Pparg-Y95C Mouse Model Strategy

-CRISPR/Cas9 technology

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Project Overview

Project Name	**Pparg-Y95C**
Project type	**Cas9-ki(PM)**
Strain background	**C57BL/6JGpt**

Technical Description

- The mouse *Pparg* gene has 7 transcripts.
- According to the structure of *Pparg* gene and requirements of customer, this project produced *Pparg*-Y95C point mutation on exon2 of the transcript of *Pparg*-201(ENSMUST00000000450.4, NM_011146.3). The 95th amino acids will be mutated from Y to C, and the corresponding codon will be mutated to TGT by the TAT.
- The mouse *Pparg*-201 transcript contains 7 exons. The translation initiation site ATG is located at exon1, and the translation termination site TAG is located at exon7, encoding 505aa.
- In this project, *Pparg* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: In vitro, sgRNA and donor vectors were constructed. Cas9, sgRNA 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.

Mutation Site

Before mutation

```
      +3                                                                                                  I   T   M
98801 TAAACTCATT CAGCTCCTCT CTATGTCTAT GAGGACTGCT CTGCCCTCCA GAACTTGACT CATATCCTGT TCTTTTTTTT CCACTTCAGA AATTACCATG
      ATTTGAGTAA GTCGAGGAGA GATACAGATA CTCCTGACGA GACGGGAGGT CTTGAACTGA GTATAGGACA AGAAAAAAAA GGTGAAGTCT TTAATGGTAC

      +3  V   D   T   E   M   P   F   W   P   T   N   F   G   I   S   S   V   D   L   S   V   M   E   D   H   S   H   S   F   D   I   K   P   F?
98901 GTTGACACAG AGATGCCATT CTGGCCCACC AACTTCGGAA TCAGCTCTGT GGACCTCTCC GTGATGGAAG ACCACTCGCA TTCCTTTGAC ATCAAGCCCT
      CAACTGTGTC TCTACGGTAA GACCGGGTGG TTGAAGCCTT AGTCGAGACA CCTGGAGAGG CACTACCTTC TGGTGAGCGT AAGGAAACTG TAGTTCGGGA

      +3  ?F  T   T   V   D   F   S   S   I   S   A   P   H   Y   E   D   I   P   F   T   R   A   D   P   M   V   A   D   Y   K   Y   D   L   K?
99001 TTACCACAGT TGATTTCTCC AGCATTTCTG CTCCACACTA TGAAGACATT CCATTCACAA GAGCTGACCC AATGGTTGCT GATTACAAAT ATGACCTGAA
      AATGGTGTCA ACTAAAGAGG TCGTAAAGAC GAGGTGTGAT ACTTCTGTAA GGTAAGTGTT CTCGACTGGG TTACCAACGA CTAATGTTTA TACTGGACTT

      +3  ?K  L   Q   E   Y   Q
99101 GCTCCAAGAA TACCAAAGTA TGTTTACTTT TCAAACTACT AGGATTGGAG TTAGACAGTT TTTTAATAAC CATTGAATAA ATGCTCCTGA GATTAGCACT
      CGAGGTTCTT ATGGTTTCAT ACAAATGAAA AGTTTGATGA TCCTAACCTC AATCTGTCAA AAAATTATTG GTAACTTATT TACGAGGACT CTAATCGTGA
```

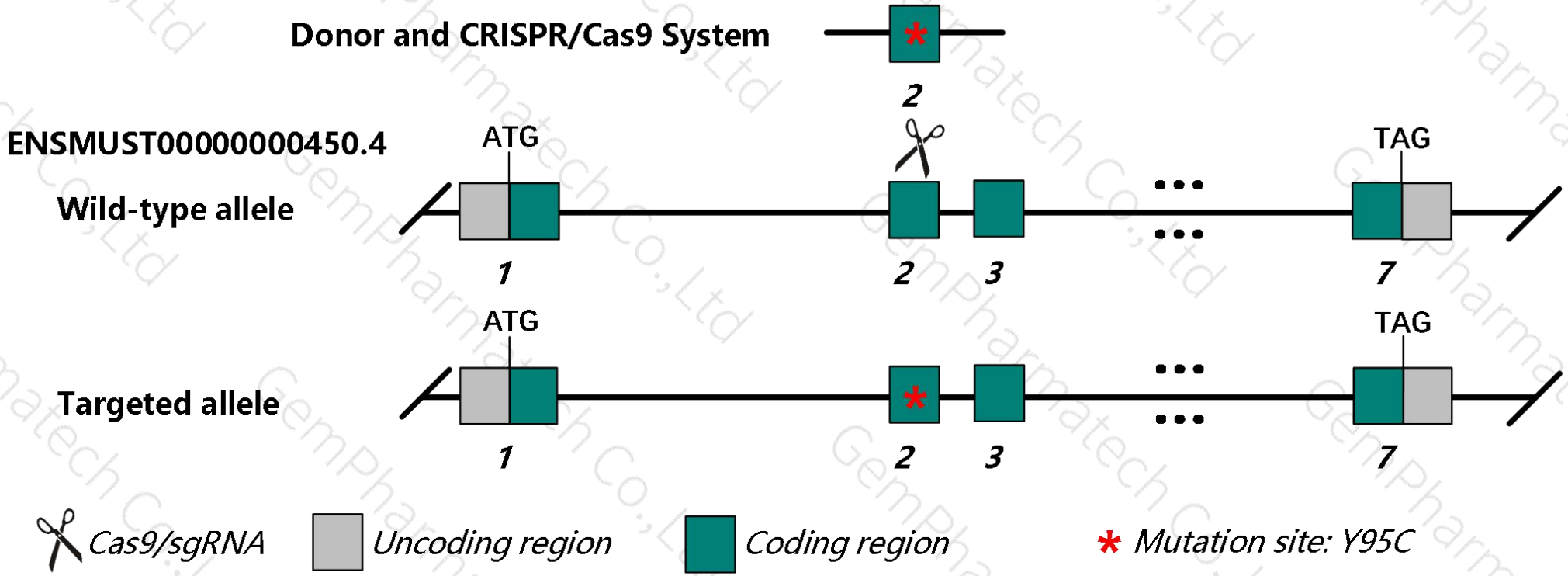
After mutation

```
      +3                                                                                                  I   T   M
98801 TAAACTCATT CAGCTCCTCT CTATGTCTAT GAGGACTGCT CTGCCCTCCA GAACTTGACT CATATCCTGT TCTTTTTTTT CCACTTCAGA AATTACCATG
      ATTTGAGTAA GTCGAGGAGA GATACAGATA CTCCTGACGA GACGGGAGGT CTTGAACTGA GTATAGGACA AGAAAAAAAA GGTGAAGTCT TTAATGGTAC

      +3  V   D   T   E   M   P   F   W   P   T   N   F   G   I   S   S   V   D   L   S   V   M   E   D   H   S   H   S   F   D   I   K   P   F?
98901 GTTGACACAG AGATGCCATT CTGGCCCACC AACTTCGGAA TCAGCTCTGT GGACCTCTCC GTGATGGAAG ACCACTCGCA TTCCTTTGAC ATCAAGCCCT
      CAACTGTGTC TCTACGGTAA GACCGGGTGG TTGAAGCCTT AGTCGAGACA CCTGGAGAGG CACTACCTTC TGGTGAGCGT AAGGAAACTG TAGTTCGGGA

      +3  ?F  T   T   V   D   F   S   S   I   S   A   P   H   Y   E   D   I   P   F   T   R   A   D   P   M   V   A   D   Y   K   C   D   L   K?
99001 TTACCACAGT TGATTTCTCC AGCATTTCTG CTCCACACTA TGAAGACATT CCATTCACAA GAGCTGACCC AATGGTTGCT GATTACAAAT GTGACCTGAA
      AATGGTGTCA ACTAAAGAGG TCGTAAAGAC GAGGTGTGAT ACTTCTGTAA GGTAAGTGTT CTCGACTGGG TTACCAACGA CTAATGTTTA CACTGGACTT

      +3  ?K  L   Q   E   Y   Q
99101 GCTCCAAGAA TACCAAAGTA TGTTTACTTT TCAAACTACT AGGATTGGAG TTAGACAGTT TTTTAATAAC CATTGAATAA ATGCTCCTGA GATTAGCACT
      CGAGGTTCTT ATGGTTTCAT ACAAATGAAA AGTTTGATGA TCCTAACCTC AATCTGTCAA AAAATTATTG GTAACTTATT TACGAGGACT CTAATCGTGA
```

The blue region is exon2 of *Pparg-201,* the yellow region represents the mutation site.

Strategy

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



Notice

- According to the data of MGI, homozygotes for targeted null mutations exhibit lethality due to placental defects. Heterozygotes show greater B cell proliferation, enhanced leptin secretion, and resistance to diet-induced adipocyte hypertrophy and insulin resistance.
- One or Two synonymous mutations of amino acids will be intronduced on exon2 of *Pparg*.
- Transcript *Pparg*-203 may be unaffected.
- There may be mutations of base in this animal model making process because of the repetition structure(polyAC) downstream of the mutation site.
- Mouse *Pparg* gene is located on Chr6. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr6, 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.

Gene name and location (NCBI)

集萃药康 GemPharmatech

Pparg peroxisome proliferator activated receptor gamma [*Mus musculus* (house mouse)]

Gene ID: 19016, updated on 17-Dec-2020

Summary

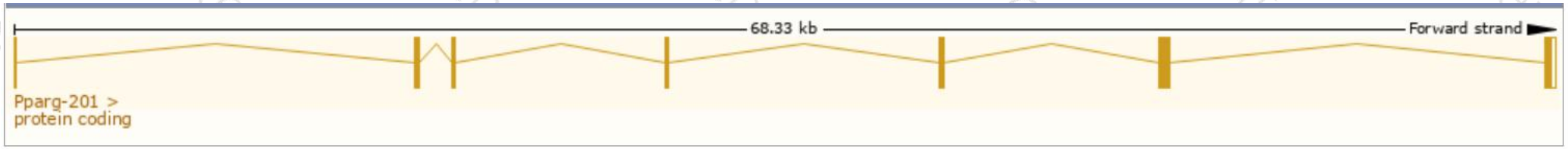
Official Symbol	Pparg provided by MGI
Official Full Name	peroxisome proliferator activated receptor gamma provided by MGI
Primary source	MGI:MGI:97747
See related	Ensembl:ENSMUSG00000000440
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Nr1; PPA; PPAR; Nr1c3; Ppar-; PPARgamma; PPAR-gamma; PPARgamma2; PPAR-gamma2
Summary	This gene encodes a nuclear receptor protein belonging to the peroxisome proliferator-activated receptor (Ppar) family. The encoded protein is a ligand-activated transcription factor that is involved in the regulation of adipocyte differentiation and glucose homeostasis. The encoded protein forms a heterodimer with retinoid X receptors and binds to DNA motifs termed "peroxisome proliferator response elements" to either activate or inhibit gene expression. Mice lacking the encoded protein die at an embryonic stage due to severe defects in placental vascularization. When the embryos lacking this gene are supplemented with healthy placentas, the mutants survive to term, but succumb to lipodystrophy and multiple hemorrhages. Alternative splicing results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Apr 2015]
Expression	Biased expression in subcutaneous fat pad adult (RPKM 32.5), mammary gland adult (RPKM 26.1) and 13 other tissues See more
Orthologs	human all

Transcript information （Ensembl）

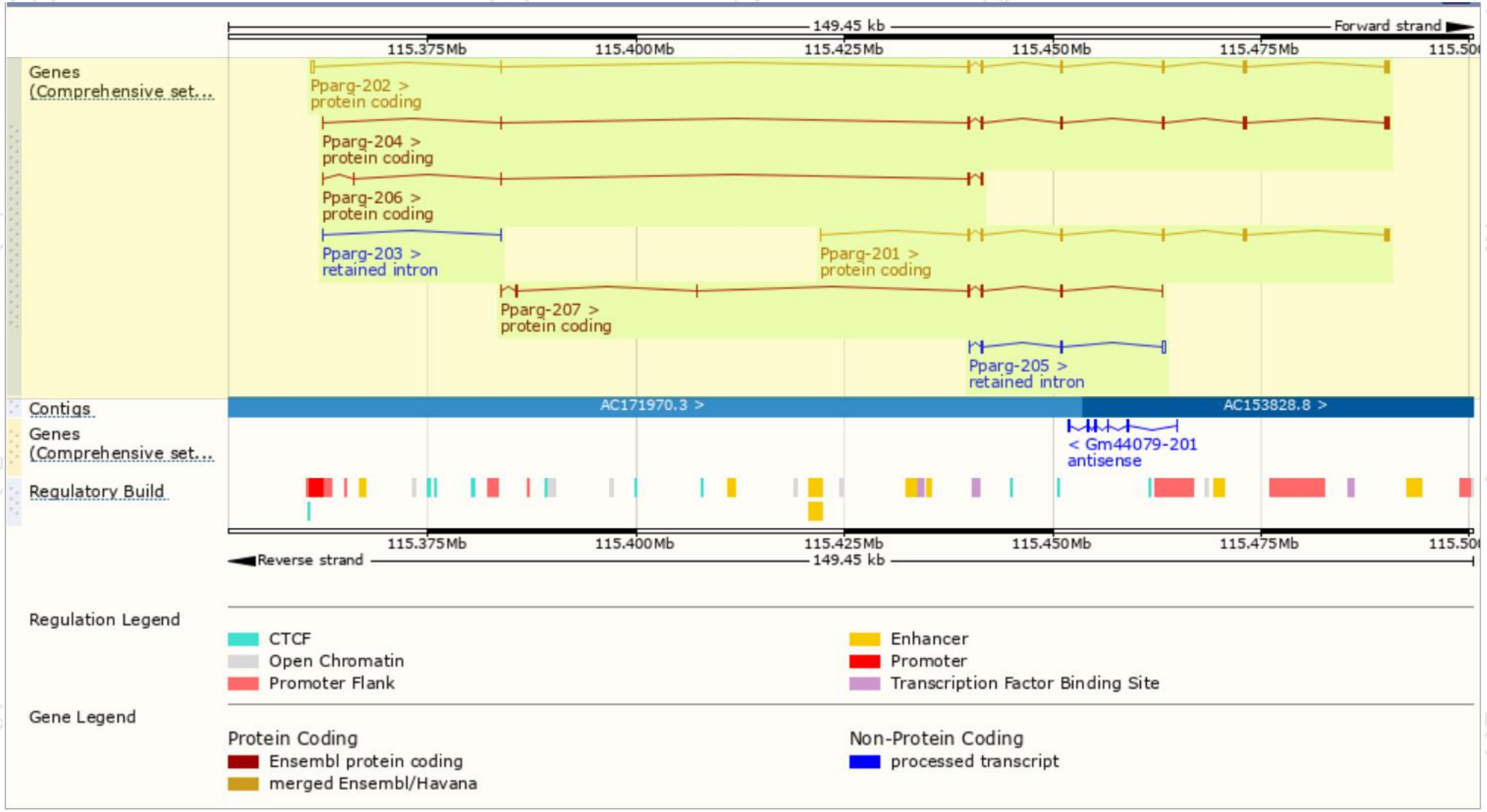
The gene has 7 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Pparg-202	ENSMUST00000171644.7	2125	475aa	Protein coding	CCDS51876	M1VPI1	TSL:1 GENCODE basic APPRIS ALT1
Pparg-204	ENSMUST00000203732.2	1826	475aa	Protein coding	CCDS51876	M1VPI1	TSL:1 GENCODE basic APPRIS ALT1
Pparg-201	ENSMUST00000000450.4	1767	505aa	Protein coding	CCDS20439	Q6GU14	TSL:1 GENCODE basic APPRIS P3
Pparg-207	ENSMUST00000205213.2	835	222aa	Protein coding	-	A0A0N4SV67	CDS 3' incomplete TSL:3
Pparg-206	ENSMUST00000204305.2	497	96aa	Protein coding	-	A0A0N4SVF8	CDS 3' incomplete TSL:3
Pparg-205	ENSMUST00000203896.1	915	No protein	Retained intron	-	-	TSL:2
Pparg-203	ENSMUST00000203308.1	213	No protein	Retained intron	-	-	TSL:5

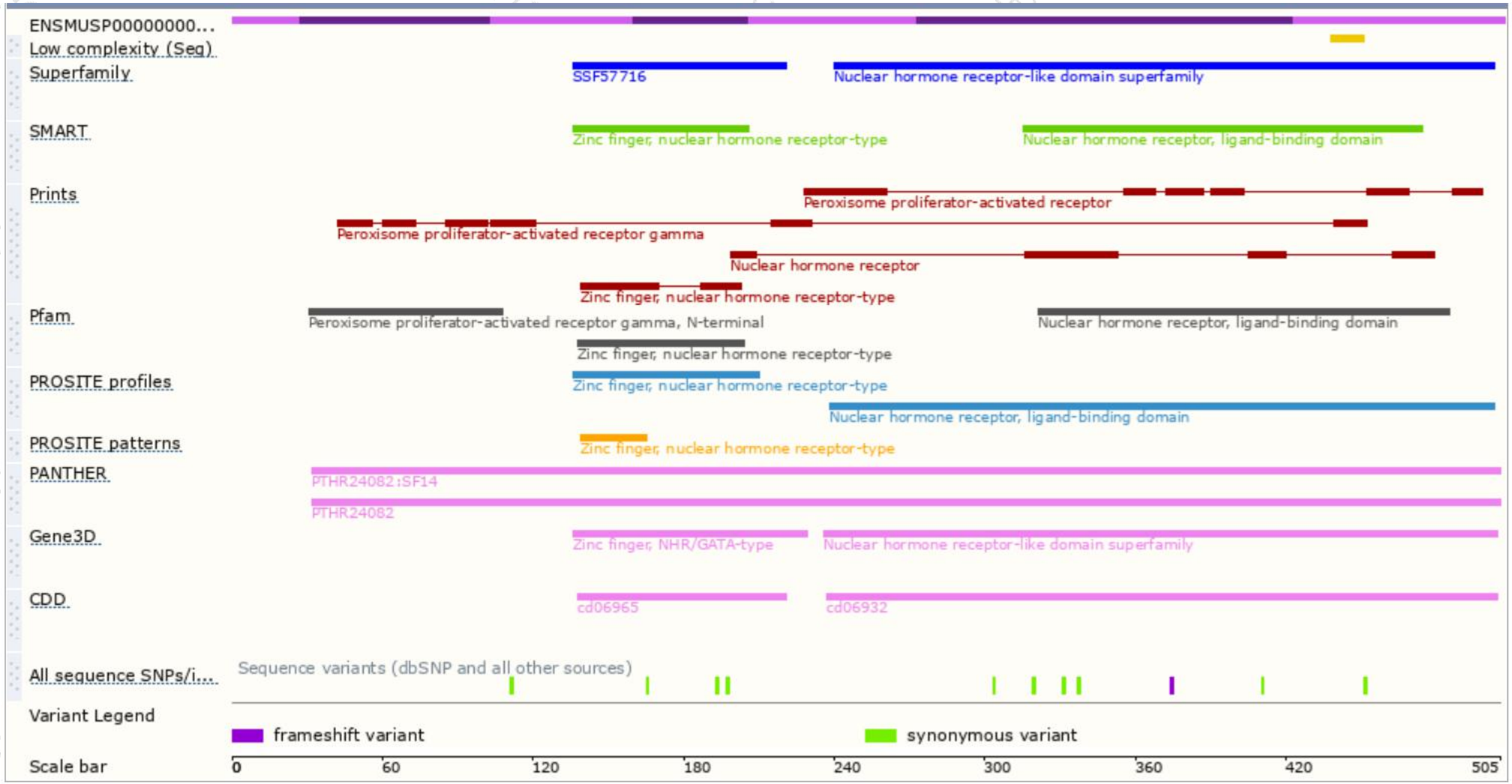
The strategy is based on the design of *Pparg*-201 transcript, the transcription is shown below:



Genomic location distribution

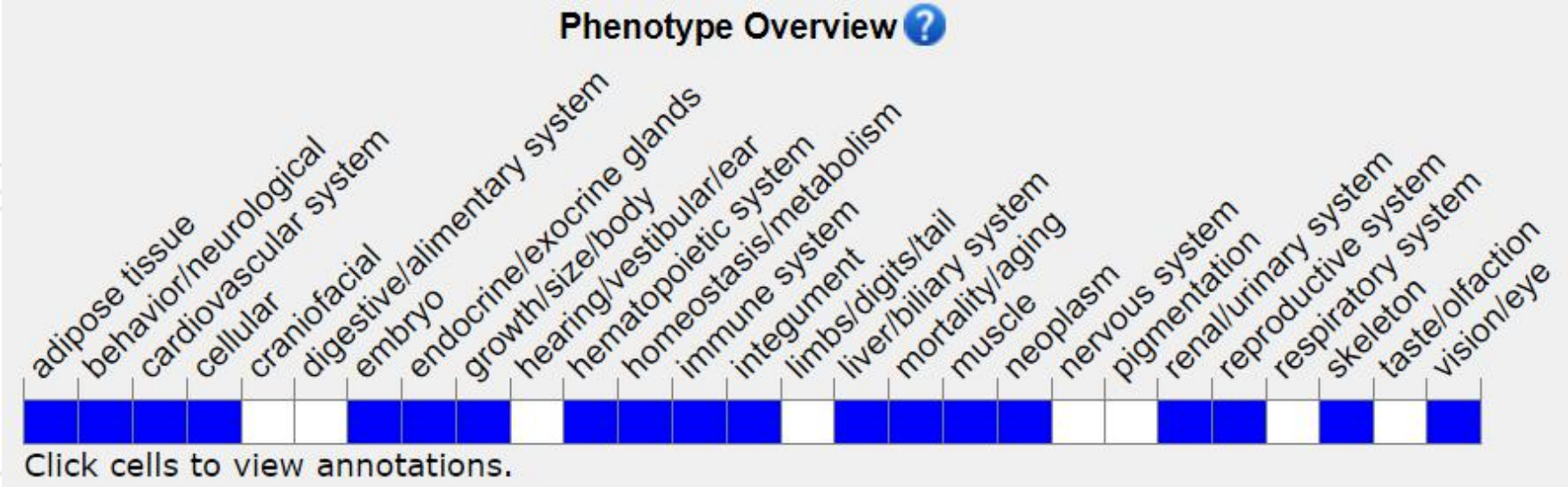


Protein domain



Mouse phenotype description(MGI)

URL link is as follows:
http://www.informatics.jax.org/marker/MGI:97747



Homozygotes for targeted null mutations exhibit lethality due to placental defects. Heterozygotes show greater B cell proliferation, enhanced leptin secretion, and resistance to diet-induced adipocyte hypertrophy and insulin resistance.

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