Glb1 Cas9-KO Strategy

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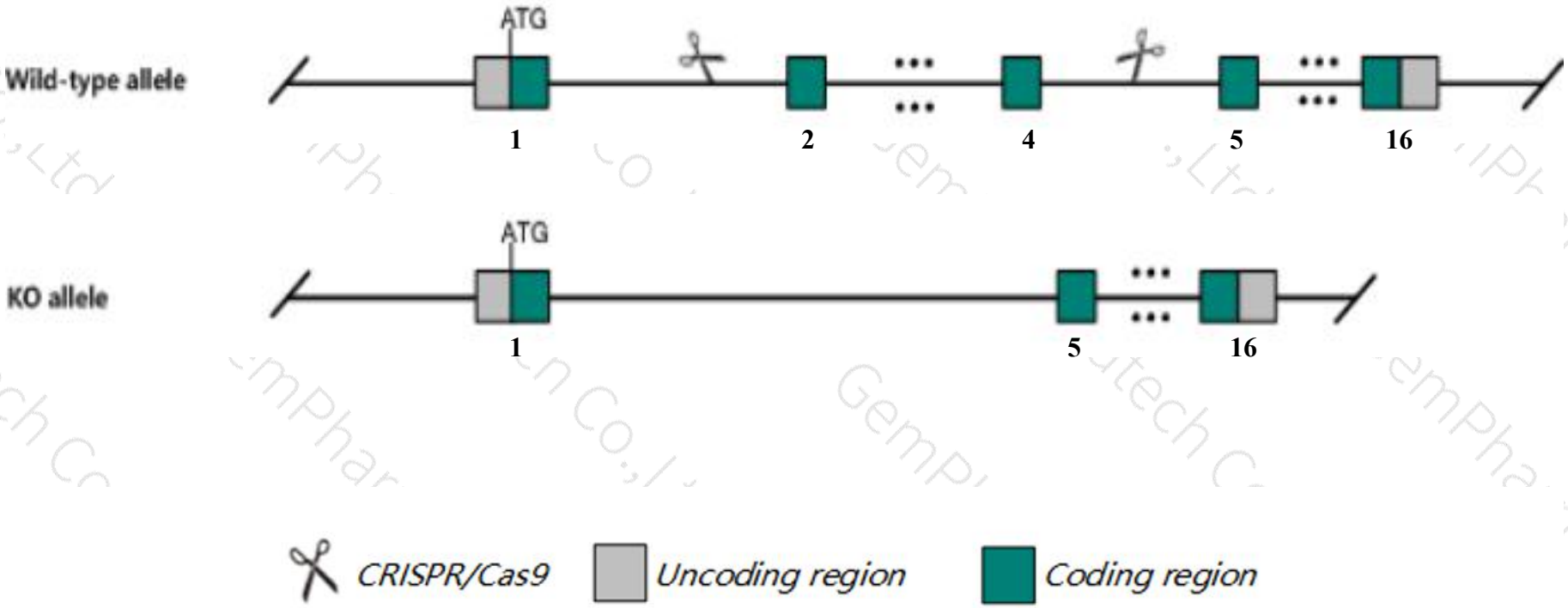
Project Overview

Project Name	***Glb1***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Glb1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Glb1* gene has 2 transcripts. According to the structure of *Glb1* gene, exon2-exon4 of *Glb1-201*(ENSMUST00000063042.10) transcript is recommended as the knockout region.The region contains 382bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Glb1* gene. The brief process is as follows: CRISPR/Cas9 system 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.

Notice

➢ According to the existing MGI data,homozygotes for a targeted null mutation exhibit progressive spastic diplegia, emaciation, and accumulation of ganglioside GM1 and asialo GM1 in brain tissue. Mutants die at 7-10 months of age.

➢ The *Glb1* 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Glb1 galactosidase, beta 1 [Mus musculus (house mouse)]

Gene ID: 12091, updated on 13-Mar-2020

Summary

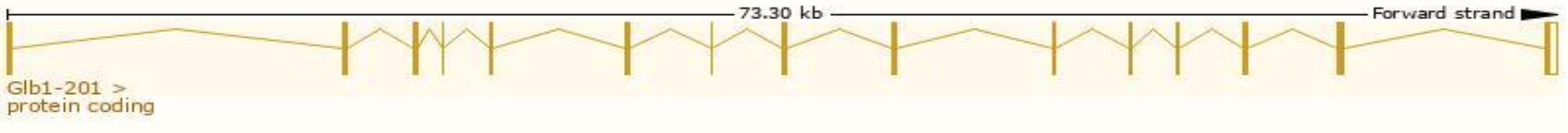
Official Symbol	Glb1 provided by MGI
Official Full Name	galactosidase, beta 1 provided byMGI
Primary source	MGI:MGI:88151
See related	Ensembl:ENSMUSG00000045594
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW125515, Bge, Bgl, Bgl-e, Bgl-s, Bgl-t, Bgs, Bgt, C130097A14Rik
Summary	This gene encodes a preproprotein that is proteolytically cleaved to yield a signal peptide and a proproptein that is subsequently processed to generate the active mature peptide. The encoded protein is a lysosomal enzyme that catalyzes the hydrolysis of terminal beta-D-galactose residues in various substrates like lactose, ganglioside GM1 and other glycoproteins. Mutations in the human gene are associated with GM1-gangliosidosis and Morquio B syndrome. Disruption of the mouse gene mirrors the symptoms of human gangliosidosis. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Dec 2013]
Expression	Ubiquitous expression in kidney adult (RPKM 57.0), genital fat pad adult (RPKM 41.2) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

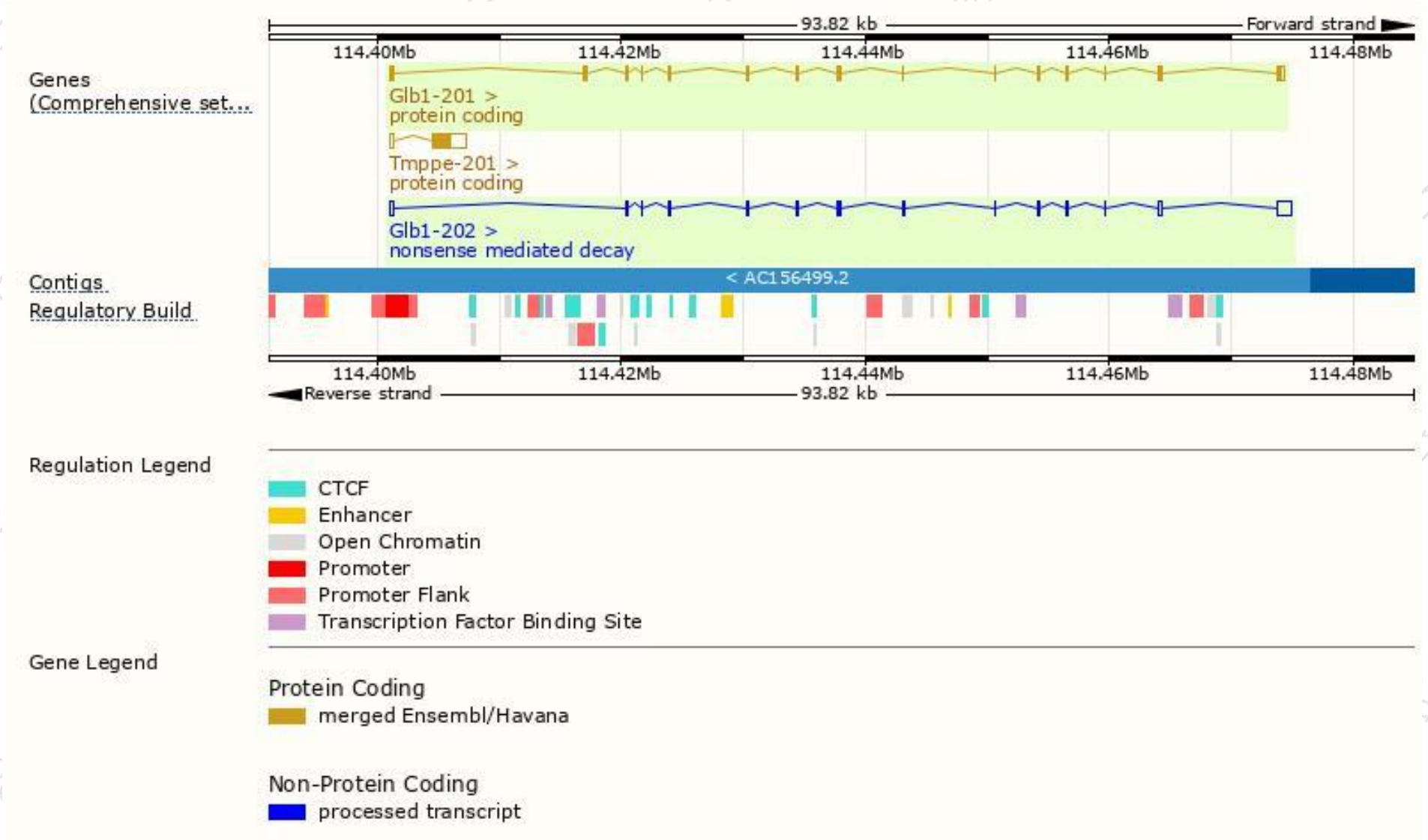
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Glb1-201	ENSMUST00000063042.10	2396	647aa	Protein coding	CCDS23593	P23780 Q3TAW7	TSL:1 GENCODE basic APPRIS P1
Glb1-202	ENSMUST00000217583.1	2806	291aa	Nonsense mediated decay	-	A0A1L1SSJ7	TSL:1

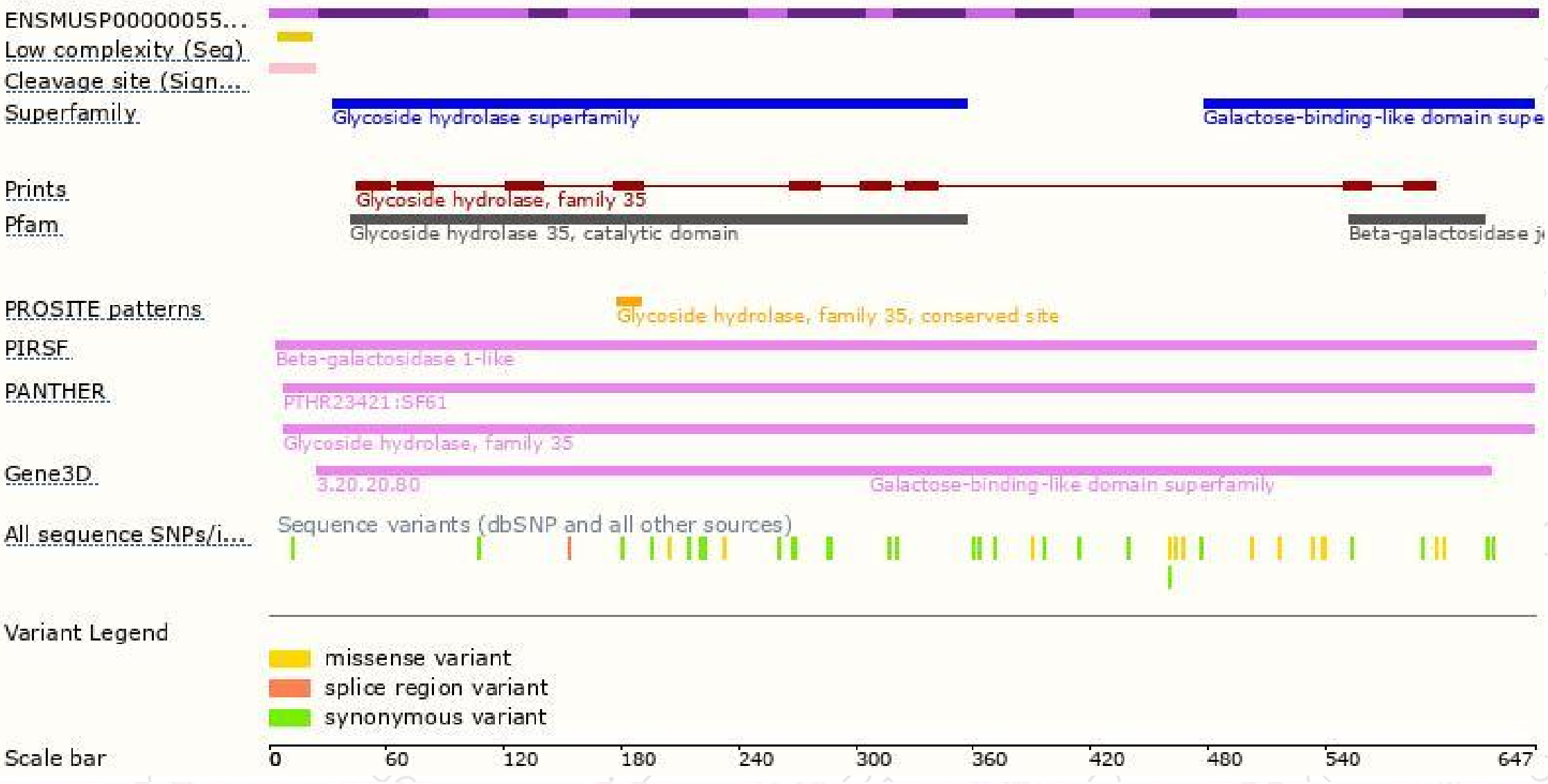
The strategy is based on the design of *Glb1-201* transcript,the transcription is shown below:



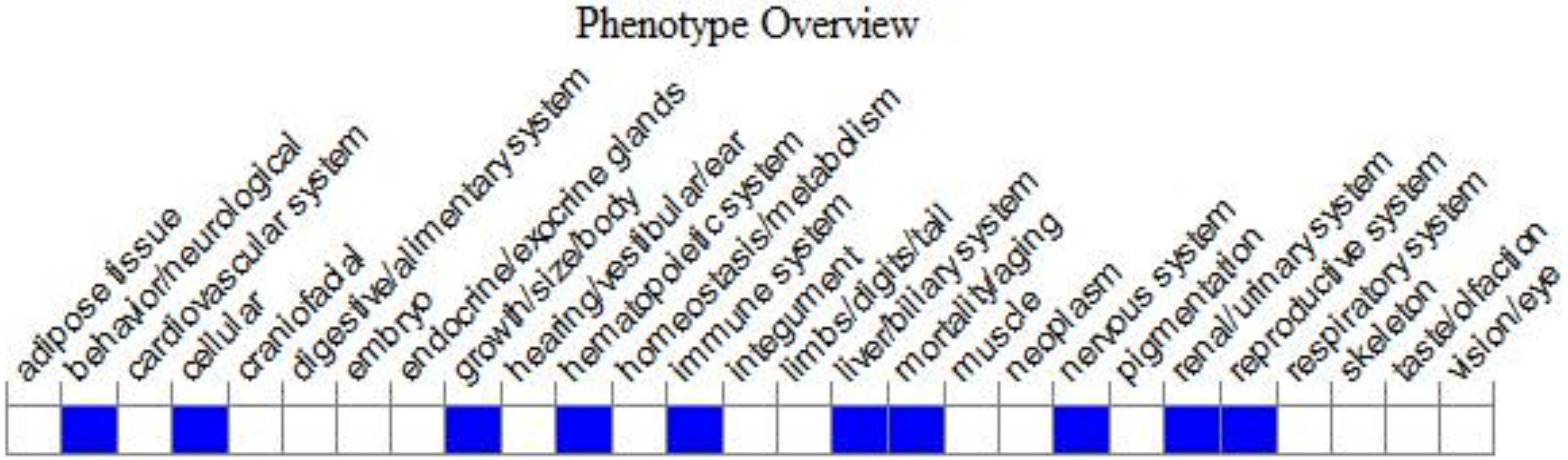
Genomic location distribution



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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