Clu Cas9-CKO Strategy

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Project Overview

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

Conditional Knockout strategy

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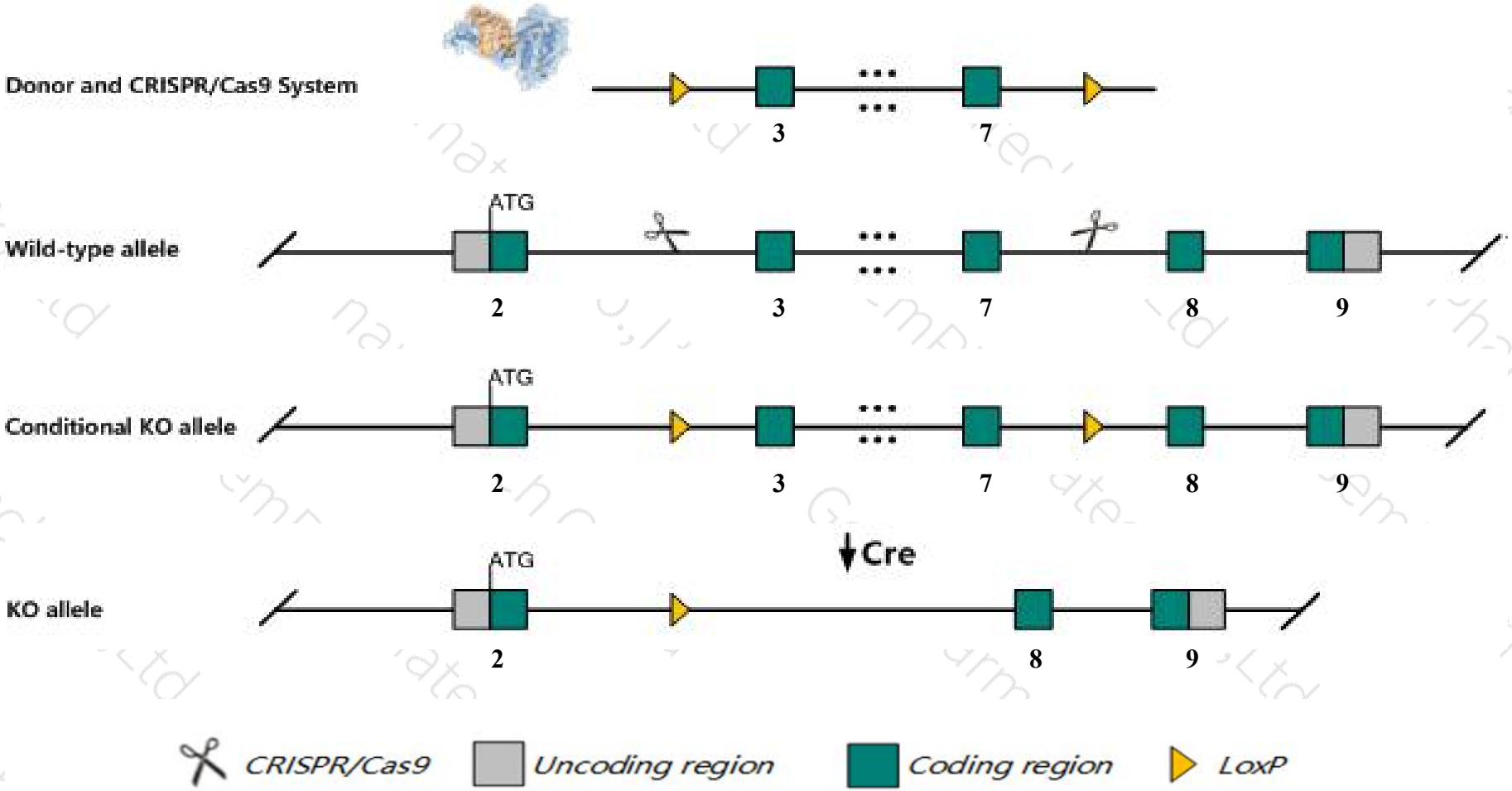
This model will use CRISPR/Cas9 technology to edit the *Clu* gene. The schematic diagram is as follows:



Technical routes

- The *Clu* gene has 9 transcripts. According to the structure of *Clu* gene, exon3-exon7 of *Clu-201* (ENSMUST00000022616.13) transcript is recommended as the knockout region.The region contains 1067bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Clu* 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,homozygous inactivation of this gene leads to progressive renal glomerulopathy and increased severity of myosin-induced autoimmune myocarditis.
- The *Clu* gene is located on the Chr14. 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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Clu clusterin [Mus musculus (house mouse)]

Gene ID: 12759, updated on 13-Mar-2020

Summary

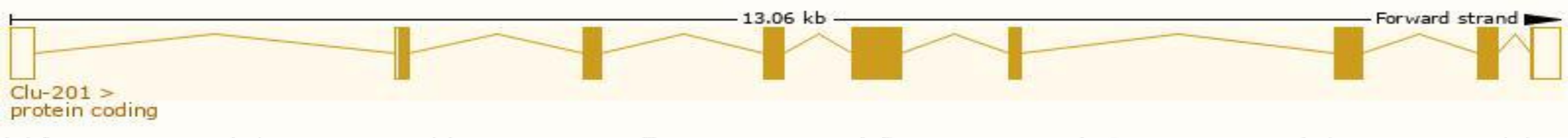
Official Symbol	Clu provided by MGI
Official Full Name	clusterin provided by MGI
Primary source	MGI:MGI:88423
See related	Ensembl:ENSMUSG00000022037
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI893575, ApoJ, Cli, D14Ucla3, SP-40, Sgp-2, Sgp2, Sugp-2
Summary	The protein encoded by this gene is a secreted chaperone that can, under some stress conditions, also be found in the cell cytosol. It has been suggested to be involved in several basic biological events such as cell death, tumor progression, and neurodegenerative disorders. The encoded preproprotein undergoes proteolytic processing to generate a disulfide-linked heterodimeric mature protein comprised of alpha and beta subunits. Mice lacking the encoded protein exhibit increased severity of autoimmune myocarditis, faster progression of the acute inflammation to myocardial scarring and decreased brain injury following neonatal hypoxic-ischemic injury. [provided by RefSeq, Nov 2015]
Expression	Biased expression in genital fat pad adult (RPKM 3056.5), ovary adult (RPKM 788.7) and 11 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

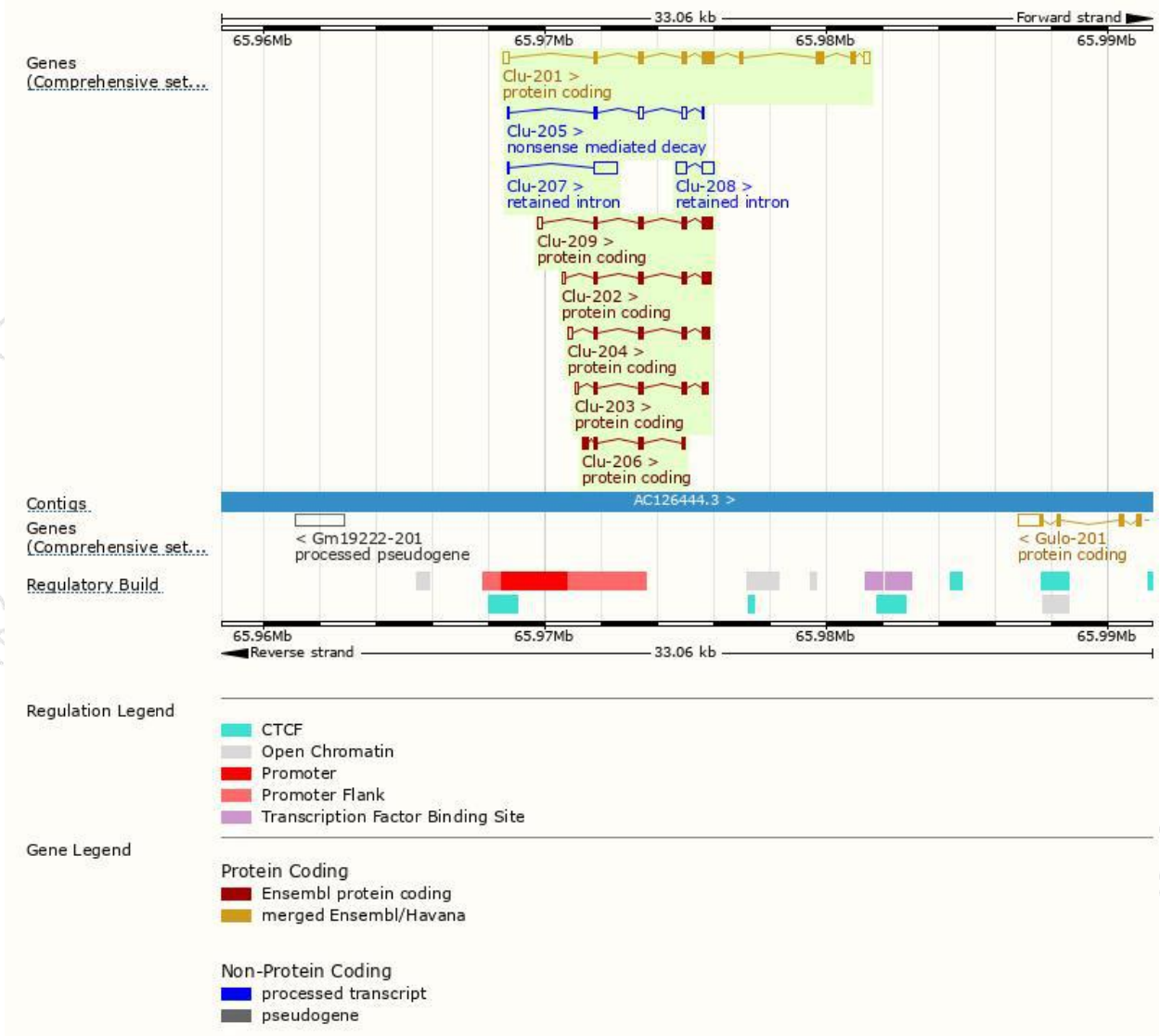
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Clu-201	ENSMUST00000022616.13	1810	448aa	Protein coding	CCDS36957	Q06890 Q549A5	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
Clu-209	ENSMUST00000153460.7	1004	265aa	Protein coding	-	E9Q8Y5	CDS 3' incomplete TSL:2
Clu-204	ENSMUST00000138191.7	844	225aa	Protein coding	-	E9PUU2	CDS 3' incomplete TSL:5
Clu-202	ENSMUST00000127387.7	811	233aa	Protein coding	-	E9PXG5	CDS 3' incomplete TSL:3
Clu-203	ENSMUST00000128539.7	756	203aa	Protein coding	-	E9Q9B8	CDS 3' incomplete TSL:2
Clu-206	ENSMUST00000144619.1	632	209aa	Protein coding	-	E9Q2G2	CDS 3' incomplete TSL:3
Clu-205	ENSMUST00000138665.1	558	39aa	Nonsense mediated decay	-	D6RFP9	TSL:5
Clu-207	ENSMUST00000146990.1	841	No protein	Retained intron	-	-	TSL:2
Clu-208	ENSMUST00000152903.1	739	No protein	Retained intron	-	-	TSL:2

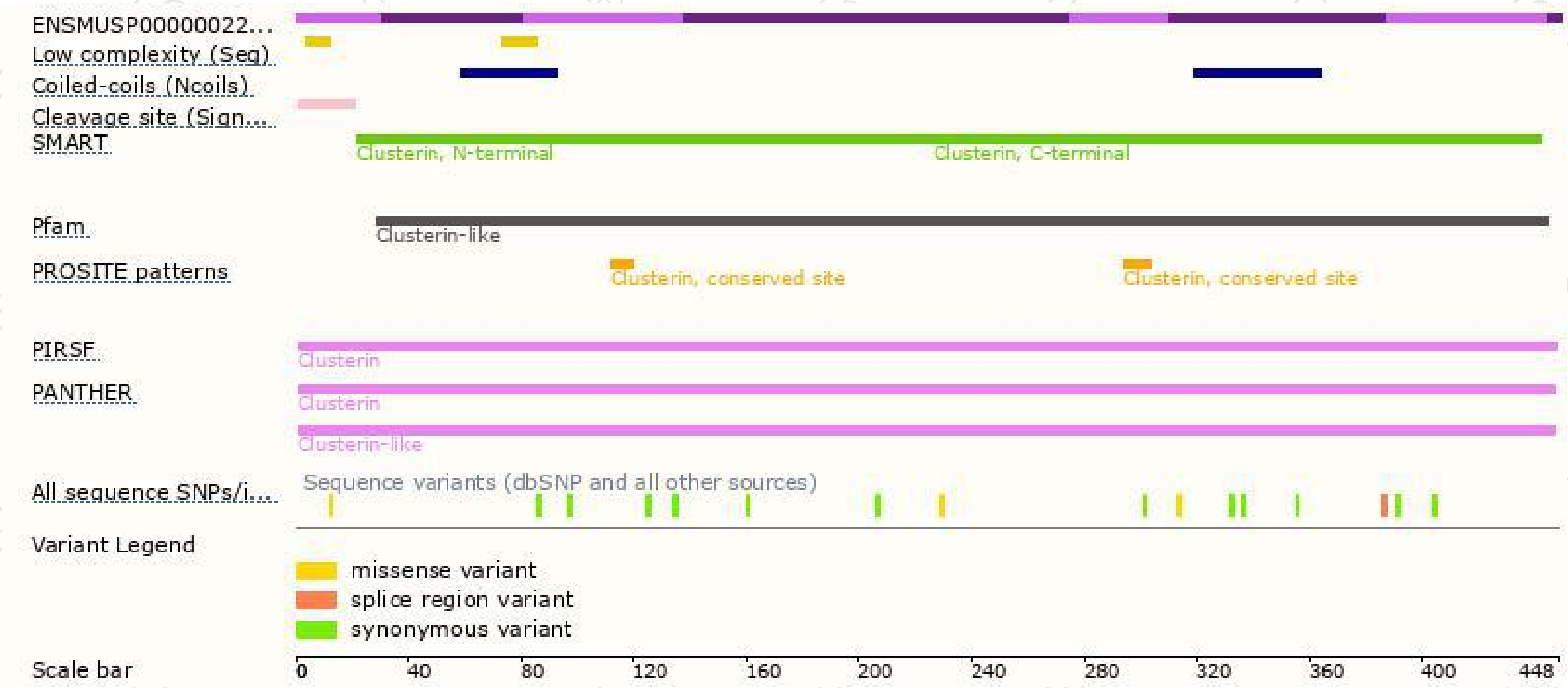
The strategy is based on the design of *Clu-201* transcript,the transcription is shown below



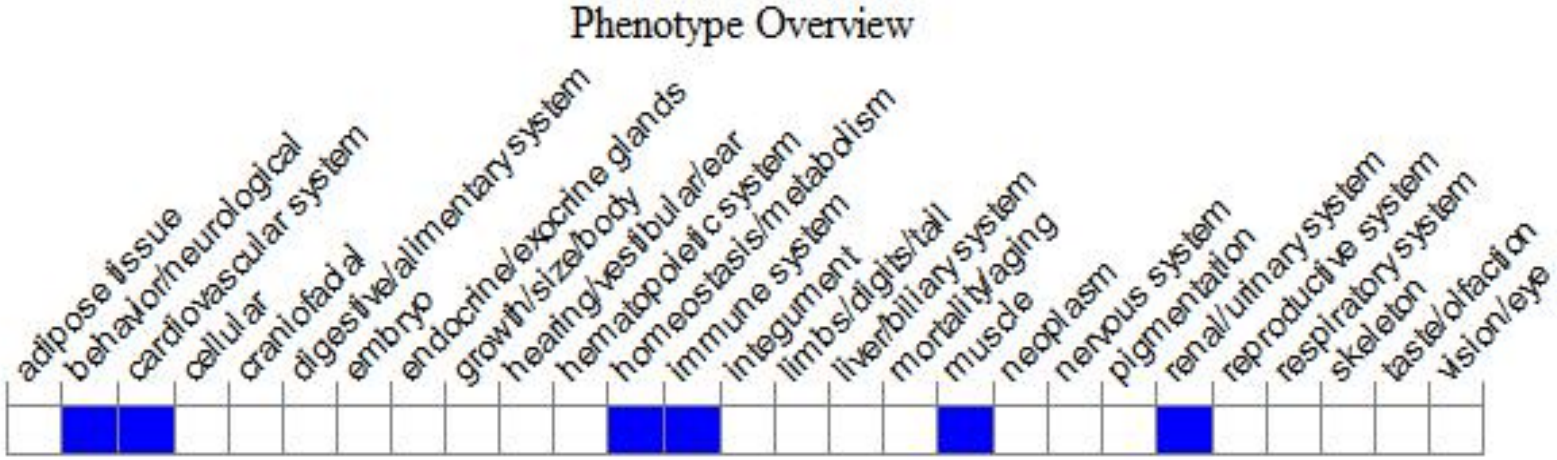
Genomic location distribution



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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