

# *Npepps* Cas9-CKO Strategy

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# Project Overview

**Project Name**

*Npepps*

**Project type**

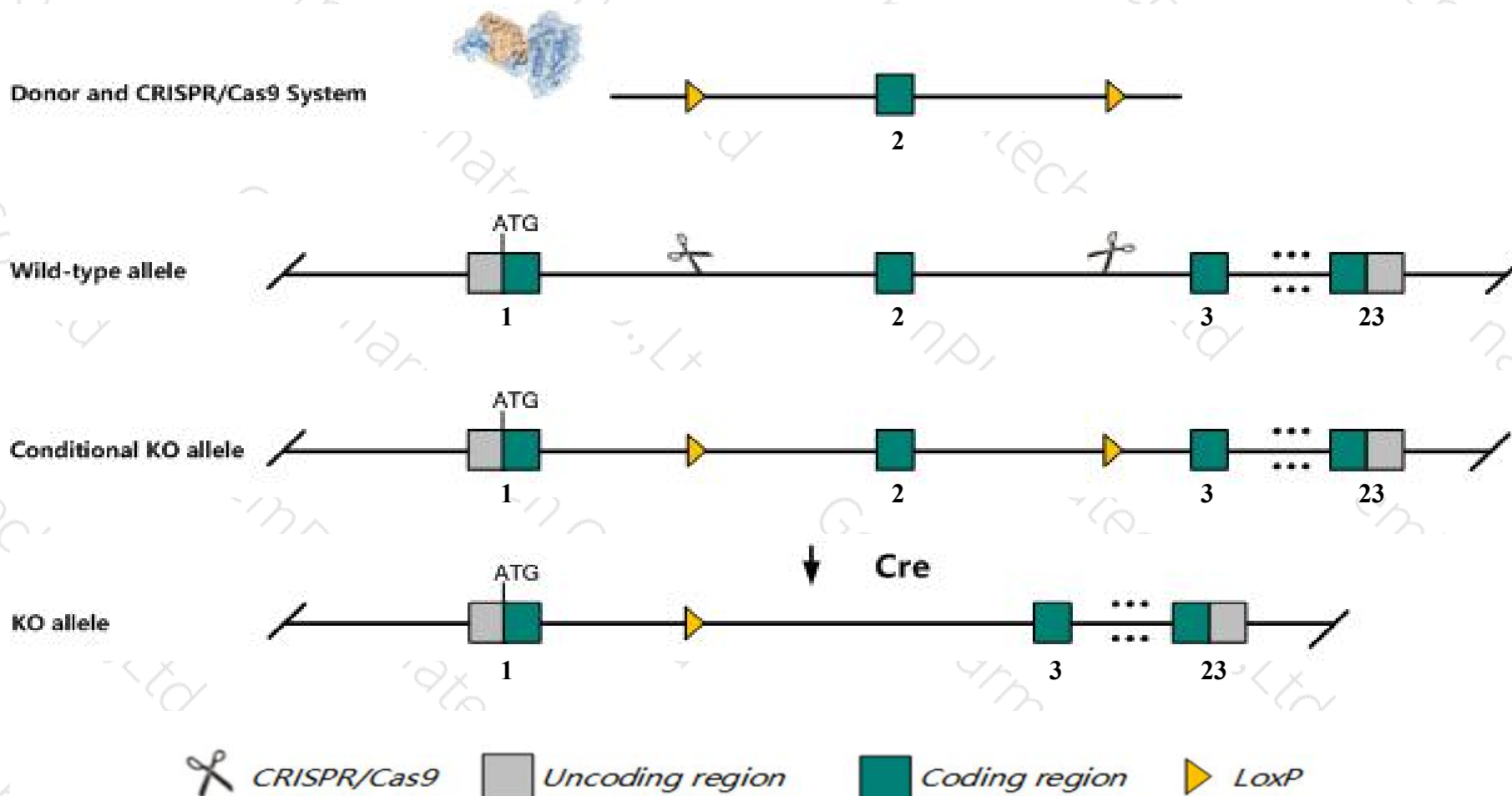
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

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



- The *Npepps* gene has 19 transcripts. According to the structure of *Npepps* gene, exon2 of *Npepps-201* (ENSMUST00000001480.13) transcript is recommended as the knockout region. The region contains 85bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Npepps* 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.

- According to the existing MGI data, Homozygotes for a gene-trapped mutation exhibit dwarfism, increased anxiety, decreased pain sensitivity, and infertility in both sexes. Females fail to produce the corpus luteum of pregnancy, while males fail to copulate and have impaired spermatogenesis.
- The *Npepps* gene is located on the Chr11. 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)

## Npepps aminopeptidase puromycin sensitive [Mus musculus (house mouse)]

Gene ID: 19155, updated on 7-Apr-2019

### Summary



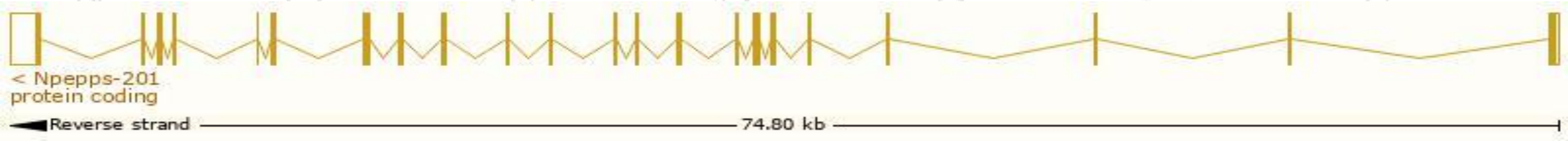
|                           |   |
|---------------------------|---|
| <b>Official Symbol</b>    | Npepps provided by <a href="#">MGI</a>  |
| <b>Official Full Name</b> | aminopeptidase puromycin sensitive provided by <a href="#">MGI</a>  |
| <b>Primary source</b>     | <a href="#">MGI:MGI:1101358</a>   |
| <b>See related</b>        | <a href="#">Ensembl:ENSMUSG00000001441</a>  |
| <b>Gene type</b>          | protein coding  |
| <b>RefSeq status</b>      | VALIDATED   |
| <b>Organism</b>           | <a href="#">Mus musculus</a>  |
| <b>Lineage</b>            | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| <b>Also known as</b>      | AAP-S, MP100, Psa, R74825, goku   |
| <b>Expression</b>         | Ubiquitous expression in CNS E18 (RPKM 20.8), CNS E14 (RPKM 19.0) and 28 other tissues <a href="#">See more</a>   |
| <b>Orthologs</b>          | <a href="#">human</a> <a href="#">all</a>   |

# Transcript information (Ensembl)

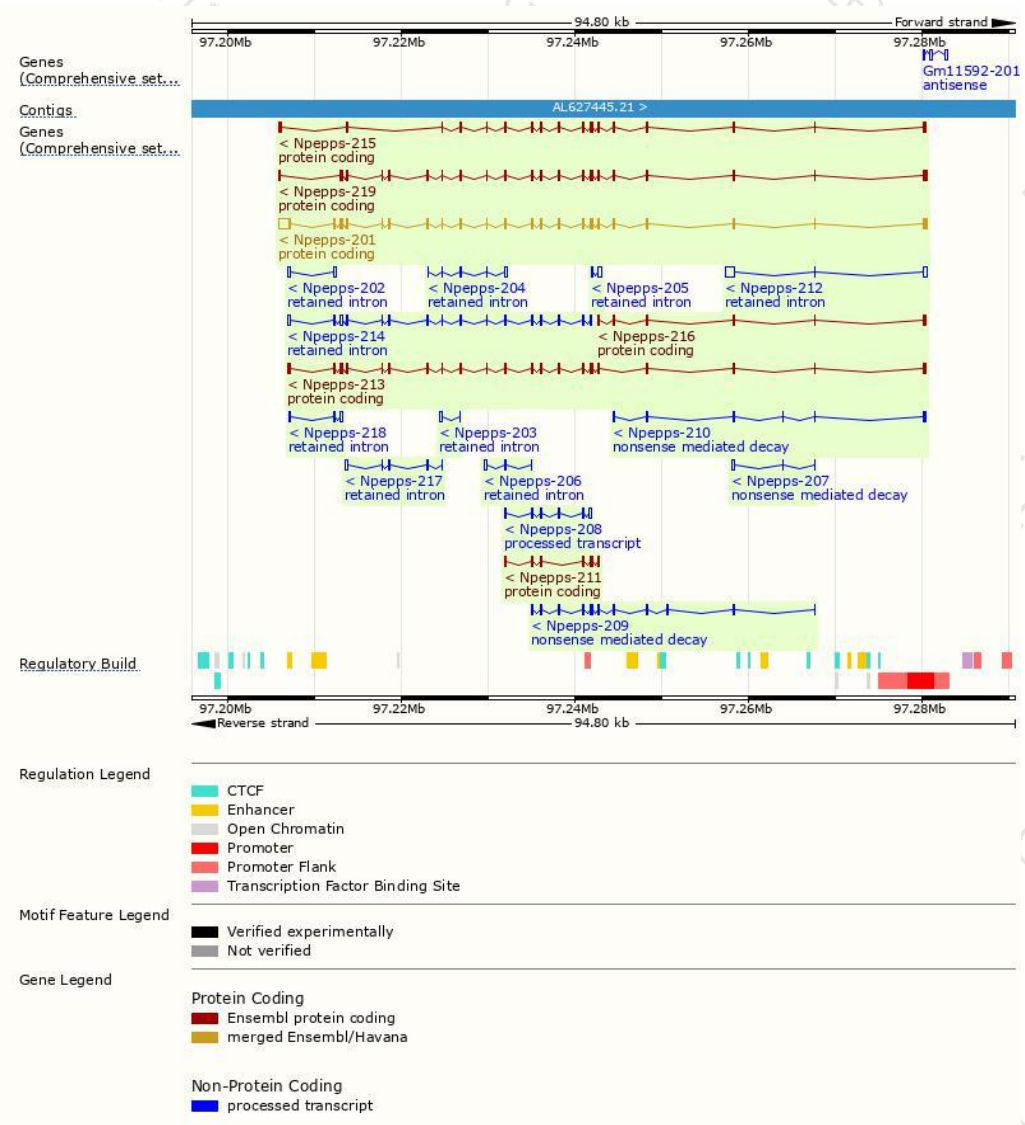
The gene has 19 transcripts,all transcripts are shown below:

| Name       | Transcript ID                        | bp   | Protein               | Biotype                 | CCDS                      | UniProt                | Flags   |
|------------|--------------------------------------|------|-----------------------|-------------------------|---------------------------|------------------------|---|
| Npepps-201 | <a href="#">ENSMUST0000001480.13</a> | 4215 | <a href="#">920aa</a> | Protein coding          | <a href="#">CCDS25317</a> | <a href="#">Q11011</a> | TSL:1 GENCODE basic APPRIS P2   |
| Npepps-219 | <a href="#">ENSMUST00000172108.7</a> | 2924 | <a href="#">889aa</a> | Protein coding          | -                         | <a href="#">E9Q039</a> | TSL:5 GENCODE basic APPRIS ALT2   |
| Npepps-213 | <a href="#">ENSMUST00000165216.7</a> | 2812 | <a href="#">876aa</a> | Protein coding          | -                         | <a href="#">F6QYF8</a> | CDS 5' incomplete TSL:5   |
| Npepps-215 | <a href="#">ENSMUST00000167806.7</a> | 2307 | <a href="#">674aa</a> | Protein coding          | -                         | <a href="#">E9Q6F4</a> | TSL:5 GENCODE basic   |
| Npepps-216 | <a href="#">ENSMUST00000168743.7</a> | 741  | <a href="#">247aa</a> | Protein coding          | -                         | <a href="#">F6V7K3</a> | 5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3 |
| Npepps-211 | <a href="#">ENSMUST00000163164.1</a> | 654  | <a href="#">218aa</a> | Protein coding          | -                         | <a href="#">F7ANF4</a> | 5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3 |
| Npepps-209 | <a href="#">ENSMUST00000154372.8</a> | 1239 | <a href="#">81aa</a>  | Nonsense mediated decay | -                         | <a href="#">F6RAU6</a> | CDS 5' incomplete TSL:5   |
| Npepps-210 | <a href="#">ENSMUST00000154917.7</a> | 794  | <a href="#">119aa</a> | Nonsense mediated decay | -                         | <a href="#">F2Z3V5</a> | TSL:3   |
| Npepps-207 | <a href="#">ENSMUST00000152546.2</a> | 463  | <a href="#">15aa</a>  | Nonsense mediated decay | -                         | <a href="#">F6Z7Y0</a> | CDS 5' incomplete TSL:2   |
| Npepps-208 | <a href="#">ENSMUST00000152820.7</a> | 764  | No protein            | Processed transcript    | -                         | -                      | TSL:3   |
| Npepps-214 | <a href="#">ENSMUST00000165489.7</a> | 2078 | No protein            | Retained intron         | -                         | -                      | TSL:5   |
| Npepps-212 | <a href="#">ENSMUST00000163950.1</a> | 1413 | No protein            | Retained intron         | -                         | -                      | TSL:1   |
| Npepps-217 | <a href="#">ENSMUST00000170506.1</a> | 865  | No protein            | Retained intron         | -                         | -                      | TSL:3   |
| Npepps-204 | <a href="#">ENSMUST00000133907.2</a> | 782  | No protein            | Retained intron         | -                         | -                      | TSL:2   |
| Npepps-205 | <a href="#">ENSMUST00000146833.1</a> | 631  | No protein            | Retained intron         | -                         | -                      | TSL:3   |
| Npepps-202 | <a href="#">ENSMUST00000107608.2</a> | 544  | No protein            | Retained intron         | -                         | -                      | TSL:2   |
| Npepps-203 | <a href="#">ENSMUST00000109294.2</a> | 455  | No protein            | Retained intron         | -                         | -                      | TSL:3   |
| Npepps-206 | <a href="#">ENSMUST00000147287.2</a> | 426  | No protein            | Retained intron         | -                         | -                      | TSL:5   |
| Npepps-218 | <a href="#">ENSMUST00000171320.1</a> | 423  | No protein            | Retained intron         | -                         | -                      | TSL:2   |

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

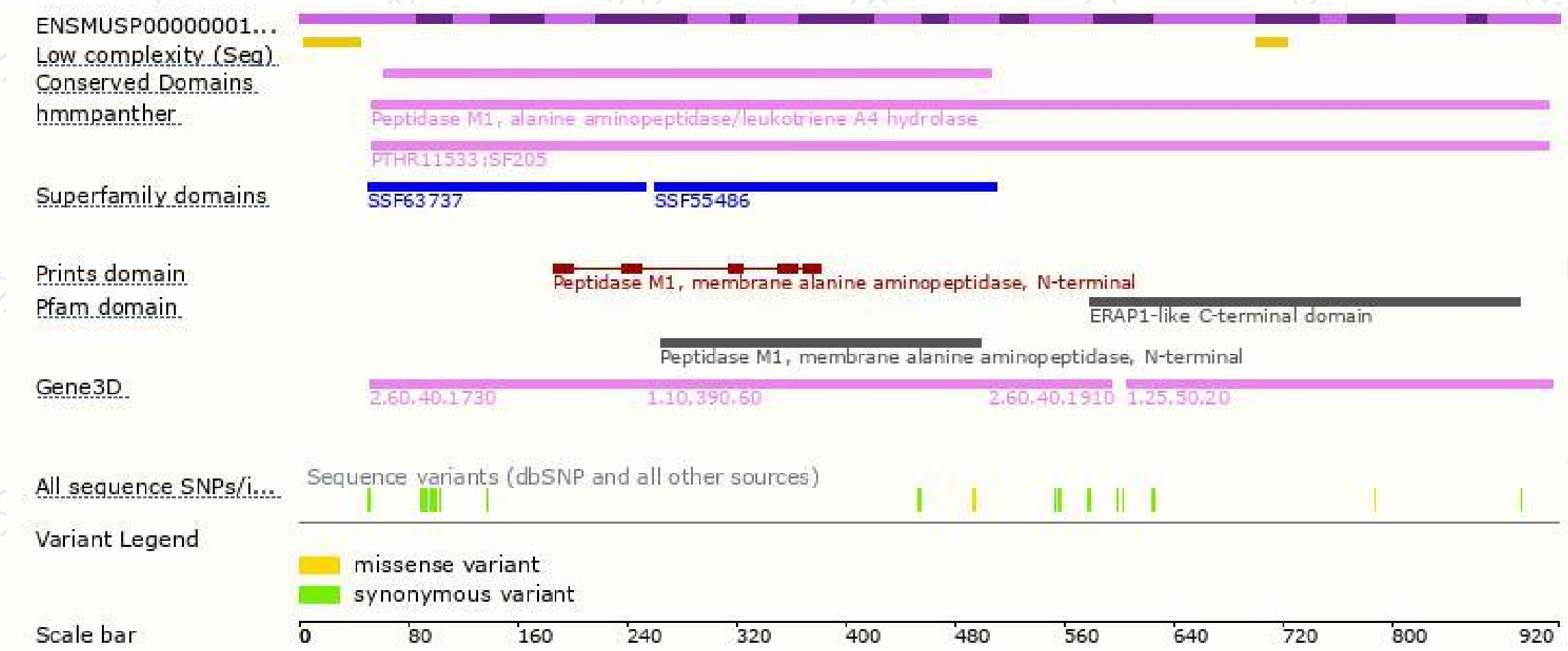


# Genomic location distribution





# 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, Homozygotes for a gene-trapped mutation exhibit dwarfism, increased anxiety, decreased pain sensitivity, and infertility in both sexes. Females fail to produce the corpus luteum of pregnancy, while males fail to copulate and have impaired spermatogenesis.

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

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