

# ***Ptbp1* Cas9-CKO Strategy**

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**Reviewer: Yanhua Shen**

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# Project Overview

**Project Name**

*Ptbp1*

**Project type**

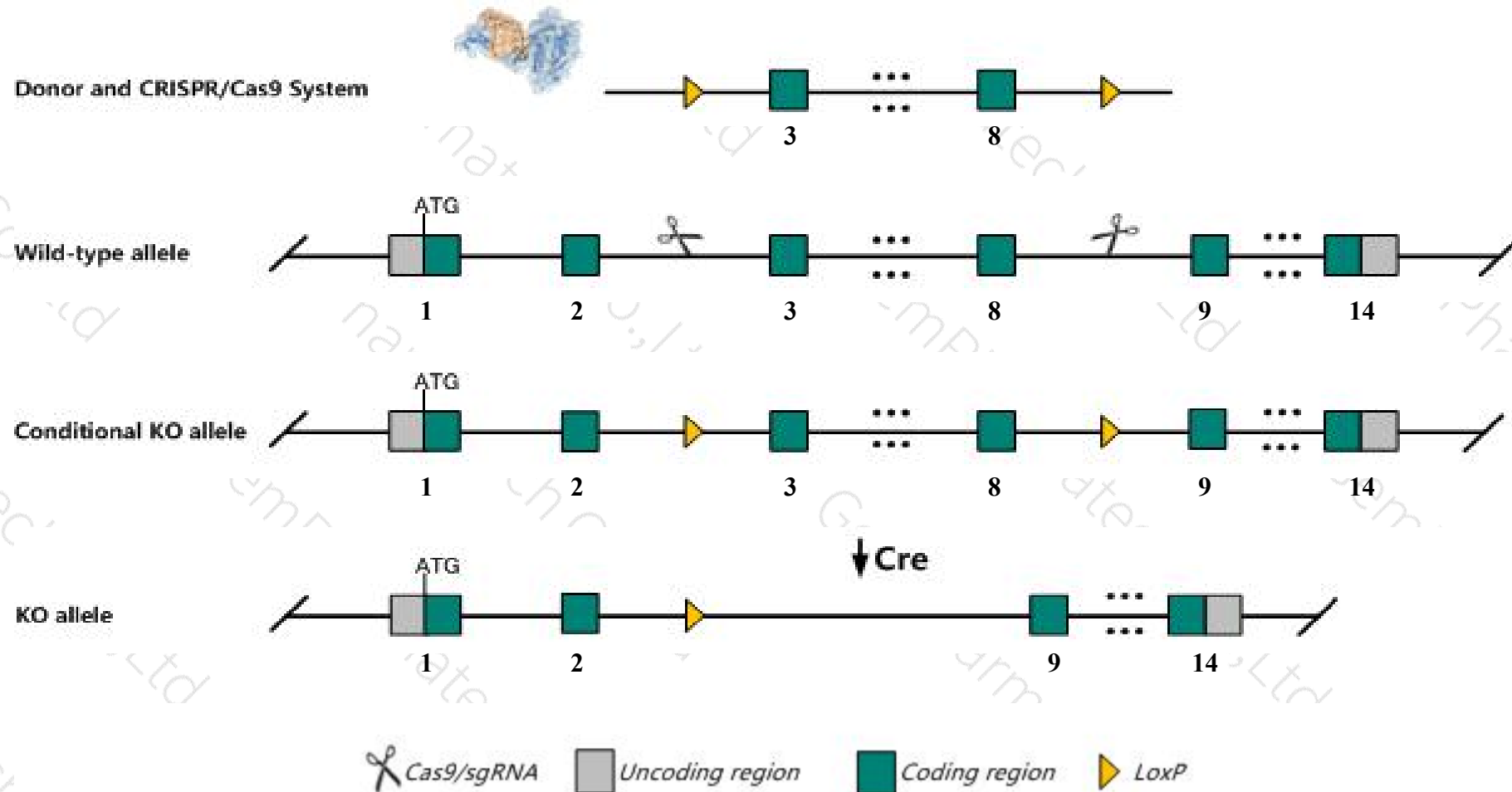
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ptbp1* gene. The schematic diagram is as follows:



# Technical routes

- The *Ptbp1* gene has 16 transcripts. According to the structure of *Ptbp1* gene, exon3-exon8 of *Ptbp1*-216(ENSMUST00000172282.7) transcript is recommended as the knockout region. The region contains 928bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ptbp1* 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/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 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.

- According to the existing MGI data, mice homozygous for a knock-out allele die before E6.5. Mice homozygous for a conditional allele activated in neuronal stem cells (NSCs) exhibit premature death, and non-obstructive hydrocephaly with loss of ependymal cells due to precocious NSC differentiation.
- The KO region was about 0.2kb away from 3-terminal of *Plppr3* gene. Knockout the region may affect the function of *Plppr3* gene.
- The *Ptbp1* gene is located on the Chr10. 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)

## Ptbp1 polypyrimidine tract binding protein 1 [ *Mus musculus* (house mouse) ]

Gene ID: 19205, updated on 8-Nov-2020

### Summary

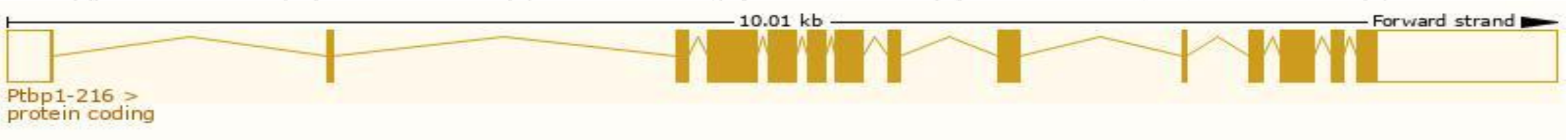
|                    |                                                                                                                                                                           |
|--------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Official Symbol    | Ptbp1 provided by <a href="#">MGI</a>                                                                                                                                     |
| Official Full Name | polypyrimidine tract binding protein 1 provided by <a href="#">MGI</a>                                                                                                    |
| Primary source     | <a href="#">MGI:MGI:97791</a>                                                                                                                                             |
| See related        | <a href="#">Ensembl:ENSMUSG00000006498</a>                                                                                                                                |
| Gene type          | protein coding                                                                                                                                                            |
| RefSeq status      | VALIDATED                                                                                                                                                                 |
| Organism           | <a href="#">Mus musculus</a>                                                                                                                                              |
| Lineage            | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| Also known as      | Pt; Ptb; PTB2; PTB3; PTB4; pPTB; HNRPI; PTB-1; D12Mgi4; AA407203; AL033359                                                                                                |
| Expression         | Ubiquitous expression in thymus adult (RPKM 115.0), limb E14.5 (RPKM 113.3) and 28 other tissues <a href="#">See more</a>                                                 |
| Orthologs          | <a href="#">human</a> <a href="#">all</a>                                                                                                                                 |

# Transcript information (Ensembl)

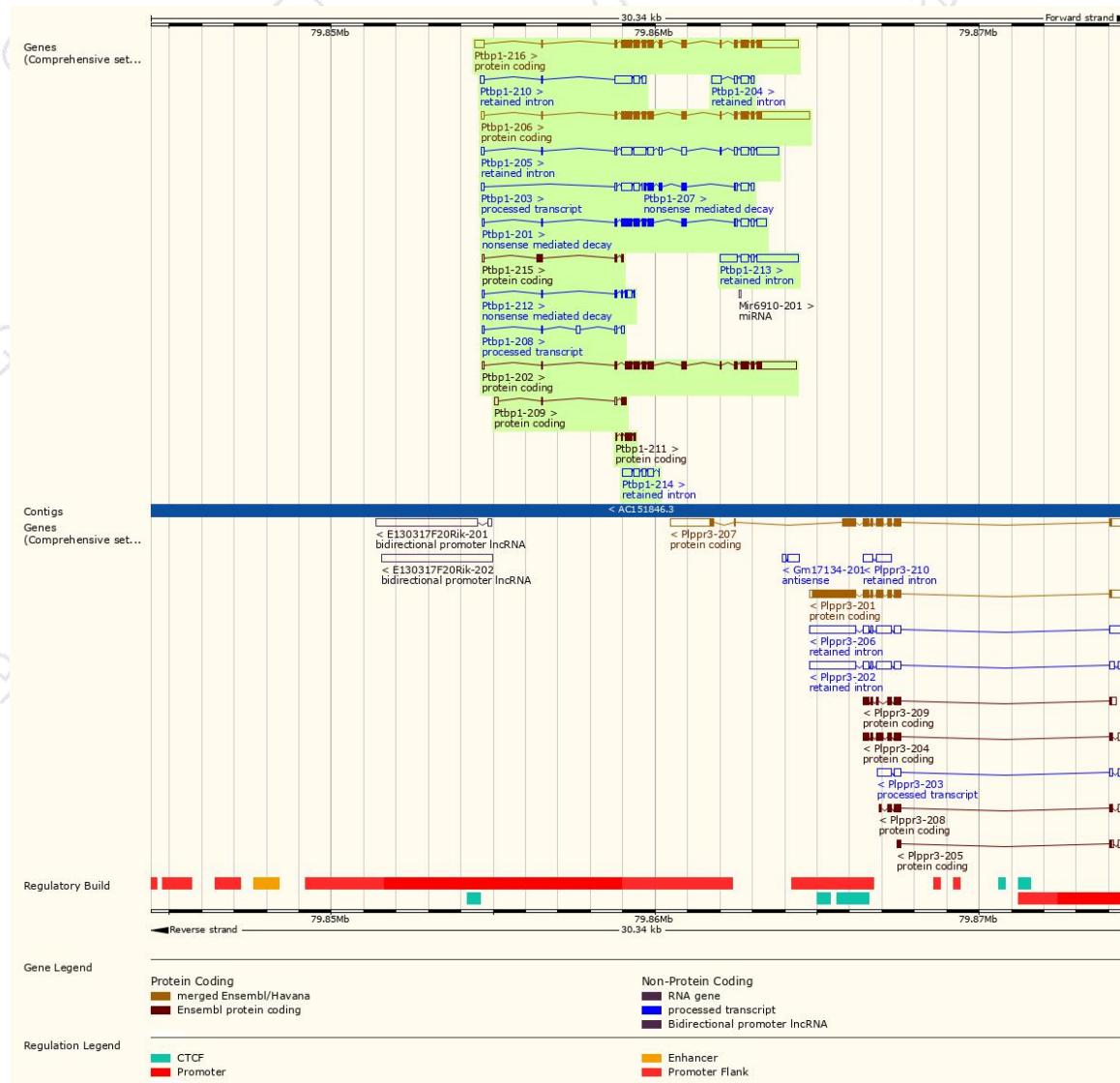
The gene has 16 transcripts,all transcripts are shown below:

| Name      | Transcript ID                         | bp   | Protein               | Biotype                 | CCDS                      | UniProt                | Flags                           |
|-----------|---------------------------------------|------|-----------------------|-------------------------|---------------------------|------------------------|---------------------------------|
| Ptbp1-206 | <a href="#">ENSMUST00000165704.7</a>  | 3169 | <a href="#">529aa</a> | Protein coding          | <a href="#">CCDS35970</a> | <a href="#">Q8BGJ5</a> | TSL:1 GENCODE basic APPRIS ALT2 |
| Ptbp1-216 | <a href="#">ENSMUST00000172282.7</a>  | 3110 | <a href="#">555aa</a> | Protein coding          | <a href="#">CCDS35969</a> | <a href="#">Q92217</a> | TSL:1 GENCODE basic APPRIS P4   |
| Ptbp1-202 | <a href="#">ENSMUST00000095457.10</a> | 2598 | <a href="#">489aa</a> | Protein coding          | <a href="#">CCDS70064</a> | <a href="#">Q8CB58</a> | TSL:1 GENCODE basic APPRIS ALT2 |
| Ptbp1-215 | <a href="#">ENSMUST00000171599.7</a>  | 389  | <a href="#">113aa</a> | Protein coding          | -                         | <a href="#">E9PZ39</a> | CDS 3' incomplete TSL:2         |
| Ptbp1-209 | <a href="#">ENSMUST00000168683.1</a>  | 375  | <a href="#">55aa</a>  | Protein coding          | -                         | <a href="#">E9Q0W3</a> | CDS 3' incomplete TSL:2         |
| Ptbp1-211 | <a href="#">ENSMUST00000169091.1</a>  | 319  | <a href="#">107aa</a> | Protein coding          | -                         | <a href="#">F7DCW4</a> | CDS 5' and 3' incomplete TSL:5  |
| Ptbp1-201 | <a href="#">ENSMUST00000057343.15</a> | 1765 | <a href="#">353aa</a> | Nonsense mediated decay | -                         | <a href="#">E9QMW9</a> | TSL:5                           |
| Ptbp1-207 | <a href="#">ENSMUST00000165724.7</a>  | 848  | <a href="#">162aa</a> | Nonsense mediated decay | -                         | <a href="#">F7AXP1</a> | CDS 5' incomplete TSL:5         |
| Ptbp1-212 | <a href="#">ENSMUST00000169483.7</a>  | 385  | <a href="#">62aa</a>  | Nonsense mediated decay | -                         | <a href="#">E9Q279</a> | TSL:3                           |
| Ptbp1-203 | <a href="#">ENSMUST00000099958.8</a>  | 657  | No protein            | Processed transcript    | -                         | -                      | TSL:3                           |
| Ptbp1-208 | <a href="#">ENSMUST00000168216.7</a>  | 369  | No protein            | Processed transcript    | -                         | -                      | TSL:3                           |
| Ptbp1-205 | <a href="#">ENSMUST00000165153.7</a>  | 2369 | No protein            | Retained intron         | -                         | -                      | TSL:5                           |
| Ptbp1-213 | <a href="#">ENSMUST00000169580.1</a>  | 2108 | No protein            | Retained intron         | -                         | -                      | TSL:2                           |
| Ptbp1-210 | <a href="#">ENSMUST00000168988.7</a>  | 950  | No protein            | Retained intron         | -                         | -                      | TSL:3                           |
| Ptbp1-214 | <a href="#">ENSMUST00000171216.1</a>  | 744  | No protein            | Retained intron         | -                         | -                      | TSL:2                           |
| Ptbp1-204 | <a href="#">ENSMUST00000164385.7</a>  | 693  | No protein            | Retained intron         | -                         | -                      | TSL:2                           |

The strategy is based on the design of *Ptbp1-216* transcript,the transcription is shown below:

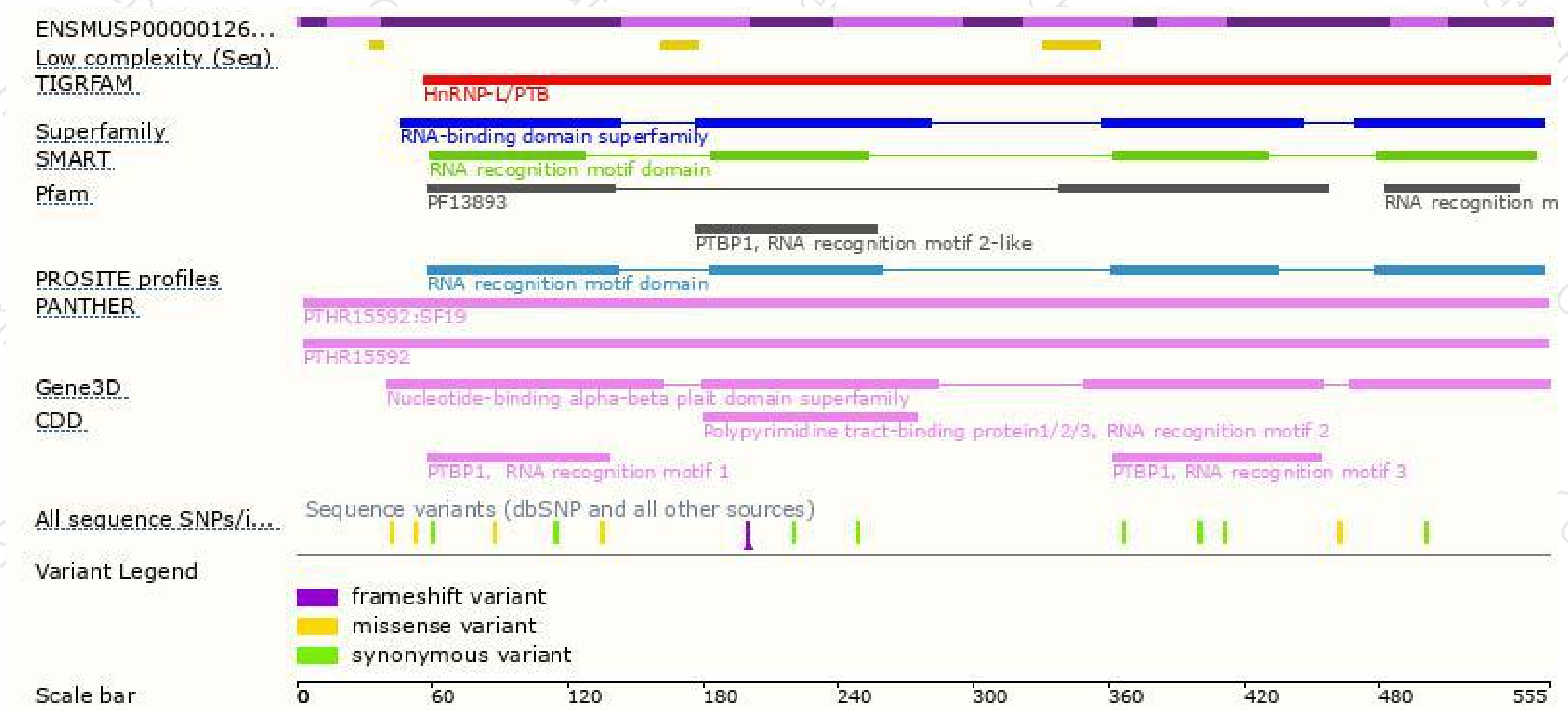


# Genomic location distribution

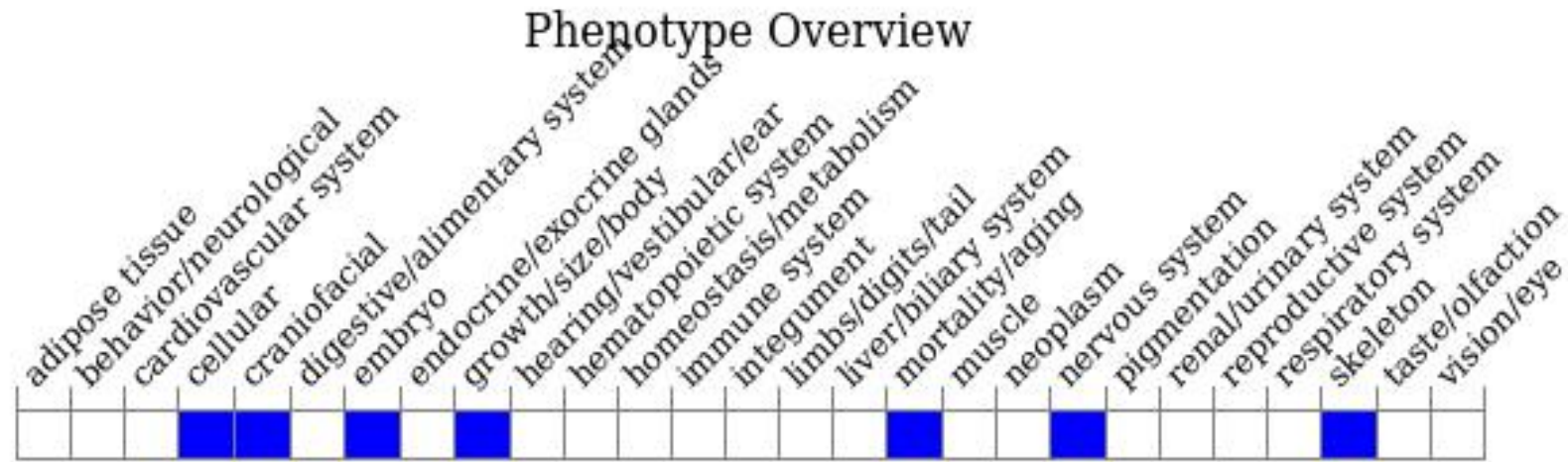




# Protein domain



# 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, mice homozygous for a knock-out allele die before E6.5. Mice homozygous for a conditional allele activated in neuronal stem cells (NSCs) exhibit premature death, and non-obstructive hydrocephaly with loss of ependymal cells due to precocious NSC differentiation.

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

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