Man2a1 Cas9-CKO Strategy

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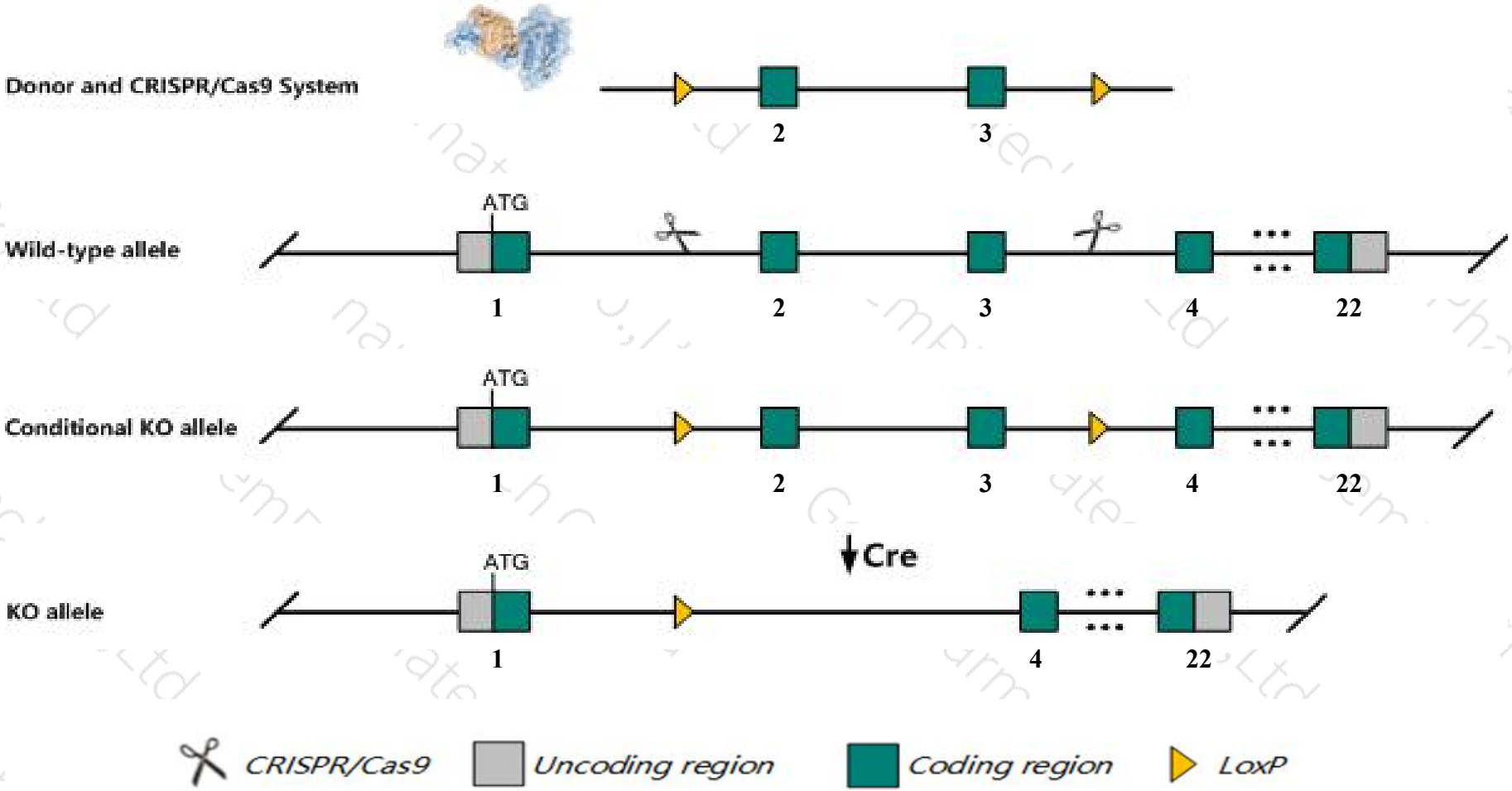
Project Overview

Project Name	***Man2a1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Man2a1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Man2a1* gene has 3 transcripts. According to the structure of *Man2a1* gene, exon2-exon3 of *Man2a1-201*(ENSMUST00000086723.9) transcript is recommended as the knockout region.The region contains 397bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Man2a1* 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 mutation of this gene results in premature death, dyserythropoiesis, systemic autoimmune disease, including an increase in serum immunoglobulins, glomerulonephritis, and hematuria,

➢ The *Man2a1* gene is located on the Chr17. 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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Man2a1 mannosidase 2, alpha 1 [Mus musculus (house mouse)]

Gene ID: 17158, updated on 13-Mar-2020

Summary

Official Symbol	Man2a1 provided by MGI
Official Full Name	mannosidase 2, alpha 1 provided byMGI
Primary source	MGI:MGI:104669
See related	Ensembl:ENSMUSG00000024085
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	Mana-2, Mana2, Map-2
Expression	Ubiquitous expression in placenta adult (RPKM 18.4), limb E14.5 (RPKM 11.1) and 28 other tissuesSee more
Orthologs	human all

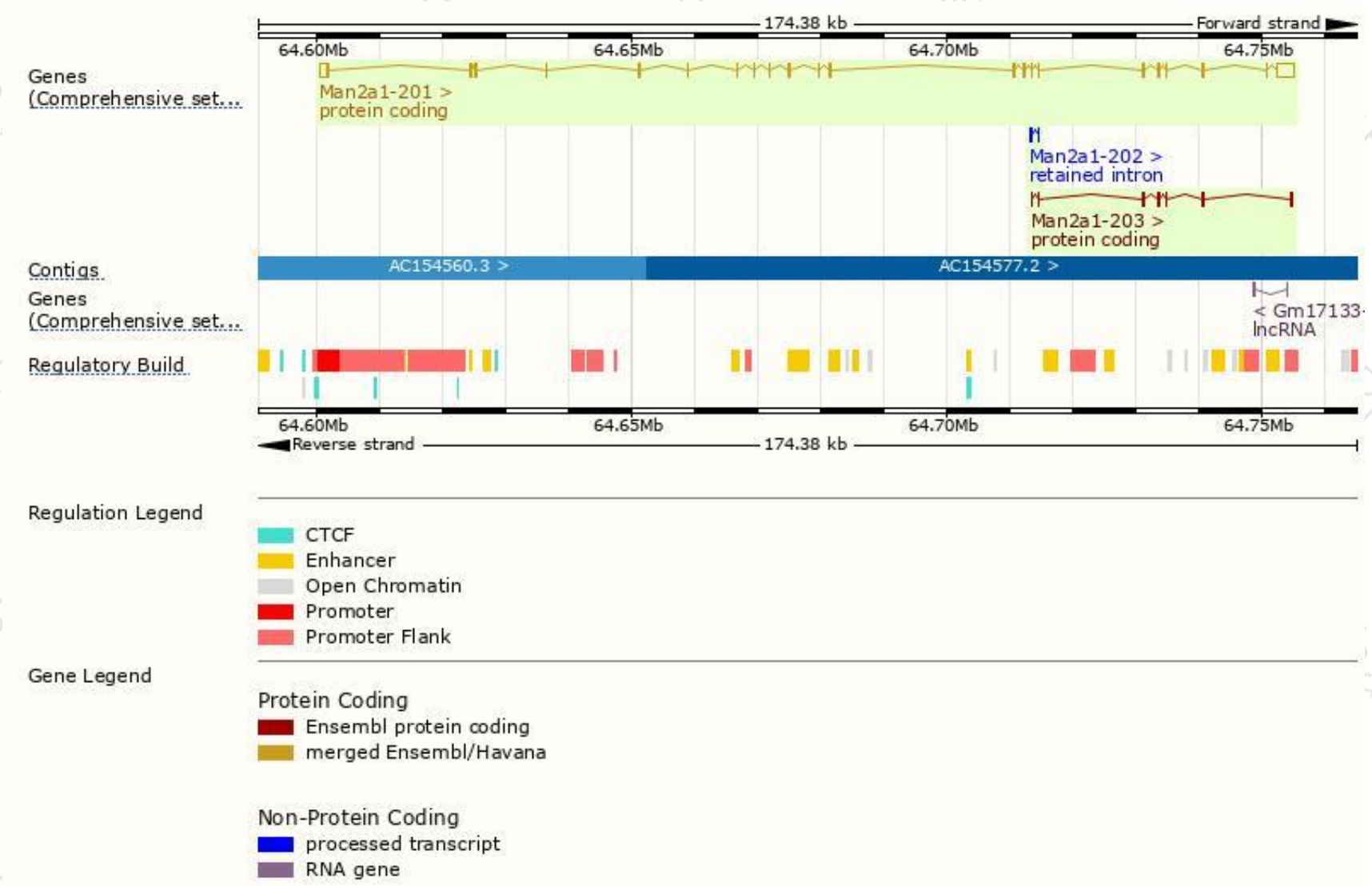
Transcript information（Ensembl）

The gene has 3 transcripts,all transcripts are shown below:

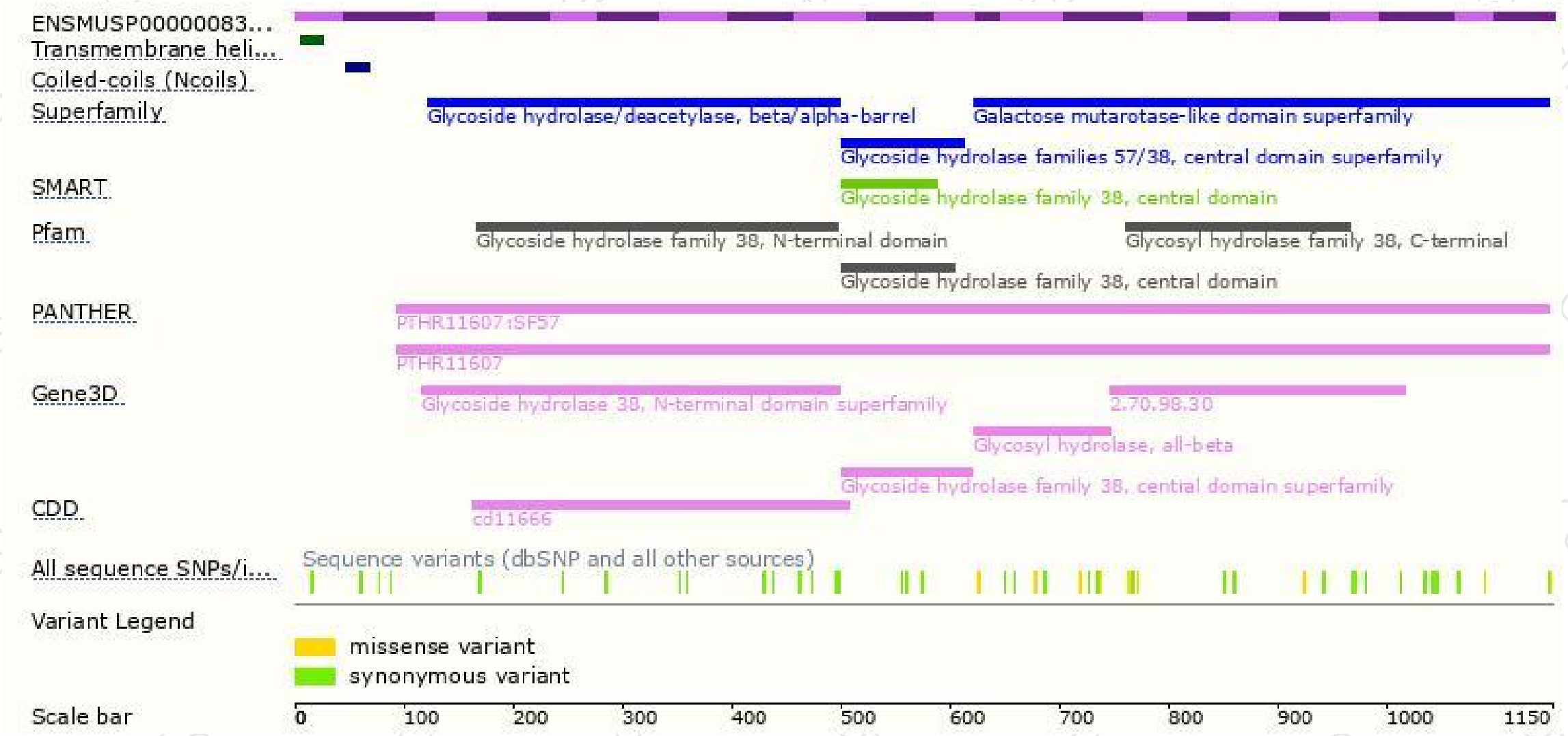
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Man2a1-201	ENSMUST00000086723.9	6991	1150aa	Protein coding	CCDS28938	P27046	TSL:1 GENCODE basic APPRIS P1
Man2a1-202	ENSMUST00000169239.1	470	No protein	Retained intron	-	-	TSL:3
Man2a1-203	ENSMUST00000169668.1	867	280aa	Protein coding	-	F6QMB7	CDS 5' incomplete TSL:5

The strategy is based on the design of *Man2a1-201* transcript,the transcription is shown below:

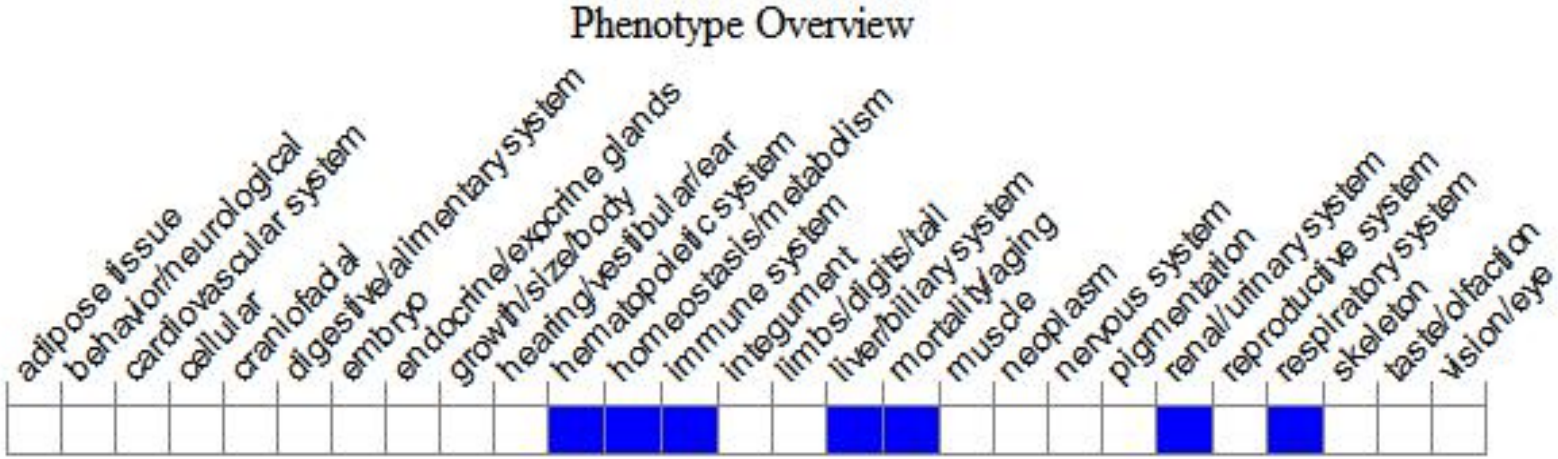
Genomic location distribution



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/).

According to the existing MGI data,homozygous mutation of this gene results in premature death, dyserythropoiesis, systemic autoimmune disease, including an increase in serum immunoglobulins, glomerulonephritis, and hematuria,

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

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