

Pdgfrb-P2A-CreERT2 Cas9-KI Strategy

Designer:

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Reviewer

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Design Date:

2018-9-5

Project Overview

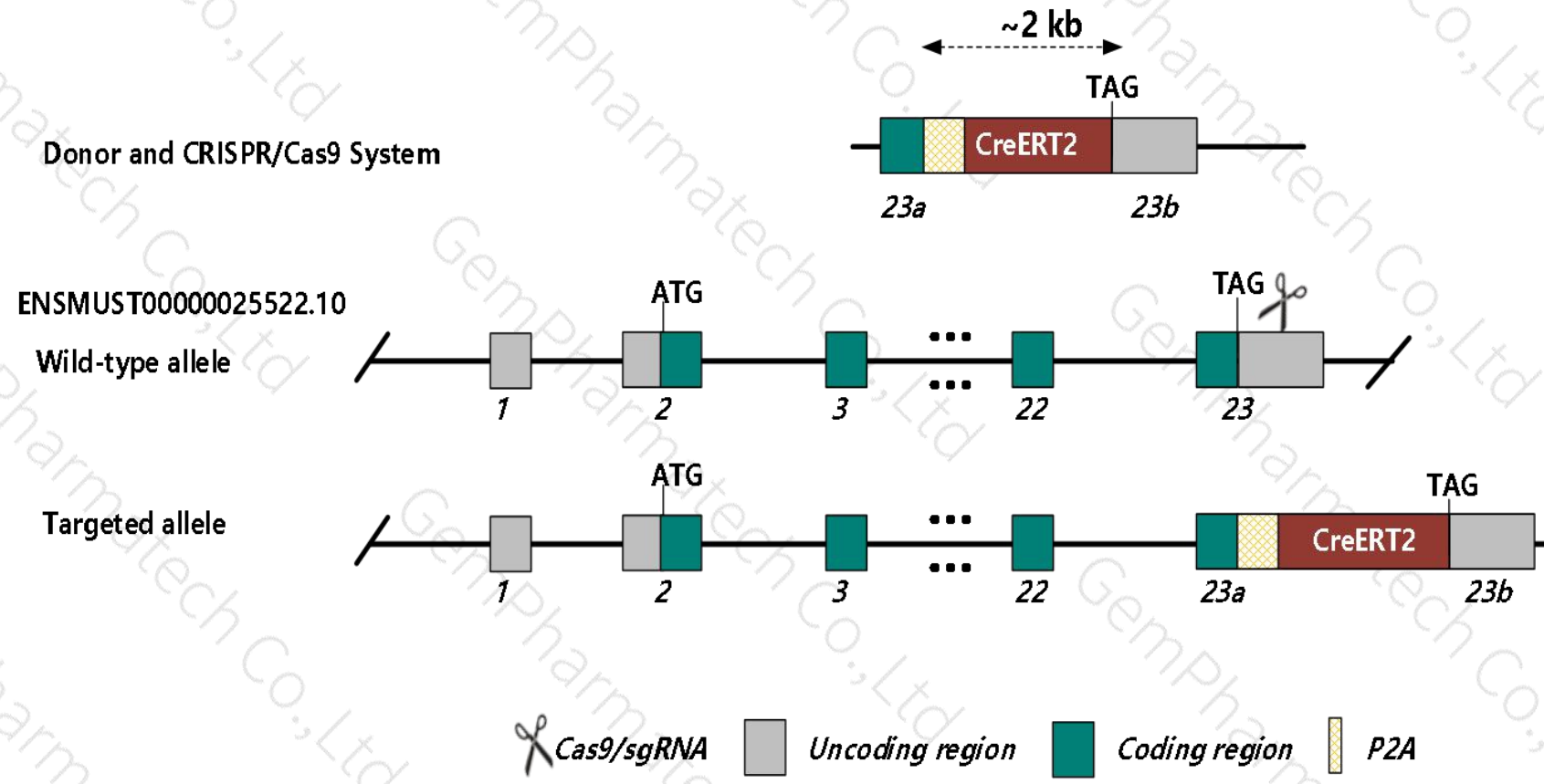
Project Name *Pdgfrb-P2A-CreERT2*

Project type **Cas9-KI**

Strain background **C57BL/6JGpt**

Knockin strategy

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



- The *Pdgfrb* gene has 2 transcripts. According to the structure of *Pdgfrb* gene, *Pdgfrb-201*(ENSMUST00000025522.10) is selected for presentation of the recommended strategy.
- *Pdgfrb-201* gene has 23 exons, with the ATG start codon in exon2 and TAG stop codon in exon23.
- We make *Pdgfrb-P2A-CreERT2* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near stop coding(TAG) of *Pdgfrb* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in P2A-CreERT2 before stop coding of *Pdgfrb* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

The Sequence of *CreERT2* (1983bp)

ATGTCCAATTTACTGACCGTACACCAAATTTGCCTGCATTACCGGTCGATGCAACGAGTGATGAGGTTTCGCAAGAACCTGATGGACATGTTTCAG
GGATCGCCAGGCGTTTTCTGAGCATACCTGGAAAATGCTTCTGTCCGTTTGCCGGTCGTGGGCGGCATGGTGCAAGTTGAATAACCGGAAATGGT
TTCCCGCAGAACCTGAAGATGTTTCGCGATTATCTTCTATATCTTCAGGCGCGCGGTCTGGCAGTAAAACTATCCAGCAACATTTGGGCCAGCTAA
ACATGCTTCATCGTCGGTCCGGGCTGCCACGACCAAGTGACAGCAATGCTGTTTCACTGGTTATGCGGGCGGATCCGAAAAGAAAACGTTGATGCC
GGTGAACGTGCAAAACAGGCTCTAGCGTTCGAACGCACACTGATTTTCGACCAGGTTTCGTTCACTCATGGAAAATAGCGATCGCTGCCAGGATATACG
TAATCTGGCATTCTGGGGATTGCTTATAACACCCTGTTACGTATAGCCGAAATTGCCAGGATCAGGGTTAAAGATATCTCACGTACTIONGACGGTGGG
AGAATGTTAATCCATATTGGCAGAACGAAAACGCTGGTTAGCACCGCAGGTGTAGAGAAGGCACCTTAGCCTGGGGGTAATAACTGGTTCGAGC
GATGGATTTCCGTCTCTGGTGTAGCTGATGATCCGAATAACTACCTGTTTTGCCGGGTCAGAAAAAATGGTGTGGCCGCGCCATCTGCCACCAGCC
AGCTATCAACTCGCGCCCTGGAAGGGATTTTTGAAGCAACTCATCGATTGATTTACGGCGCTAAGGATGACTCTGGTCAGAGATACCTGGCCTGGT
CTGGACACAGTGCCCGTGTTCGGAGCCGCGCGAGATATGGCCCGCGCTGGAGTTTCAATACCGGAGATCATGCAAGCTGGTGGCTGGACCAATGT
AAATATTGTCATGAACTATATCCGTAACCTGGATAGTGAAACAGGGGCAATGGTGCGCCTGCTGGAAGATGGCGATCTCGAGCCATCTGCTGGAG
ACATGAGAGCTGCCAACCTTTGGCCAAGCCCGCTCATGATCAAACGCTCTAAGAAGAACAGCCTGGCCTTGTCCCTGACGGCCGACCAGATGGT
CAGTGCCTTGTTGGATGCTGAGCCCCCATACTCTATTCCGAGTATGATCCTACCAGACCCTTCAGTGAAGCTTCGATGATGGGCTTACTGACCAA
CCTGGCAGACAGGGAGCTGGTTCACATGATCAACTGGGCGAAGAGGGTGGCAGGCTTTGTGGATTTGACCCTCCATGATCAGGTCCACCTTCTAG
AATGTGCCTGGCTAGAGATCCTGATGATTGGTCTCGTCTGGCGCTCCATGGAGCACCCAGTGAAGCTACTGTTTGCTCCTAACTTGCTCTTGGACA
GGAACCAGGGAAAATGTGTAGAGGGCATGGTGGAGATCTTCGACATGCTGCTGGCTACATCATCTCGGTTCCGCATGATGAATCTGCAGGGAGAG
GAGTTTGTGTGCCTCAAATCTATTATTTTGTCTAATTCTGGAGTGTACACATTTCTGTCCAGCACCTGAAGTCTCTGGAAGAGAAGGACCATATCC
ACCGAGTCCTGGACAAGATCACAGACACTTTGATCCACCTGATGGCCAAGGCAGGCCTGACCCTGCAGCAGCAGCACCAGCGGCTGGCCCAGC
TCCTCCTCATCTCTCCACATCAGGCACATGAGTAACAAAGGCATGGAGCATCTGTACAGCATGAAGTGCAAGAACGTGGTGGCCCTCTATGAC
CTGCTGCTGGAGGGCGGCGGACGCCACCCTACATGCGCCCACTAGCCGTGGAGGGGCATCCGTGGAGGAGACGGACCAAAGCCACTTGGCC
ACTGCGGGCTCTACTTCATCGCATTCTTGCAAAAGTATTACATCACGGGGGAGGCAGAGGGTTTCCCTGCCACAGCTTAA

- According to the existing MGI data, homozygous null mutants die perinatally with internal bleeding, thrombocytopenia, anemia and kidney defects. A frameshift mutation results in neonatal lethals with edema and hemorrhaging; several point mutations show cardiovascular abnormalities.
- The *Pdgfrb* gene is located on the Chr18. If the knockin 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

Pdgfrb platelet derived growth factor receptor, beta polypeptide [*Mus musculus* (house mouse)]

Gene ID: 18596, updated on 24-Apr-2018

Summary

Official Symbol Pdgfrb provided by [MGI](#)
Official Full Name platelet derived growth factor receptor, beta polypeptide provided by [MGI](#)
Primary source [MGI:MGI:97531](#)
See related [Ensembl:ENSMUSG00000024620](#) [Vega:OTTMUSG00000014920](#)
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 Pdgfr; CD140b; PDGFR-1; AI528809
Expression Broad expression in lung adult (RPKM 46.3), subcutaneous fat pad adult (RPKM 35.2) and 24 other tissues [See more](#)
Orthologs human all

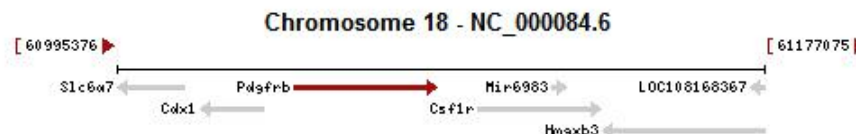
Genomic context

Location: 18 E1; 18 34.41 cM

See Pdgfrb in [Genome Data Viewer](#)

Exon count: 24

Annotation release	Status	Assembly	Chr	Location
106	current	GRCm38.p4 (GCF_000001635.24)	18	NC_000084.6 (61045150..61085067)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	18	NC_000084.5 (61204804..61244721)

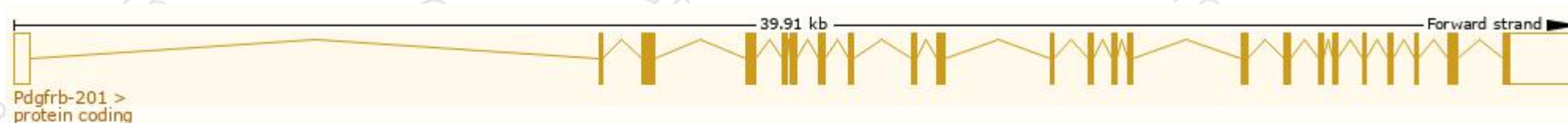


Transcript information (Ensembl)

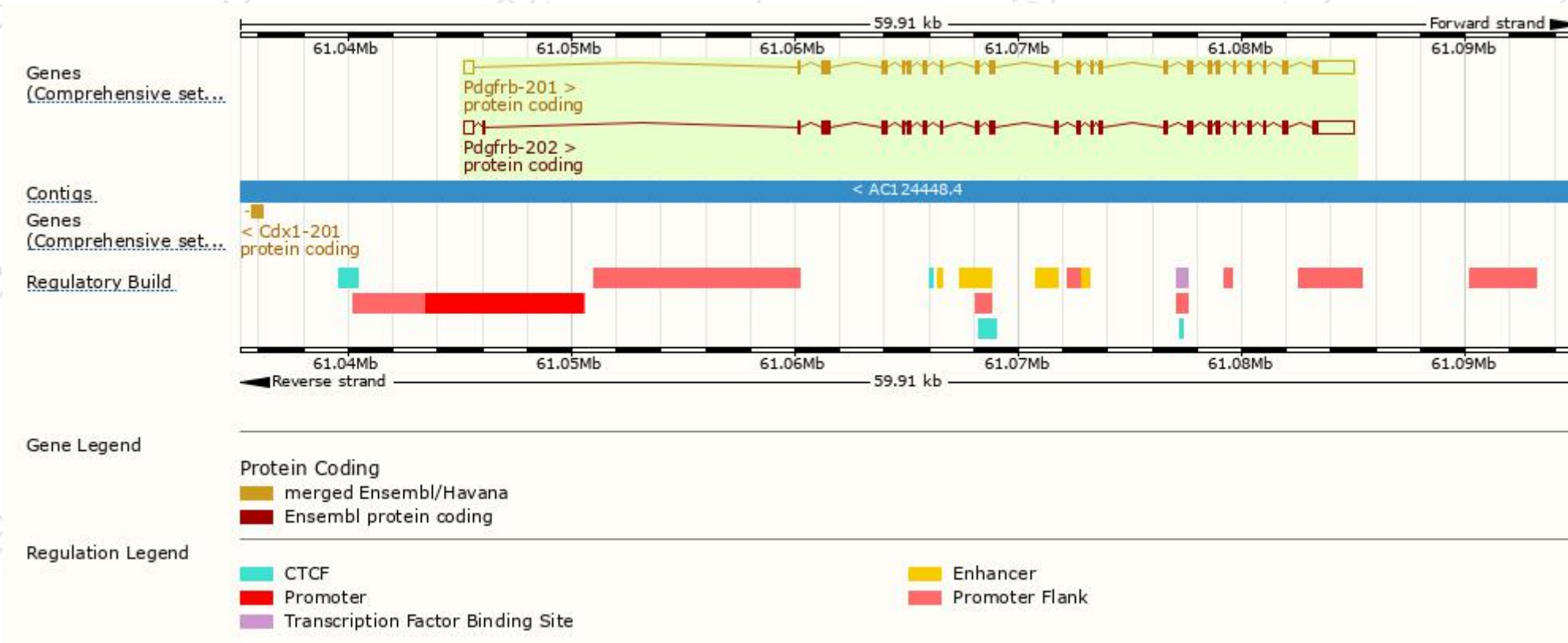
The gene has 2 transcripts, and all transcripts are shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags
Pdgfrb-201	ENSMUST00000025522.10	5407	1099aa	Protein coding	CCDS50300	E9QPE2	NM_001146268 NM_008809 NP_001139740 NP_032835	TSL:1 GENCODE basic APPRIS P2
Pdgfrb-202	ENSMUST00000115274.1	5423	1103aa	Protein coding	-	E9QN12	-	TSL:1 GENCODE basic APPRIS ALT2

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

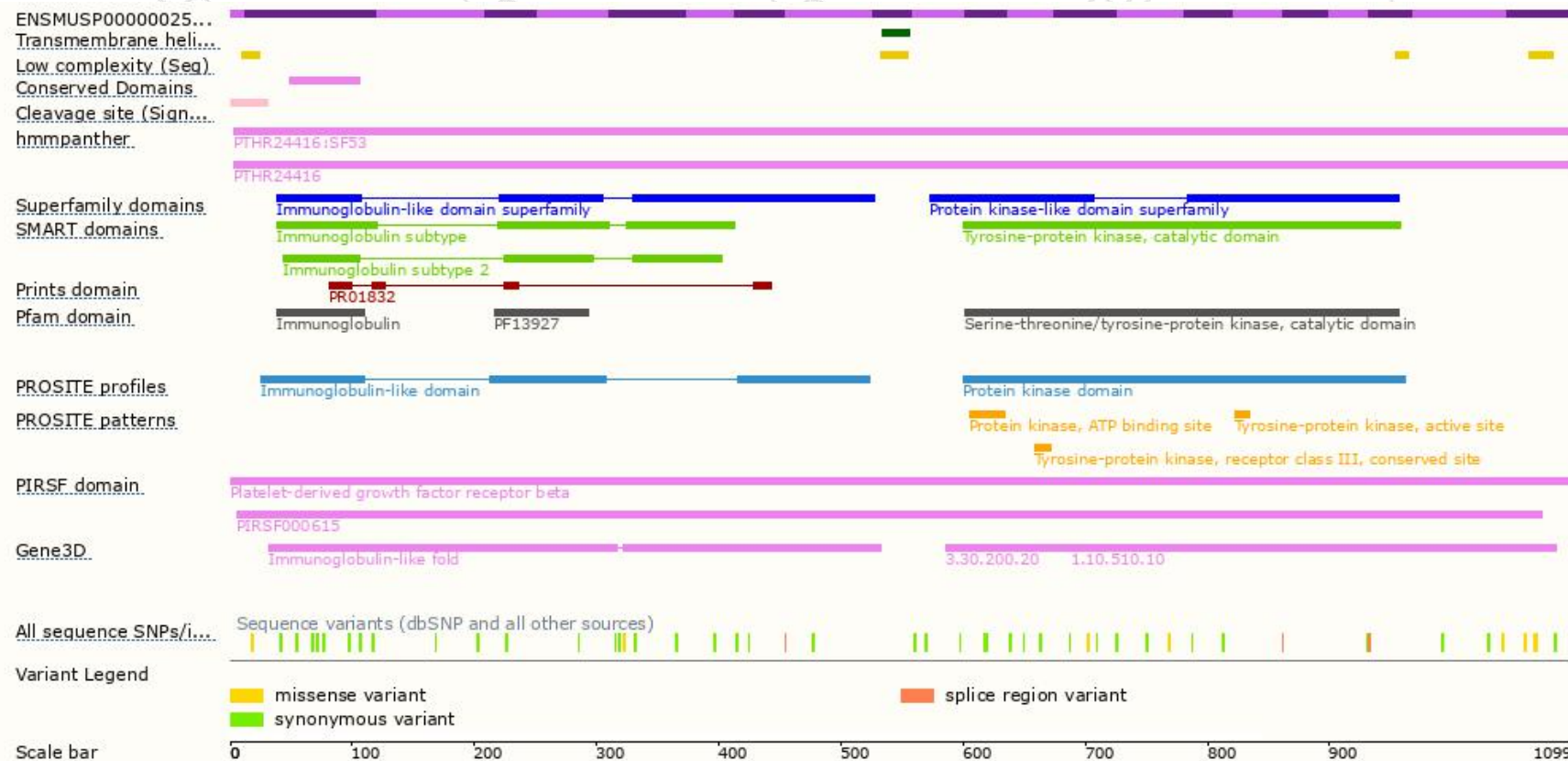


Genomic location distribution

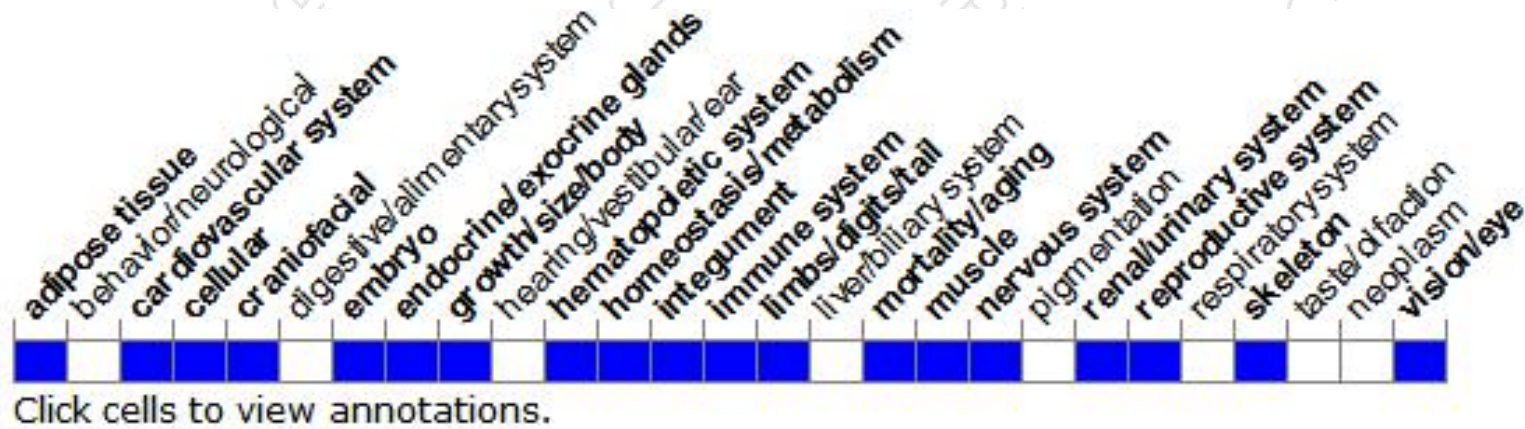


Protein domain

Protein domains for ENSMUSP00000025522.4



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(<http://www.informatics.jax.org/marker/MGI:97531>).

Homozygous null mutants die perinatally with internal bleeding, thrombocytopenia, anemia and kidney defects. A frameshift mutation results in neonatal lethals with edema and hemorrhaging; several point mutations show cardiovascular abnormalities.