Selenot Cas9-CKO Strategy

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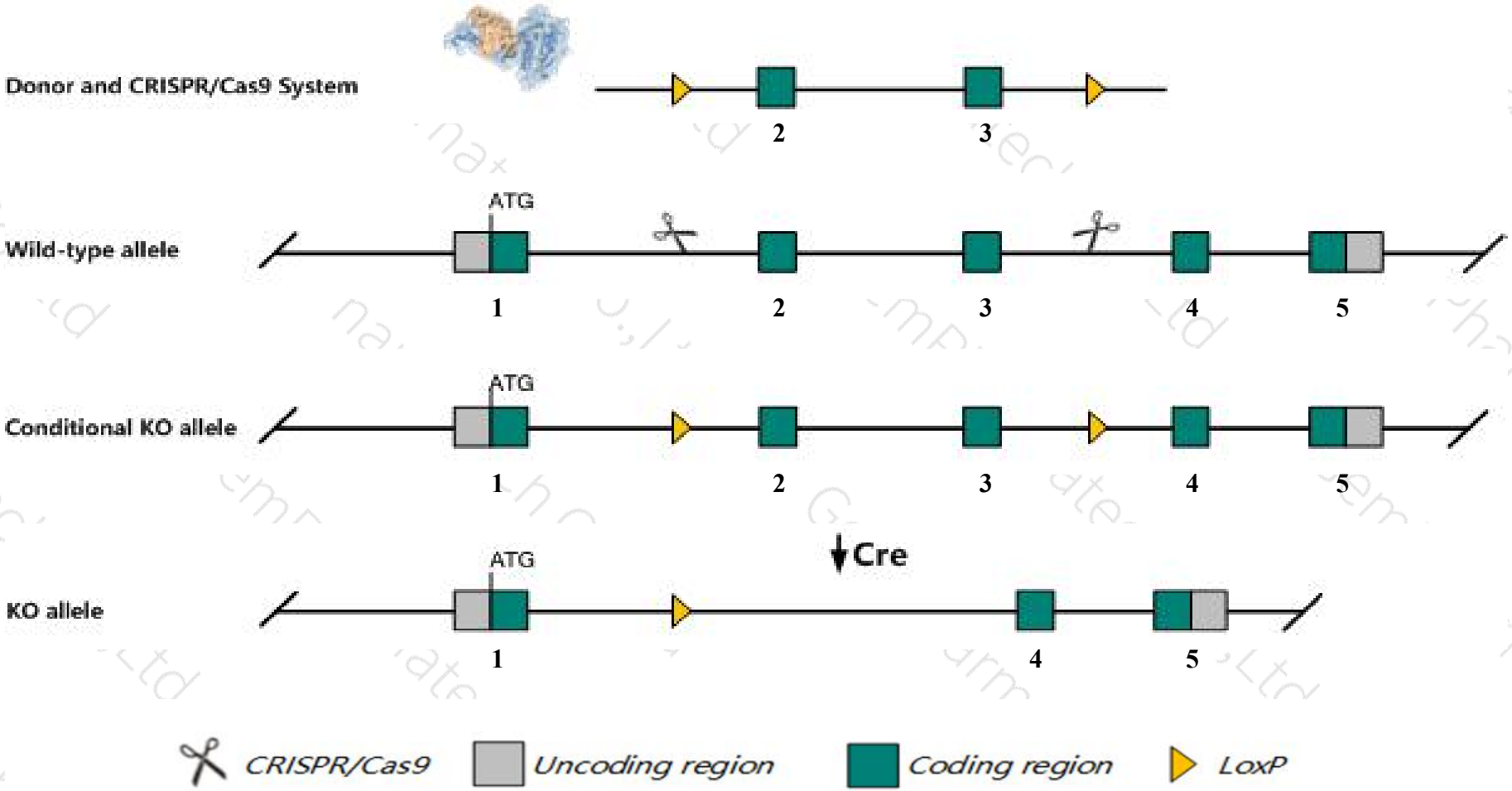
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Selenot* gene has 2 transcripts. According to the structure of *Selenot* gene, exon2-exon3 of *Selenot-201* (ENSMUST00000107924.1) transcript is recommended as the knockout region.The region contains 238bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Selenot* 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 conditional allele activated in beta cells exhibit impaired glucose tolerance, increased circulating glucose levels, decreased circulating insulin levels, decreased insulin secretion and an increase in smaller islets.
- The *Selenot* 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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Selenot selenoprotein T [Mus musculus (house mouse)]

Gene ID: 69227, updated on 13-Mar-2020

Summary

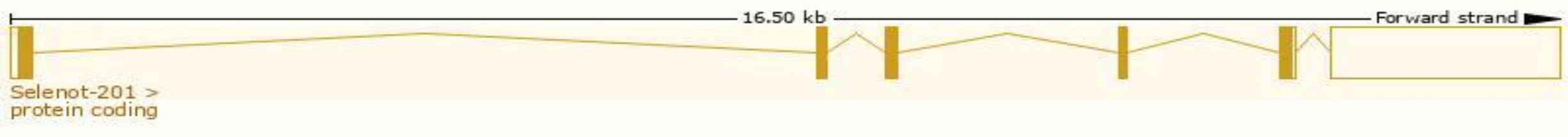
Official Symbol	Selenot provided by MGI
Official Full Name	selenoprotein T provided byMGI
Primary source	MGI:MGI:1916477
See related	Ensembl:ENSMUSG00000075700
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2810407C02Rik, 5730408P04Rik, Selt
Summary	This gene encodes a selenoprotein, containing a selenocysteine (Sec) residue at the active site. Sec is encoded by the UGA codon that normally signals translation termination. The 3' UTRs of selenoprotein mRNAs contain a conserved stem-loop structure, the Sec insertion sequence (SECIS) element, that is necessary for the recognition of UGA as a Sec codon rather than as a stop signal. This protein is localized in the endoplasmic reticulum. It belongs to the SelWTH family that possesses a thioredoxin-like fold and a conserved CxxU (C is cysteine, U is Sec) motif found in several redox active proteins. Studies in mice indicate a crucial role for this gene in the protection of dopaminergic neurons against oxidative stress in Parkinson's disease, and in the control of glucose homeostasis in pancreatic beta-cells. A pseudogene of this locus has been identified on chromosome 8. [provided by RefSeq, Aug 2017]
Expression	Ubiquitous expression in kidney adult (RPKM 32.3), placenta adult (RPKM 31.3) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

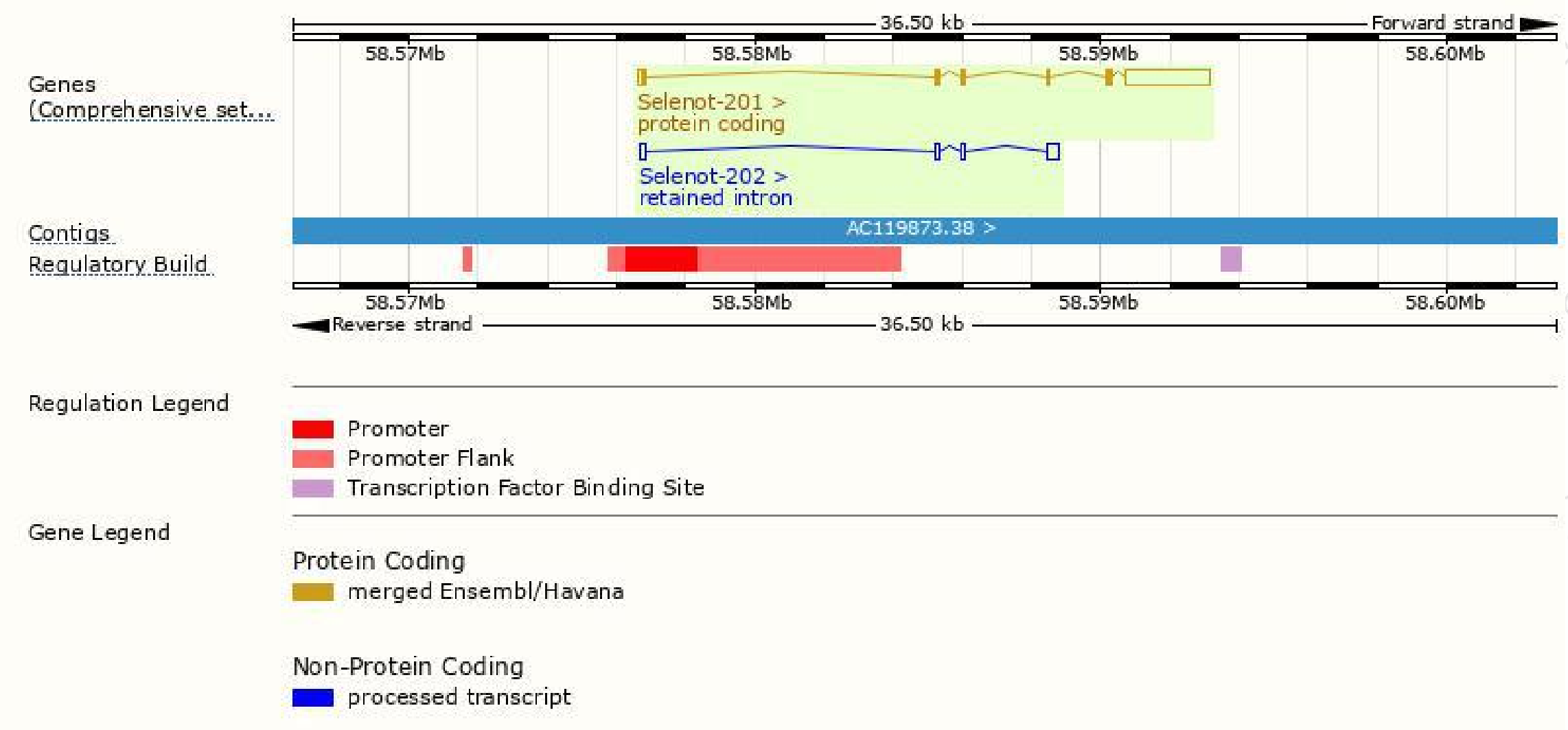
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Selenot-201	ENSMUST00000107924.1	3157	195aa	Protein coding	CCDS38437	P62342	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
Selenot-202	ENSMUST00000125815.1	781	No protein	Retained intron	-	-	TSL:2

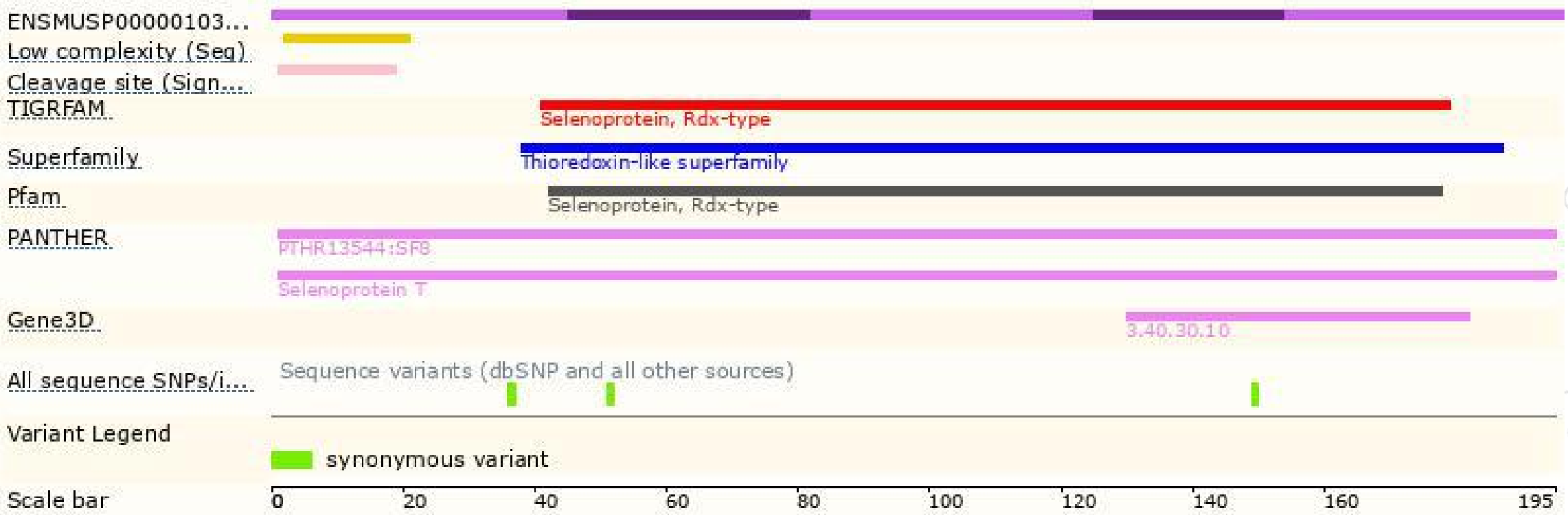
The strategy is based on the design of *Selenot-201* transcript,the transcription is shown below:



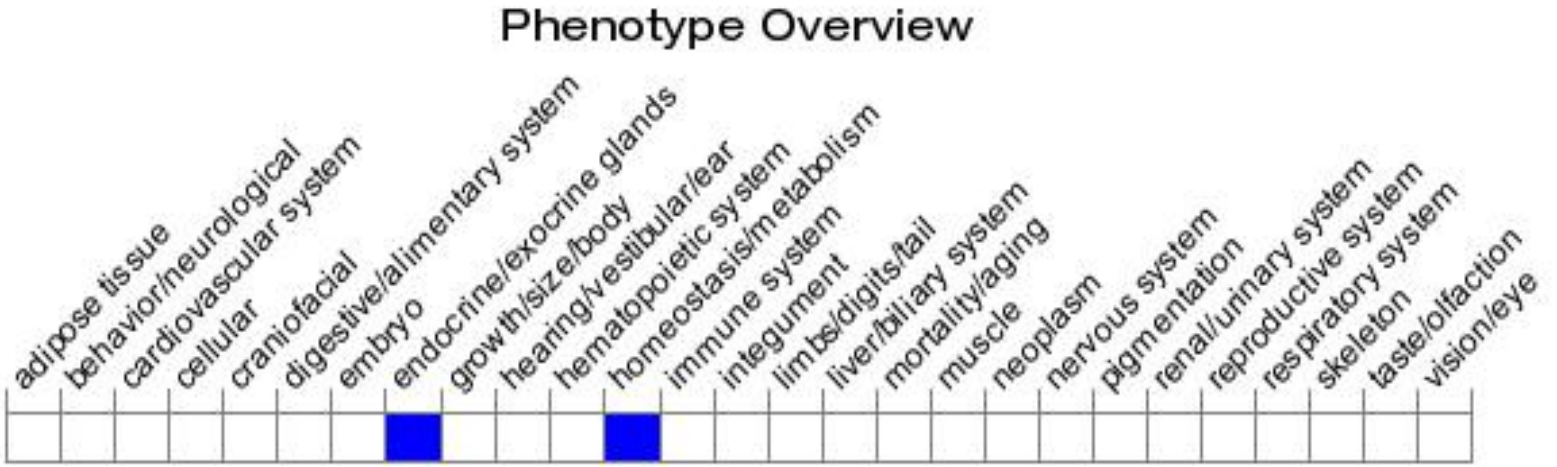
Genomic location distribution



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,mice homozygous for a conditional allele activated in beta cells exhibit impaired glucose tolerance, increased circulating glucose levels, decreased circulating insulin levels, decreased insulin secretion and an increase in smaller islets.

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

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