Ghrhr Cas9-CKO Strategy

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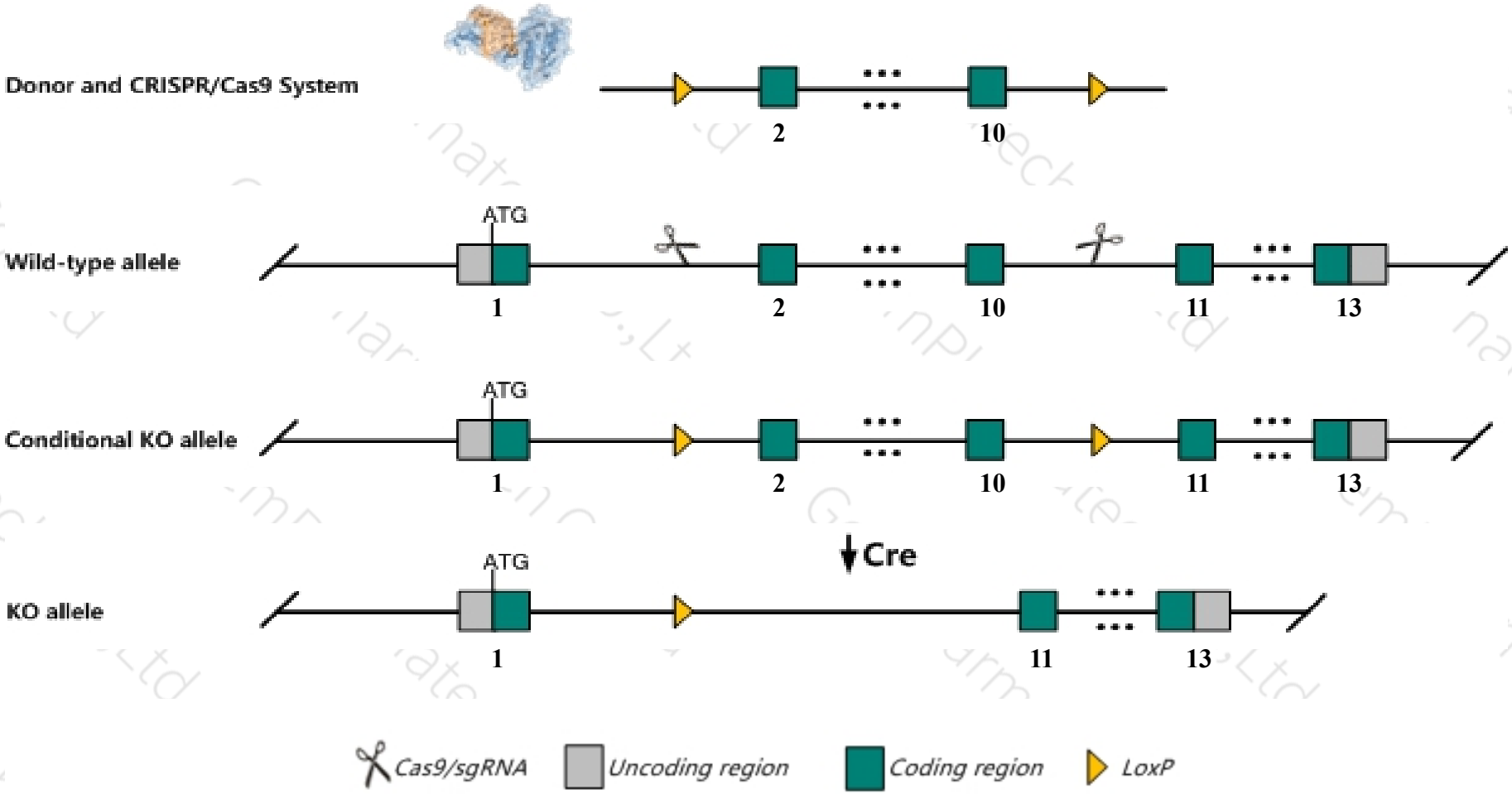
Project Overview

Project Name	***Ghrhr***
Project type	**Cas9-CKO**
Strain background	**C57BL/6J**

Conditional Knockout strategy

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



Technical routes

- The *Ghrhr* gene has 3 transcripts. According to the structure of *Ghrhr* gene, exon2-exon10 of *Ghrhr-201* (ENSMUST00000063578.5) transcript is recommended as the knockout region.The region contains 917bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Ghrhr* 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/6J 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/6J 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,Homozygotes for a spontaneous null mutation exhibit reduced growth from 2 weeks of age, impaired growth hormone synthesis and release, obesity, decreased bone mass, reduced fertility in males, impaired first lactations, and extended life span.

- The *Ghrhr* 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）

集萃药康 GemPharmatech

Ghrhr growth hormone releasing hormone receptor [Mus musculus (house mouse)]

Gene ID: 14602, updated on 19-Mar-2019

Summary

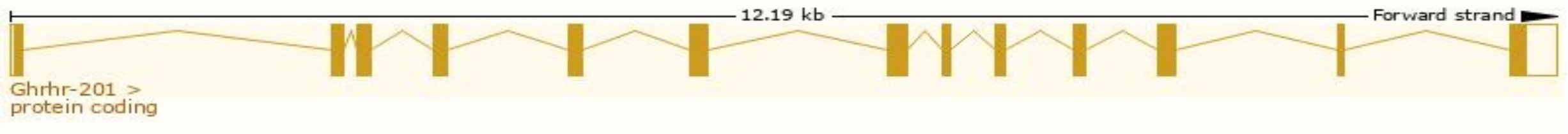
Official Symbol	Ghrhr provided by MGI
Official Full Name	growth hormone releasing hormone receptor provided by MGI
Primary source	MGI:MGI:95710
See related	Ensembl:ENSMUSG00000004654
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Ghrfr, lit, little
Expression	Low expression observed in reference dataset See more
Orthologs	human all

Transcript information（Ensembl）

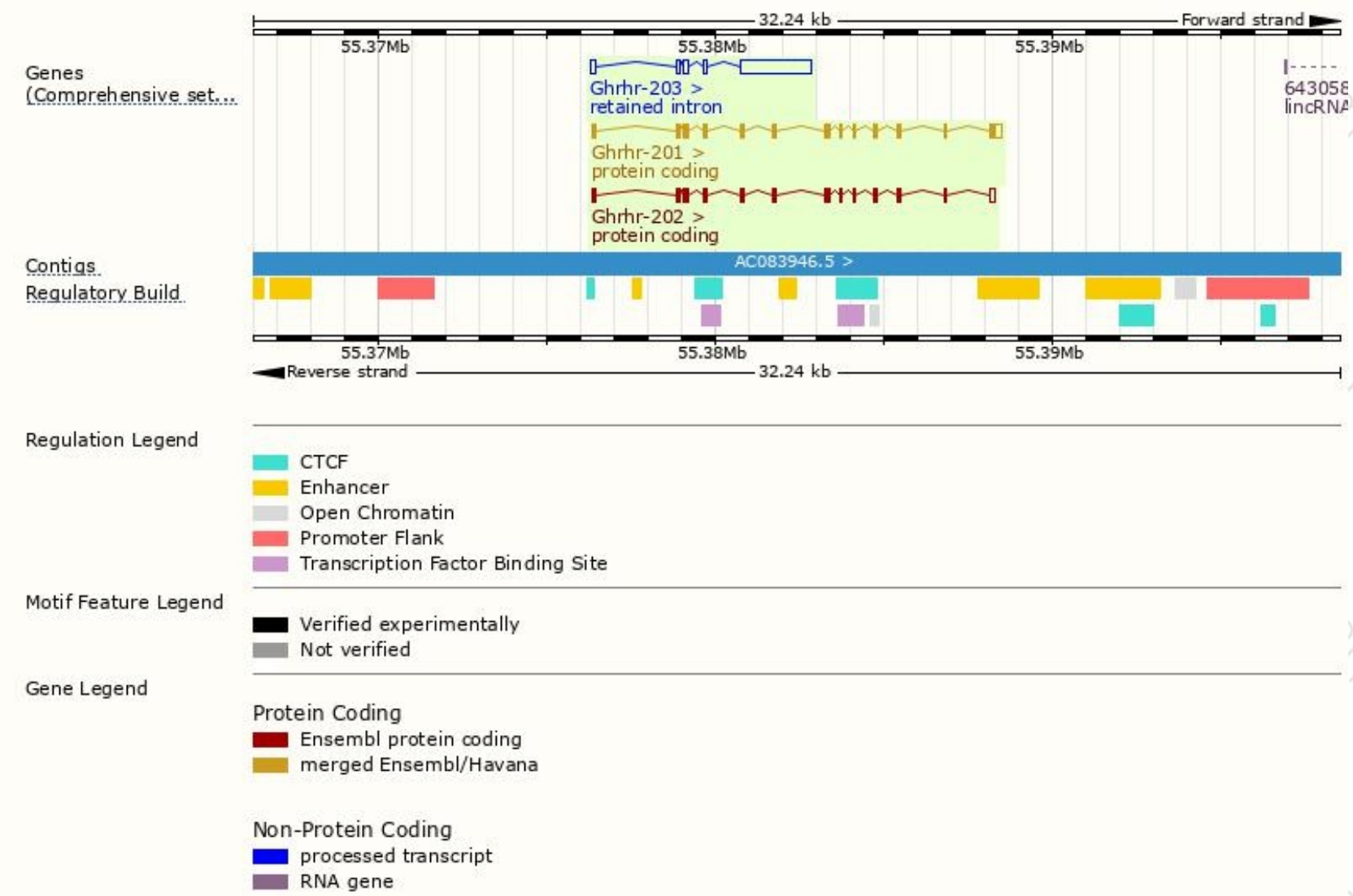
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ghrhr-201	ENSMUST00000063578.5	1550	423aa	Protein coding	CCDS20165	P32082	TSL:1 GENCODE basic APPRIS P1
Ghrhr-202	ENSMUST00000203241.1	1328	386aa	Protein coding	-	A0A0N4SVS4	TSL:1 GENCODE basic
Ghrhr-203	ENSMUST00000204988.1	2565	No protein	Retained intron	-	-	TSL:2

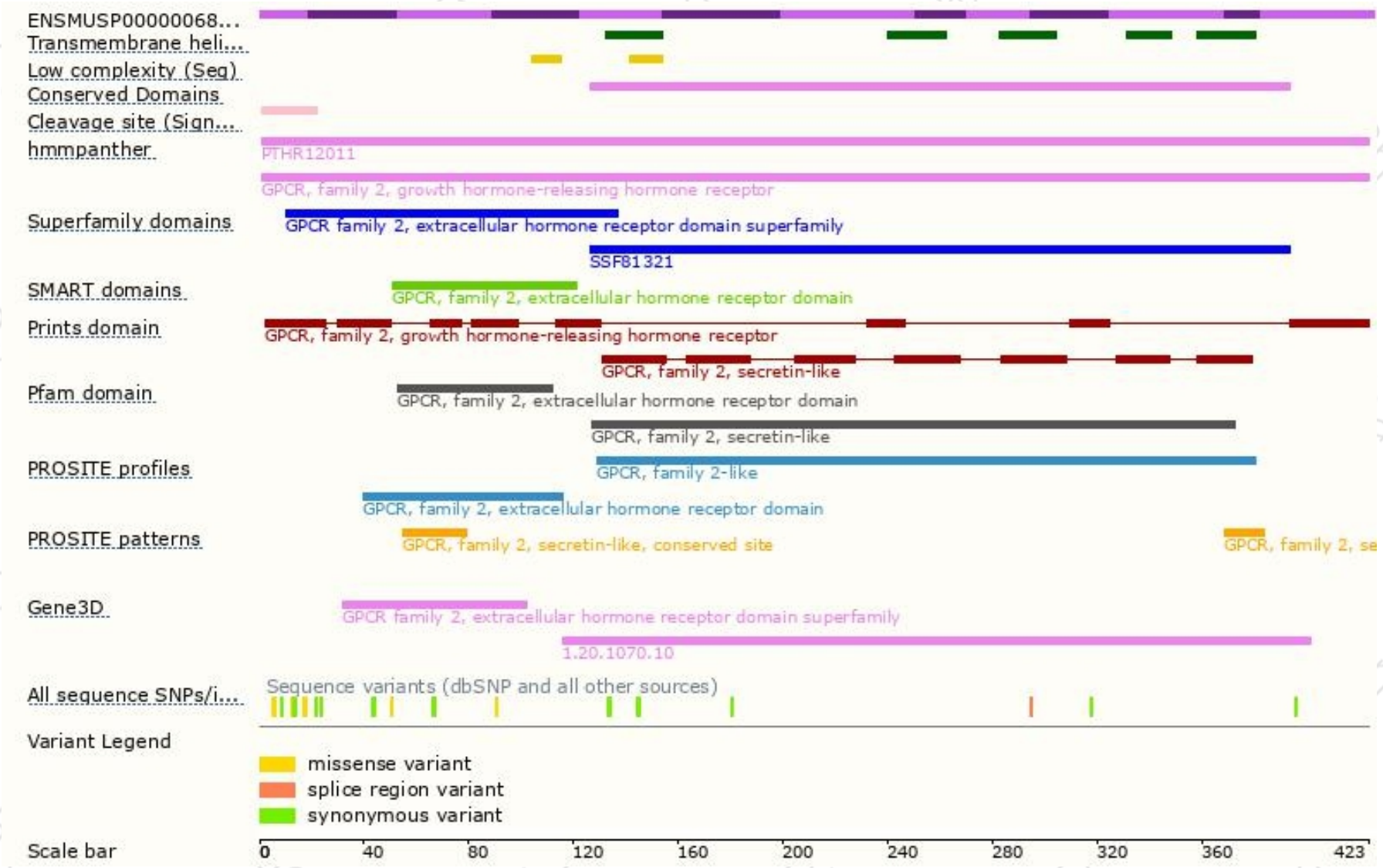
The strategy is based on the design of *Ghrhr-201* transcript,The transcription is shown below



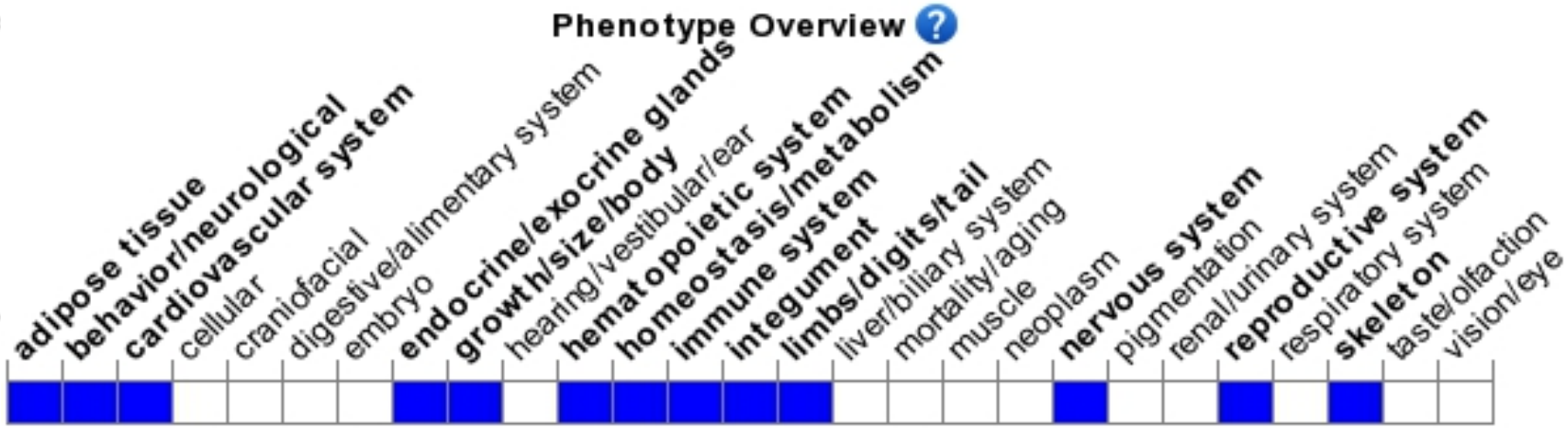
Genomic location distribution



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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