Lep Cas9-KO Strategy

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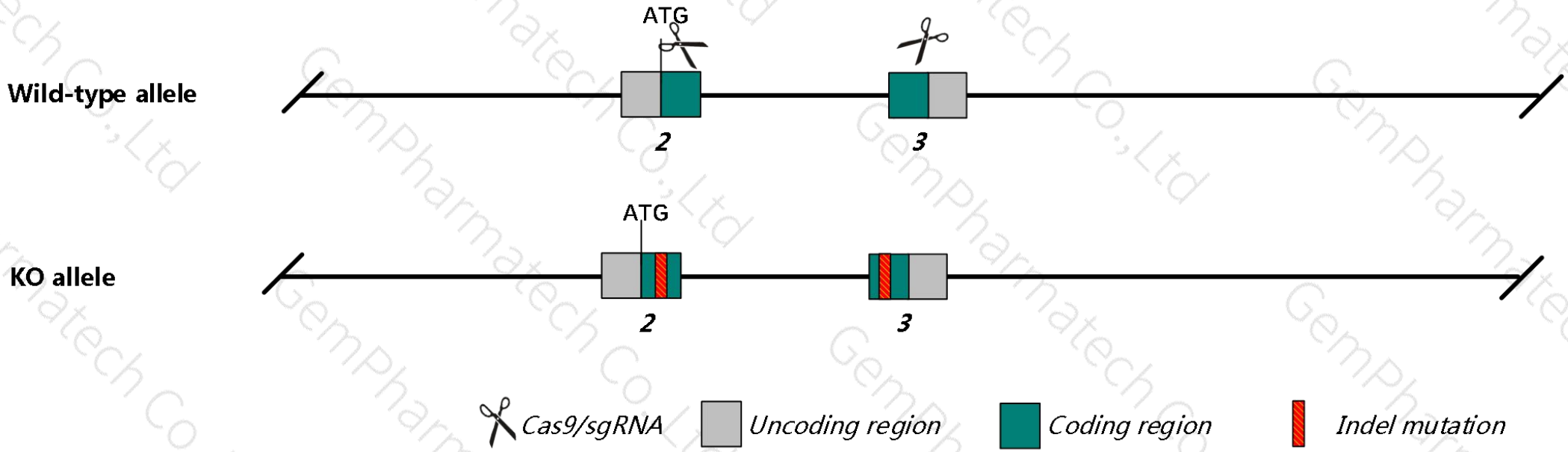
Project Overview

Project Name	***Lep***
Project type	**Cas9-KO**
Strain background	**C57BL/6J**

Knockout strategy

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



Technical routes

➢ The *Lep* gene has 2 transcripts. According to the structure of *Lep* gene, partial exon2 and exon3 of *Lep-201* (ENSMUST00000069789.11) transcript is recommended as the knockout region, result in indel mutation and disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Lep* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

Notice

- According to the existing MGI data,Homozygotes are obese, hyperphagic, have low activity, high metabolic efficiency, impaired thermogenesis, infertility and short lifespan in addition to varying other abnormalities. Strain background affects severity and course of diabetes. Heterozygotes survive fasting longer than control mice.

- The *Lep* 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 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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Lep leptin [Mus musculus (house mouse)]

Gene ID: 16846, updated on 2-Apr-2019

Summary

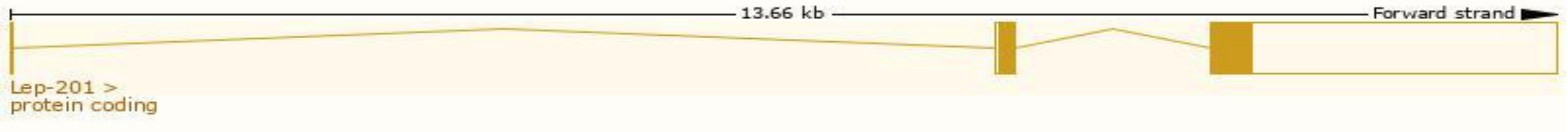
Official Symbol	Lep provided by MGI
Official Full Name	leptin provided by MGI
Primary source	MGI:MGI:104663
See related	Ensembl:ENSMUSG00000059201
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	ob, obese
Expression	Biased expression in subcutaneous fat pad adult (RPKM 267.7), genital fat pad adult (RPKM 183.5) and 1 other tissue See more
Orthologs	human all

Transcript information（Ensembl）

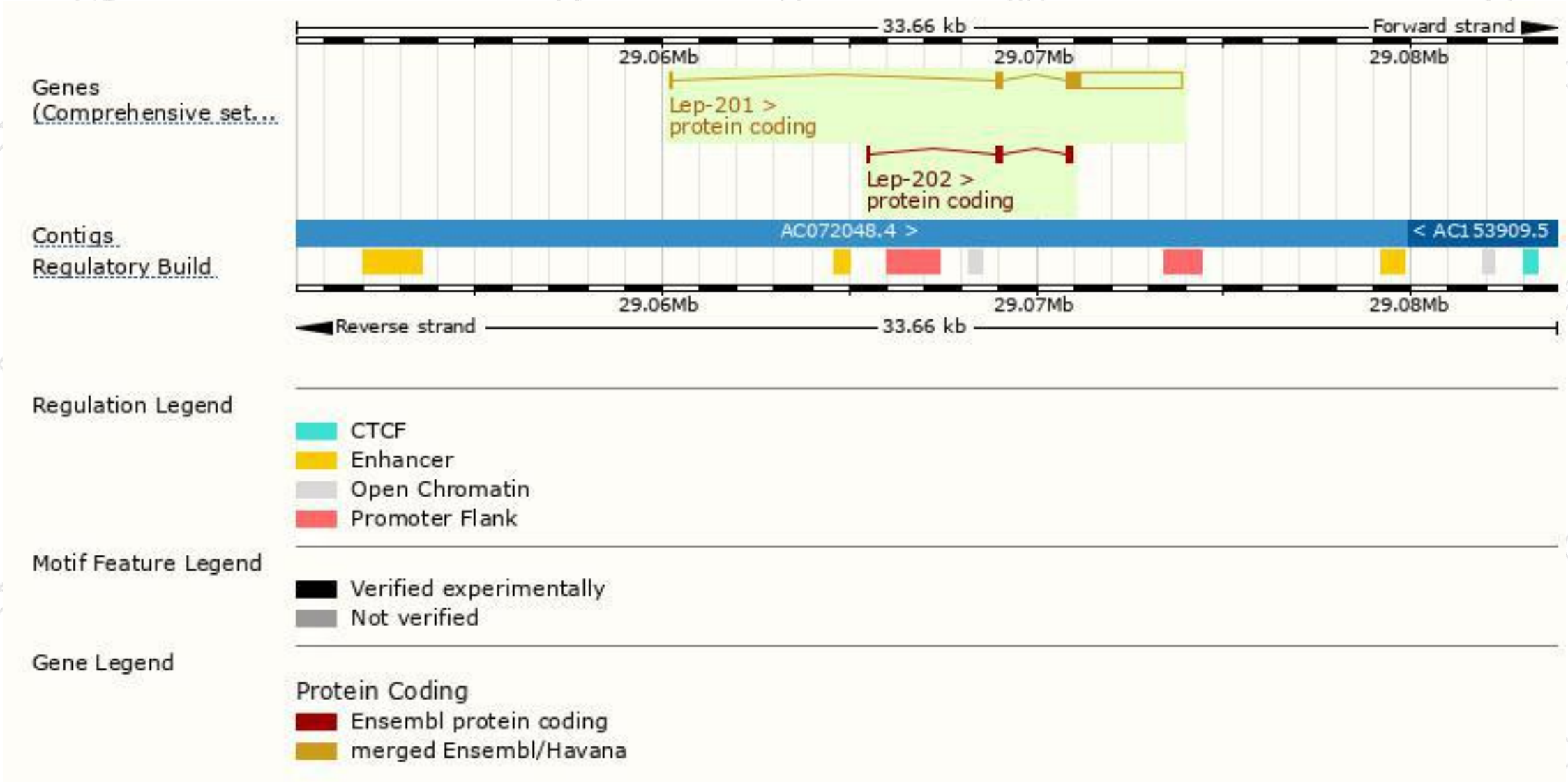
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lep-201	ENSMUST00000069789.11	3259	167aa	Protein coding	CCDS19955	P41160 Q544U0	TSL:1 GENCODE basic APPRIS P1
Lep-202	ENSMUST00000169505.1	420	98aa	Protein coding	-	E9Q7Z0	CDS 3' incomplete TSL:3

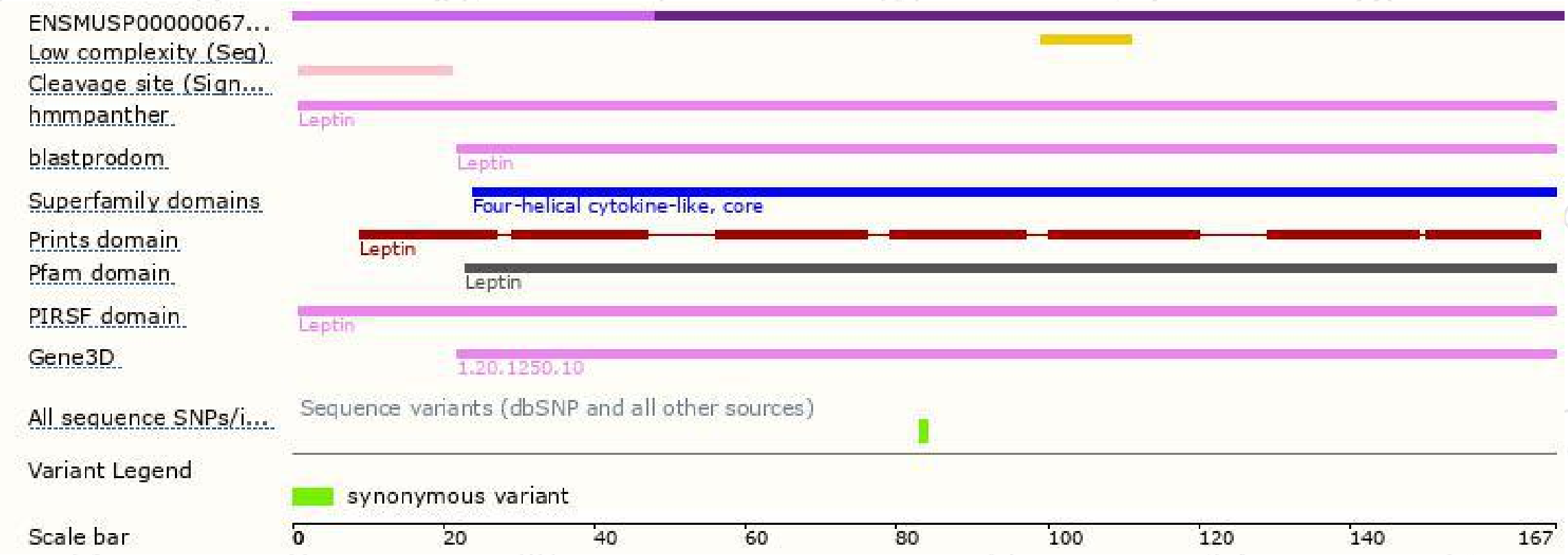
The strategy is based on the design of *Lep-201* transcript,The transcription is shown below



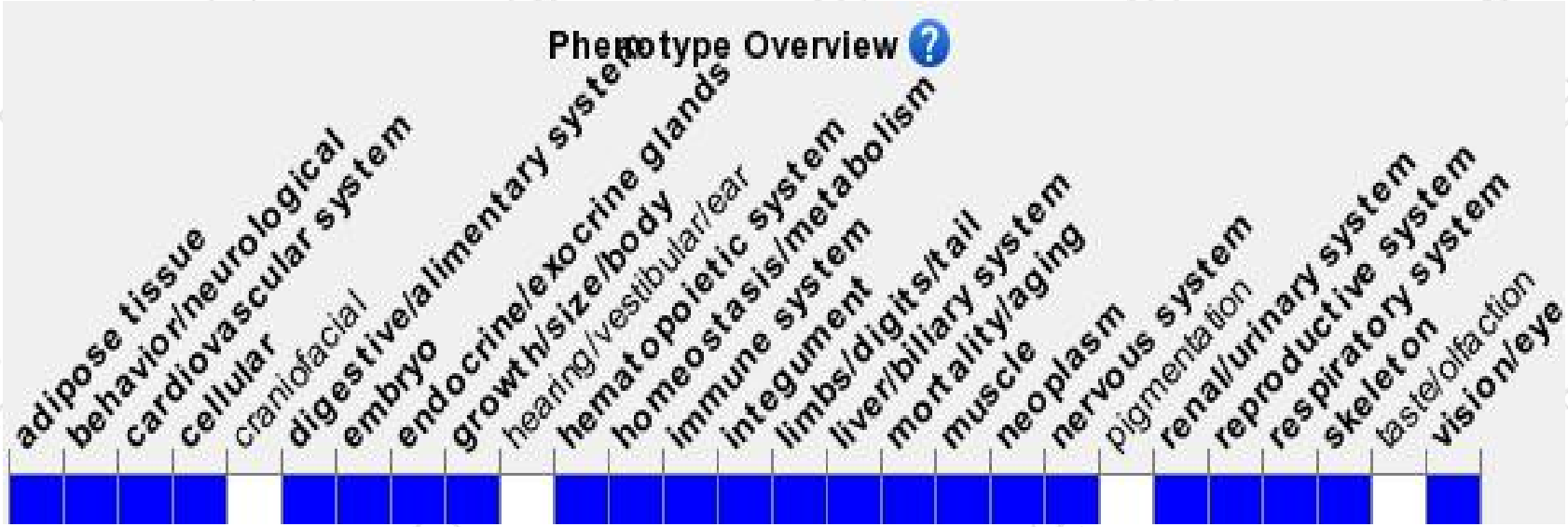
Genomic location distribution



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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