Gdf3 Cas9-CKO Strategy

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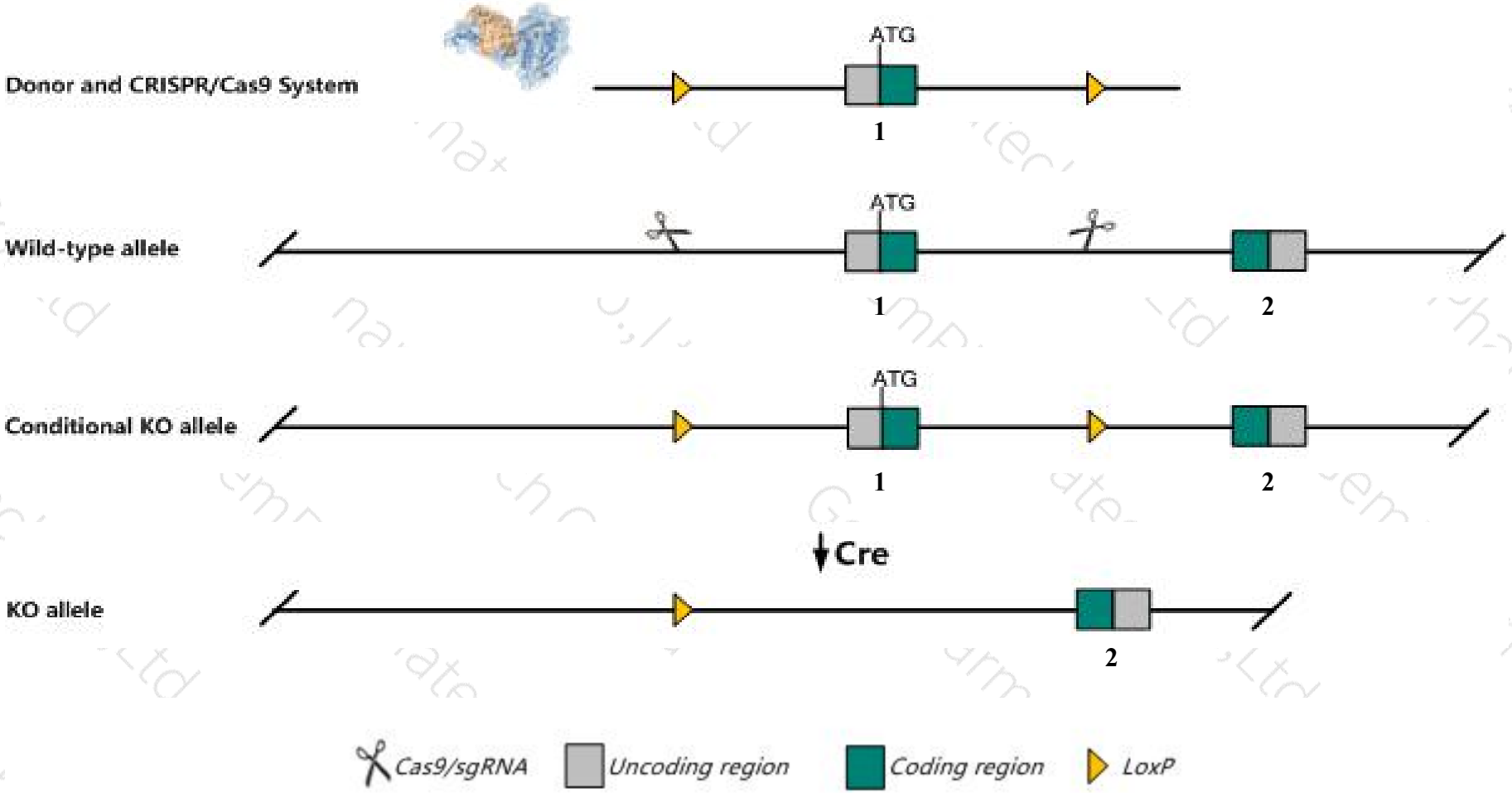
Project Overview

Project Name	***Gdf3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Gdf3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Gdf3* gene has 2 transcripts. According to the structure of *Gdf3* gene, exon1 of *Gdf3-201*(ENSMUST00000032211.4) transcript is recommended as the knockout region.The region contains start codon ATG.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Gdf3* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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 null allele exhibit prenatal lethality and resistance to diet-induced obesity.

➢ The *Gdf3* gene is located on the Chr6. 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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Gdf3 growth differentiation factor 3 [Mus musculus (house mouse)]

Gene ID: 14562, updated on 13-Mar-2020

Summary

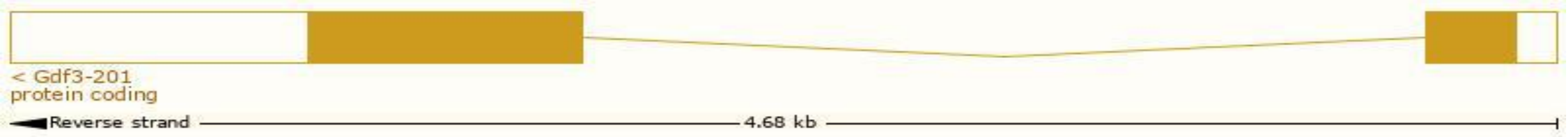
Official Symbol	Gdf3 provided by MGI
Official Full Name	growth differentiation factor 3 provided by MGI
Primary source	MGI:MGI:95686
See related	Ensembl:ENSMUSG00000030117
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	C78318, Gdf-3, Vgr-2, Vgr2, ecat9
Summary	This gene encodes a secreted ligand of the TGF-beta (transforming growth factor-beta) superfamily of proteins. Ligands of this family bind various TGF-beta receptors leading to recruitment and activation of SMAD family transcription factors that regulate gene expression. The encoded preproprotein is proteolytically processed to generate each subunit of the disulfide-linked homodimer. This protein is important in embryogenesis and likely plays a role ocular and skeletal development. Mice lacking a functional copy of this gene exhibit defects in early embryonic development resulting in embryonic lethality. [provided by RefSeq, Aug 2016]
Expression	Low expression observed in reference dataset See more
Orthologs	human all

Transcript information（Ensembl）

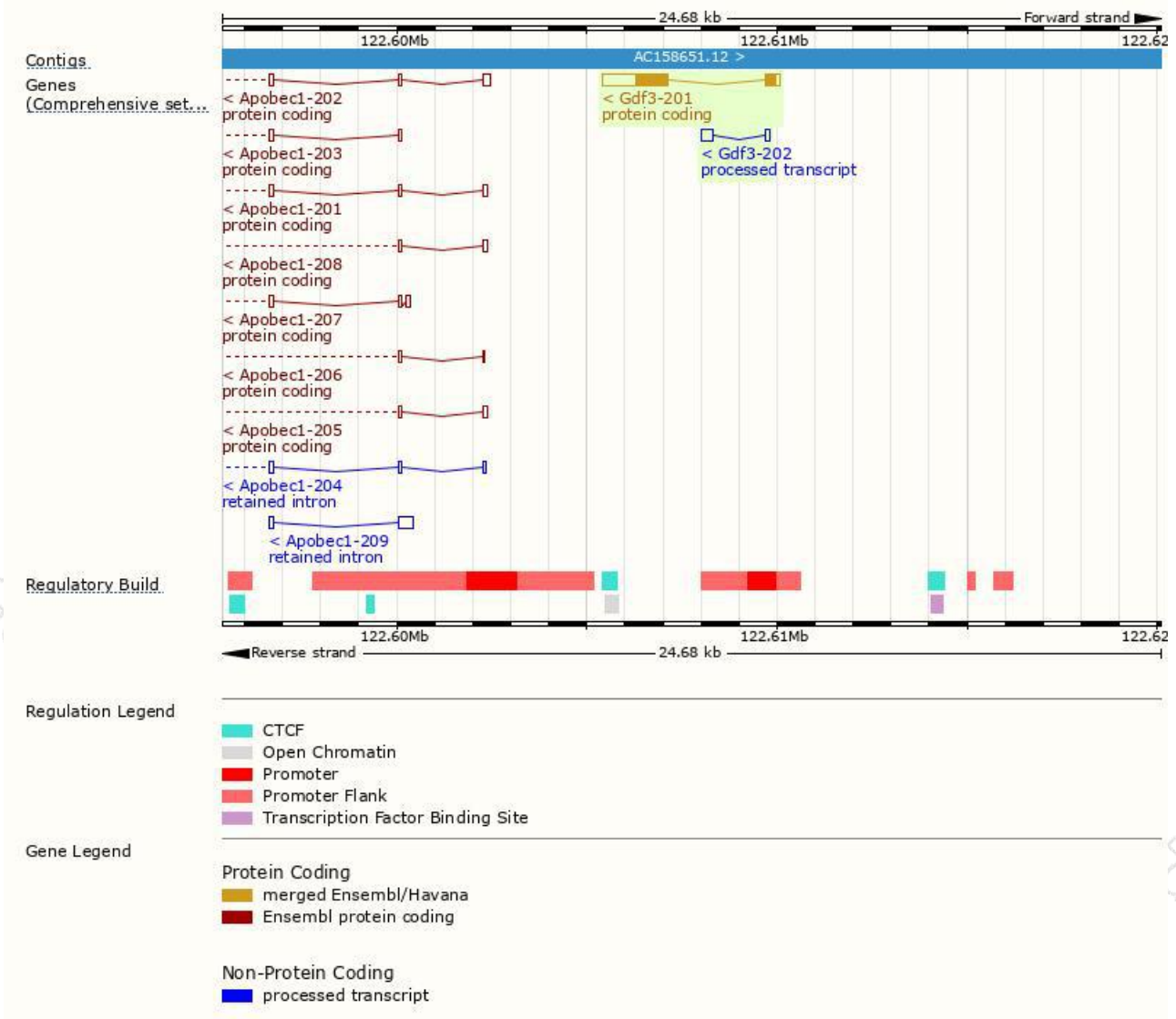
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gdf3-201	ENSMUST00000032211.4	2125	366aa	Protein coding	CCDS20499	Q07104	TSL:1 GENCODE basic APPRIS P1
Gdf3-202	ENSMUST00000204882.1	427	No protein	Processed transcript	-	-	TSL:2

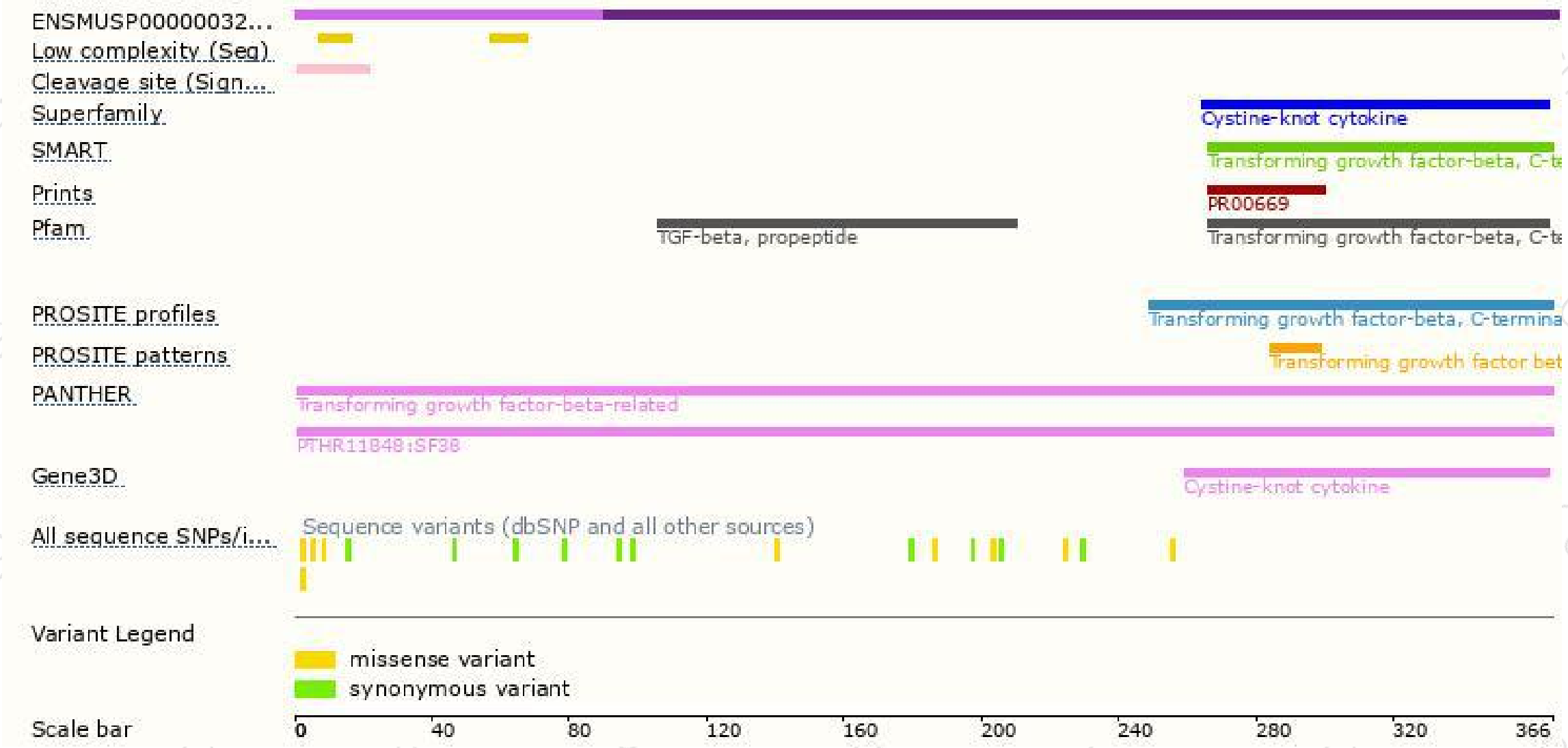
The strategy is based on the design of *Gdf3-201* transcript,the transcription is shown below:



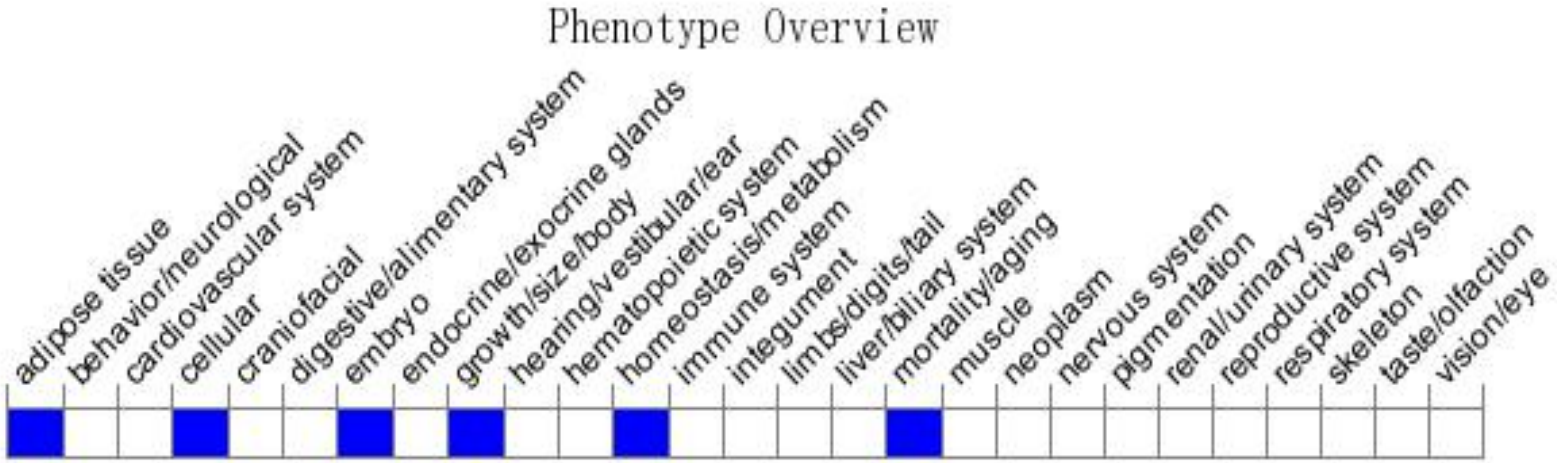
Genomic location distribution



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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