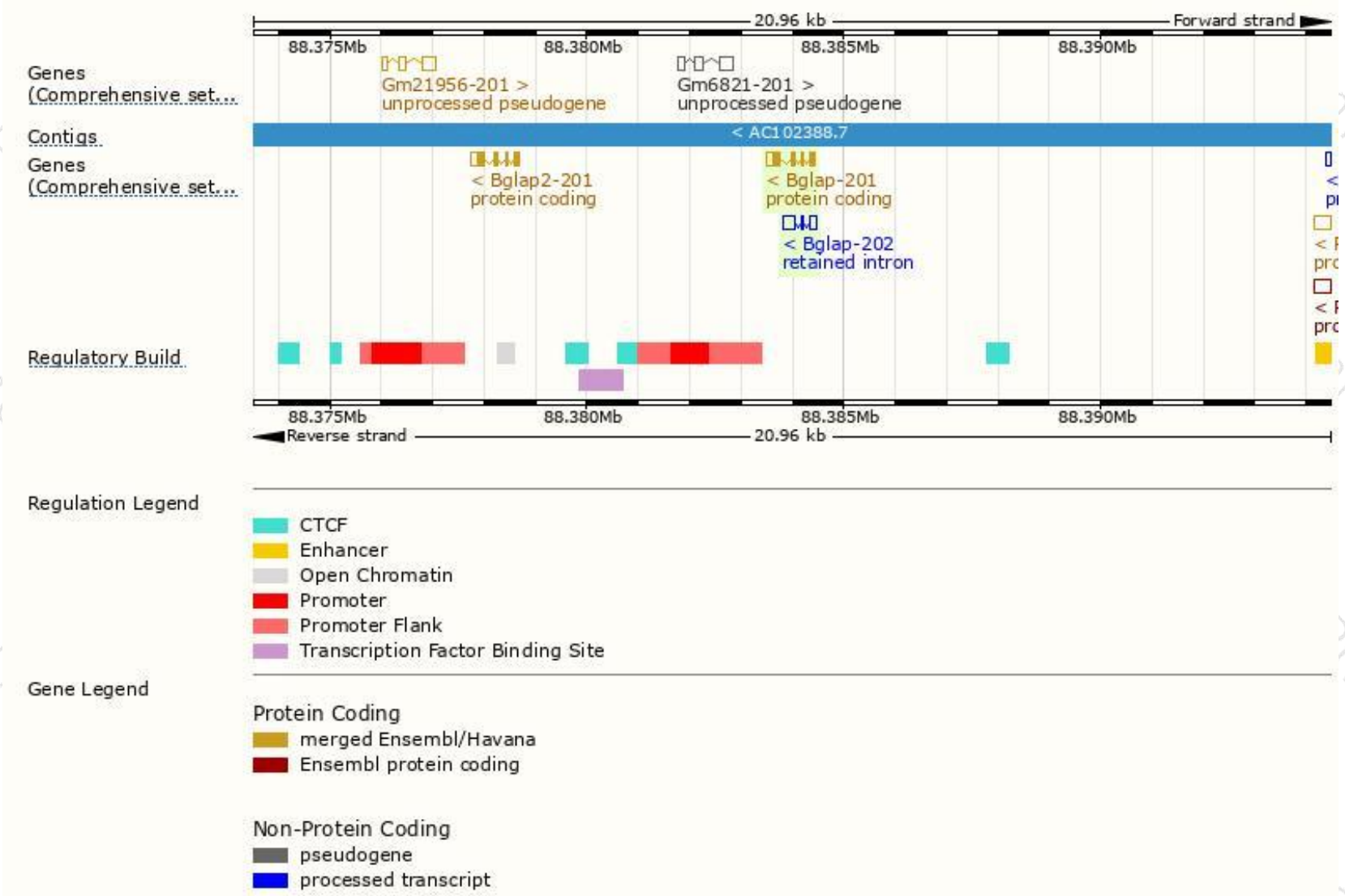
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Bglap-201	ENSMUST00000076048.4	440	95aa	Protein coding	CCDS17474	P86546	TSL:1 GENCODE basic APPRIS P1
Bglap-202	ENSMUST00000128732.1	351	No protein	Retained intron	-	-	TSL:2

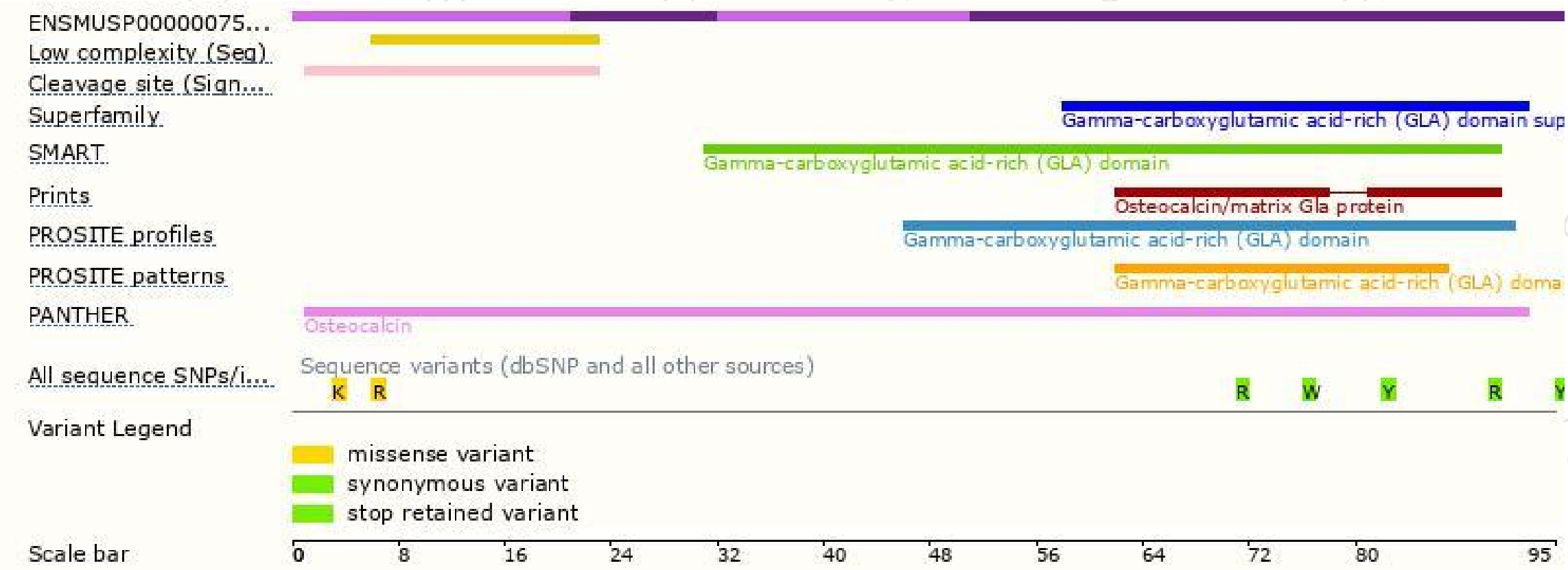
The strategy is based on the design of *Bglap-201* transcript,the transcription is shown below



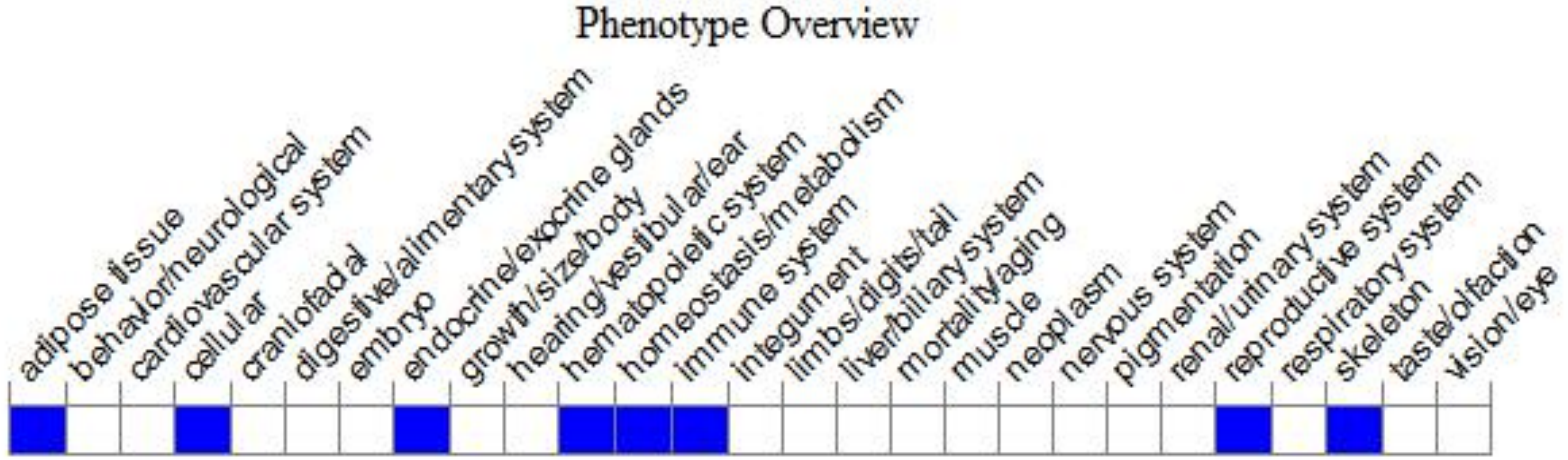
Genomic location distribution



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/).

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

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