Lepr-p.Y985F&Y1077F&Y1138F cas9-ki Mouse Model Strategy

-CRISPR/Cas9 technology

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Reviewer: Yanhua Shen

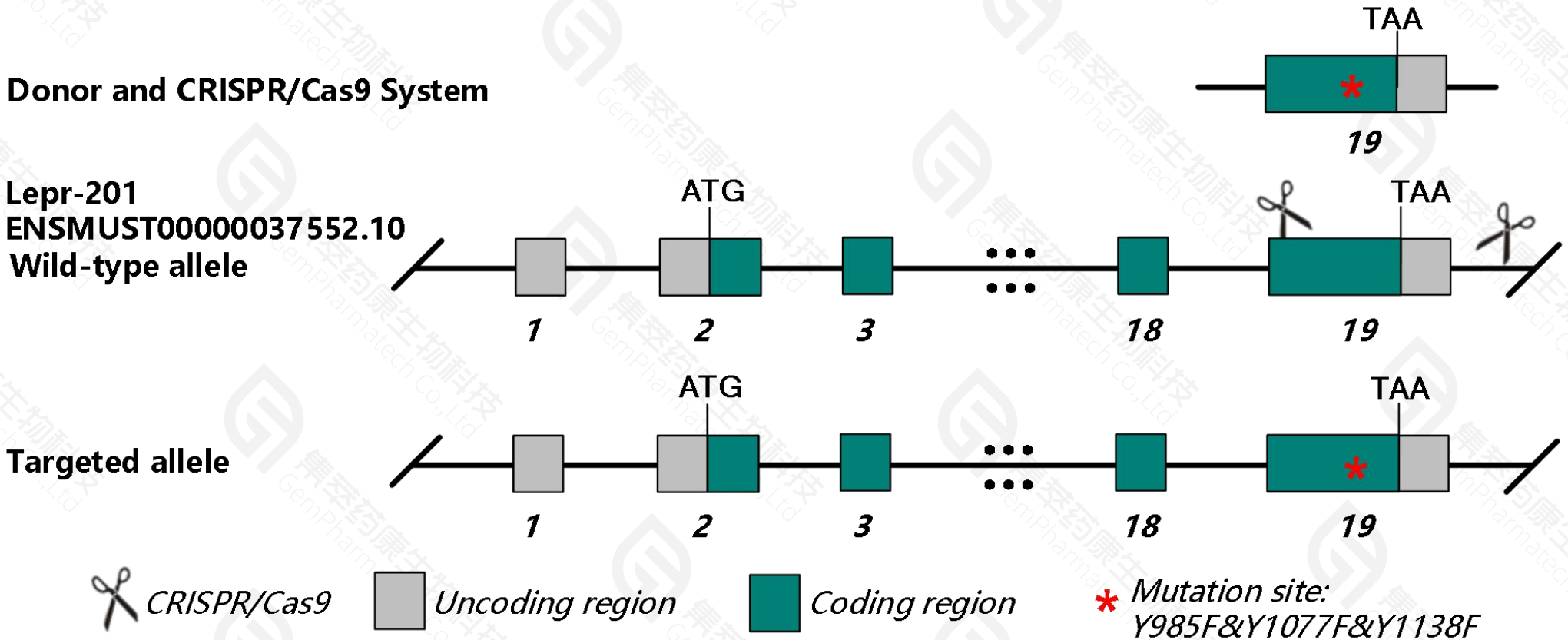
Design Date: 2021-9-8

Project Overview

Project Name	**Lepr-p.Y985F&Y1077F&Y1138F**
Project type	**cas9-ki**
Strain background	**C57BL/6JGpt**

Strategy

This model uses CRISPR/Cas9 technology to edit the *Lepr* gene and the schematic diagram is as follow:



Technical Description

- According to Ensembl data, the mouse *Lepr* gene has 7 transcripts.
- According to the structure of *Lepr* gene and requirements of customer, this project produced *Lepr*-p.Y985F&Y1077F&Y1138F point mutations on exon19 of the transcript of *Lepr*-201(ENSMUST00000037552.10, NM_146146.3→NP_666258.2). The 985th amino acids will be mutated from Y to F, and the corresponding codon will be mutated from TAT to TTC. The 1077th amino acids will be mutated from Y to F, and the corresponding codon will be mutated from TAT to TTC. The 1138th amino acids will be mutated from Y to F, and the corresponding codon will be mutated from TAC to TTC.
- The mouse *Lepr*-201 transcript contains 19 exons. The translation initiation site ATG is located at exon2, and the translation termination site TAA is located at exon19, encoding 1162aa.
- In this project, *Lepr* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: CRISPR/Cas9 system and donor were injected into the fertilized eggs of C57BL/6JGpt mice for homologous recombination, and obtained positive F0 mice identified by PCR and sequencing analysis. The stable inheritable positive F1 mice model was obtained by mating F0 mice with C57BL/6JGpt mice.

Notice

- According to the data of MGI, homozygous of this model is female and male infertility.
- The 3’ gRNA will be located on about 600bp downstream of *Lepr*-201.
- One or two synonymous mutations of amino acids may be intronduced on exon19 of *Lepr*-201.
- Two or four base mutations may be intronduced on downstream of *Lepr*-201.
- The effect of *Lepr*-202, *Lepr*-203 and *Lepr*-205 transcripts is unknown in this strategy. *Lepr*-204, *Lepr*-206 and *Lepr*-207 transcripts may not be affected.
- Mouse *Lepr* gene is located on Chr4. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr4, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Reference

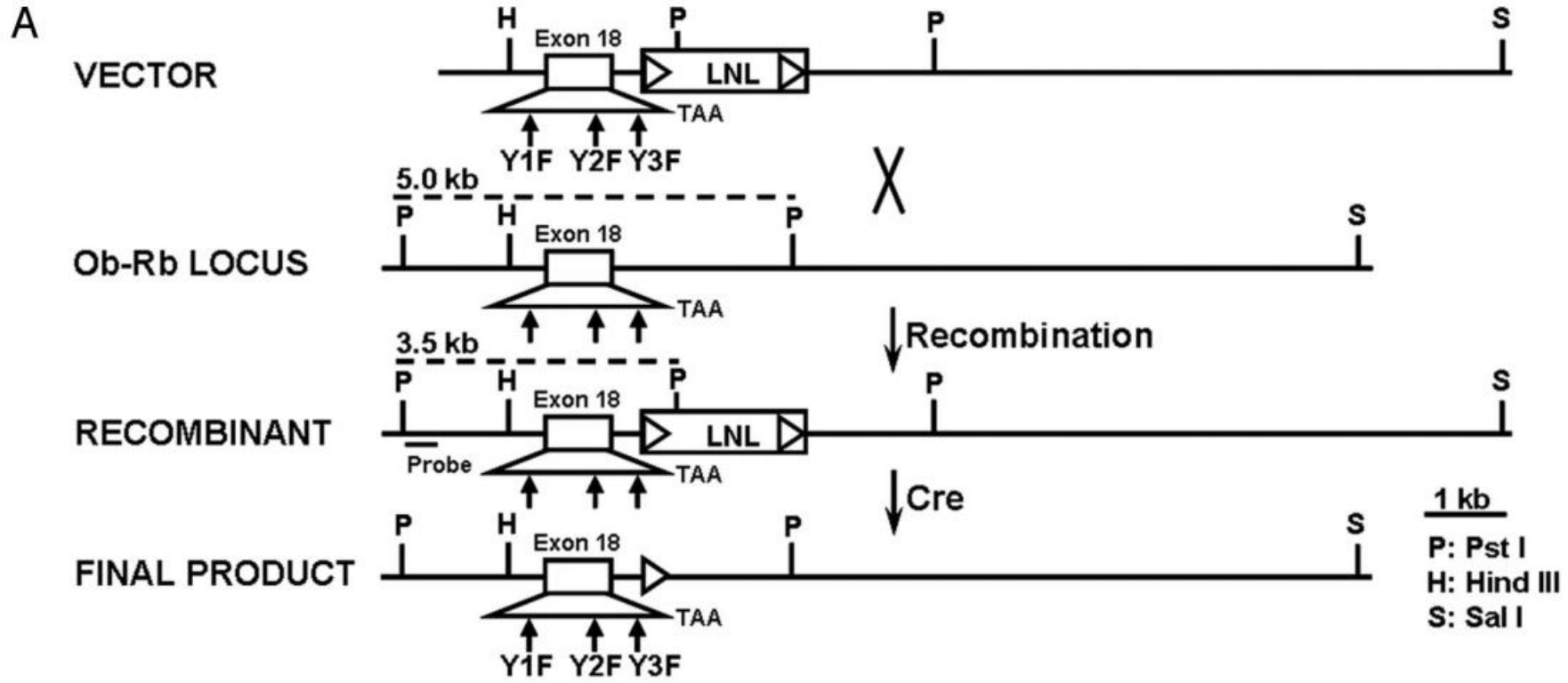


Fig. 1. Abrogation of hypothalamic STAT3 activation in Y3F and Y123F mice. (*A*) Schematic diagram showing the strategy of homologous gene targeting to replace exon 18 of the leptin receptor gene with the mutant exon 18 (Y123F) harboring phenylalanine (F) substitutions for all of the 3 tyrosine residues at positions 985, 1077, and 1138. The selection marker (LNL) cassette was subsequently removed by *cre* excision. The Y3F knockin mice were likewise generated. (*B*) Chimeric

Tyrosine-dependent and -independent actions of leptin receptor in control of energy balance and glucose homeostasis. Proc Natl Acad Sci U S A. 2008 Nov 25;105(47):18619-24.

Mutation Site

集萃药康 GemPharmatech

Before mutation

The red region represents the mutation site.

Mutation Site

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After mutation

The red region represents the mutation site.

Gene name and location(NCBI)

Lepr leptin receptor [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 16847, updated on 31-Aug-2021

Summary

Official Symbol	Lepr provided by MGI
Official Full Name	leptin receptor provided by MGI
Primary source	MGI:MGI:104993
See related	Ensembl:ENSMUSG00000057722
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	O; LE; db; OB-; Obr; obl; Modb; Leprb; Modb1; LEPROT; obese-; OB-RGRP; diabetes; obese-like
Expression	Broad expression in bladder adult (RPKM 5.0), placenta adult (RPKM 3.3) and 18 other tissues See more
Orthologs	human all

NEW **Try the new Gene table**

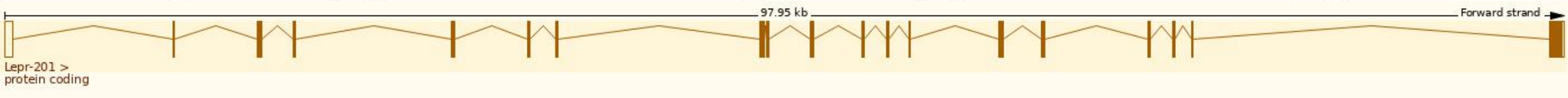
Try the new Transcript table

Transcript information (Ensembl)

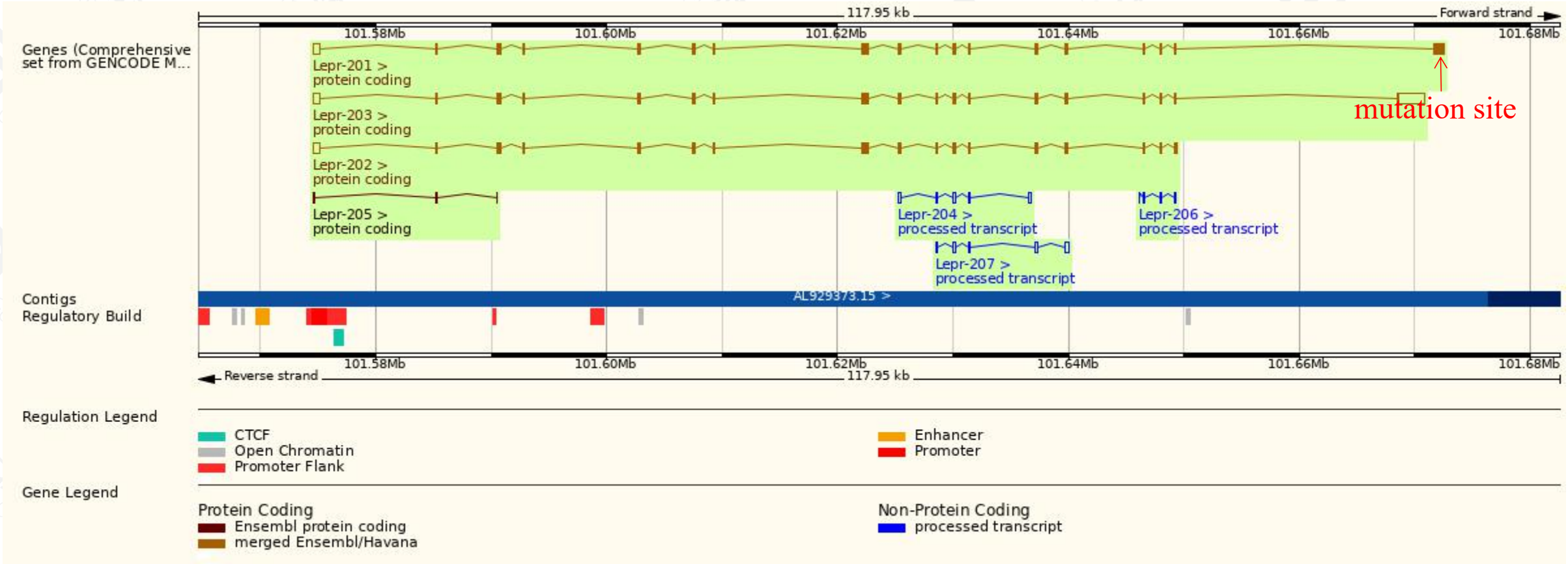
The gene has 7 transcripts, and all transcripts are shown below:

Name ▲	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Lepr-201	ENSMUST00000037552.10	4127	1162aa	Protein coding	CCDS51240	P48356-1	GENCODE basic APPRIS ALT2 TSL:1
Lepr-202	ENSMUST00000102777.10	3410	892aa	Protein coding	CCDS18397	P48356-3	GENCODE basic APPRIS P3 TSL:1
Lepr-203	ENSMUST00000106921.9	5542	894aa	Protein coding	CCDS51239	P48356-2	GENCODE basic APPRIS ALT2 TSL:1
Lepr-204	ENSMUST00000128948.8	787	No protein	Processed transcript	-	-	TSL:3
Lepr-205	ENSMUST00000145024.2	208	30aa	Protein coding	-	A2AV66	TSL:5 CDS 3' incomplete
Lepr-206	ENSMUST00000151733.2	415	No protein	Processed transcript	-	-	TSL:3
Lepr-207	ENSMUST00000156402.2	889	No protein	Processed transcript	-	-	TSL:2

The strategy is based on the *Lepr*-201 transcript, the transcription is shown below:

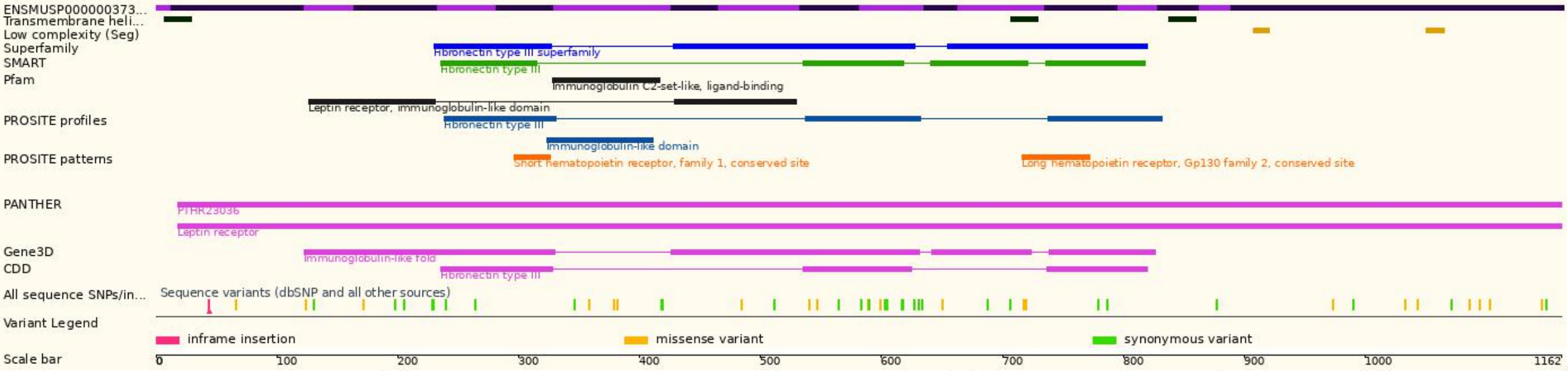


Genomic location distribution



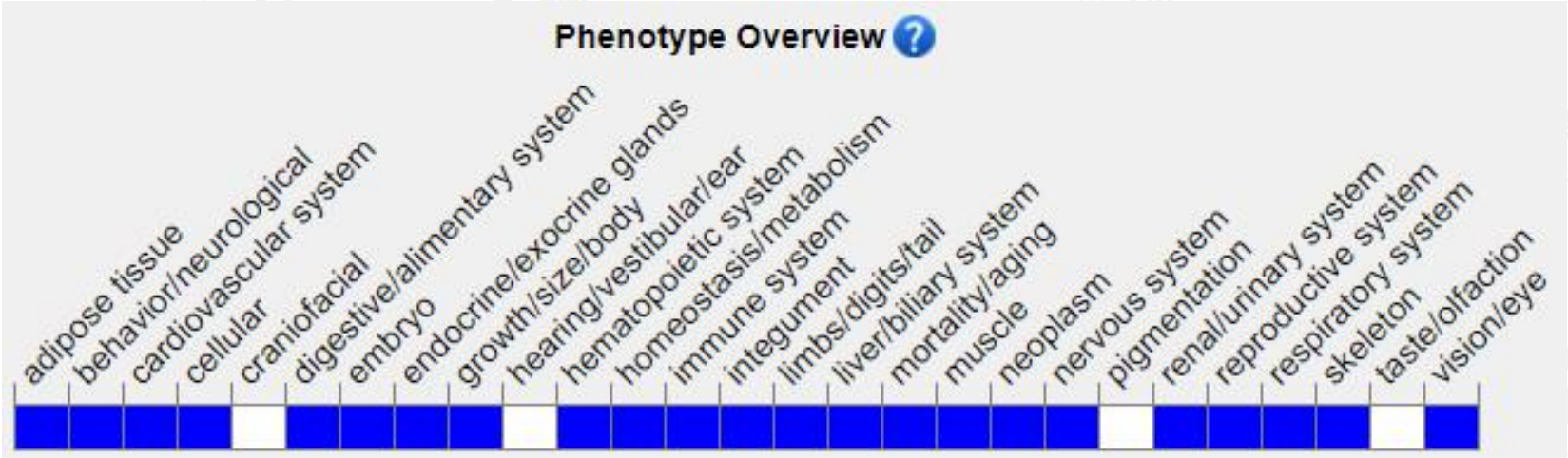
Protein domain

Protein domains for ENSMUSP00000037385.4



Mouse phenotype description(MGI)

URL link is as follows:
http://www.informatics.jax.org/marker/MGI:104993



Homozygous mutants are hyperphagic, low-activity, poorly cold-adapted, sterile and have enhanced fat conversion. They are obese, hyperinsulinemic and, on certain strains, severely hyperglycemic. Heterozygotes are normal but resistant to prolonged fasting.

If you have any questions, please feel free to contact us.
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