

# ***Kit-p.D808E* cas9-ki(PM) Mouse Model Strategy** **-CRISPR/Cas9 technology**

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# Project Overview

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**Project Name**

**Kit-p.D808E**

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**Project type**

**cas9-ki(PM)**

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**Strain background**

**C57BL/6JGpt**

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# Technical Description

- The mouse *Kit* gene has 8 transcripts. According to the structure of *Kit* gene and requirements of customer, this project produced *Kit*-p.D808E point mutation on exon17 of the transcript of *Kit*-204 (ENSMUST00000144270.8) . The 808th amino acids will be mutated from D to E, and the corresponding codon will be mutated to GAT by the GAA.
- The mouse *Kit*-204 transcript contains 21 exons. The translation initiation site ATG is located at exon1, and the translation termination site TGA is located at exon21, encoding 975aa.
- In this project, *Kit* 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

C I H?  
TTCATGTAAA CACCATTGTA GTATTAAAAT CATCTTCTCT CGGAGAGCTG AAATGAATGG CTGTTGCTGT CTTTCCTTTT CTCCCCAAC AGTGTATTCA  
AAGTACATTT GTGGTAACAT CATAATTTTA GTAGAAGAGA GCCTCTCGAC TTTACTTACC GACAACGACA GAAAGGAAAA GAGGGGGTTG TCACATAAGT  
H R D L A A R N I L L T H G R I T K I C D F G L A R D I R N D S N Y  
CAGAGATTTG GCAGCCAGGA ATATCCTCCT CACTCACGGG CGGATCACAA AGATTTCGA TTTCGGGCTA GCCAGAGACA TCAGGAATGA TTCGAATTAC  
GTCTCTAAAC CGTCGGTCCT TATAGGAGGA GTGAGTGCCC GCCTAGTGTT TCTAAACGCT AAAGCCCGAT CGGTCTCTGT AGTCCTTACT AAGCTTAATG  
V V K G N  
GTGGTCAAAG GAAATGTGAG TACCTTTCTC CATCTCATGA GTCTACCCAG GGTGCTTTGG TATCCAGTCT TGATTCTAAA TTGTTTTCTA TGATCATTAC  
CACCAGTTTC CTTTACACTC ATGGAAAGAG GTAGAGTACT CAGATGGGTC CCACGAAACC ATAGGTCAGA ACTAAGATTT AACAAAAGAT ACTAGTAATG

D808位点

## After mutation

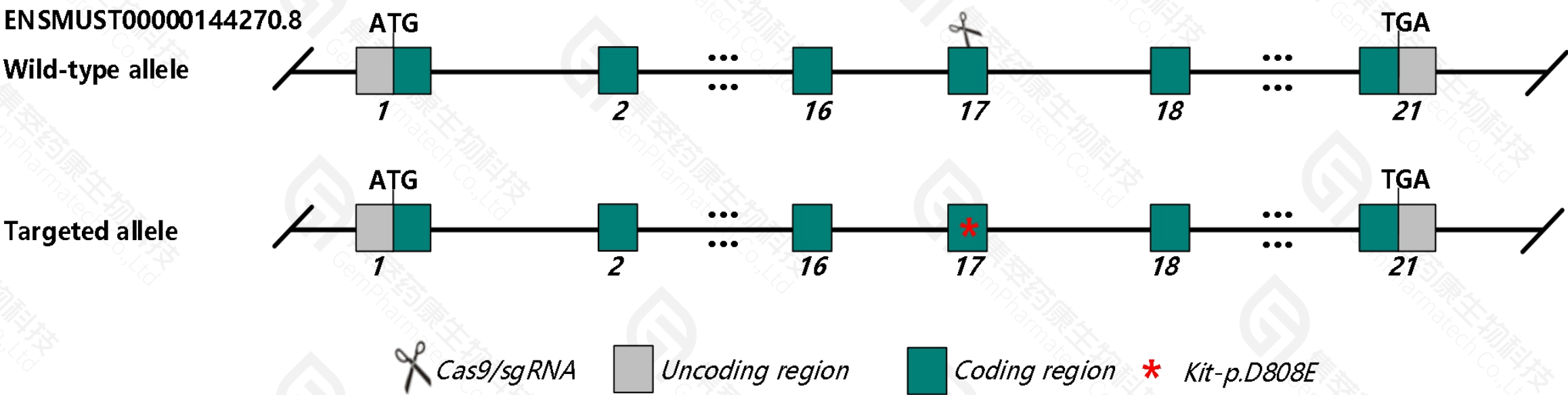
C I H?  
TTCATGTAAA CACCATTGTA GTATTAAAAT CATCTTCTCT CGGAGAGCTG AAATGAATGG CTGTTGCTGT CTTTCCTTTT CTCCCCAAC AGTGTATTCA  
AAGTACATTT GTGGTAACAT CATAATTTTA GTAGAAGAGA GCCTCTCGAC TTTACTTACC GACAACGACA GAAAGGAAAA GAGGGGGTTG TCACATAAGT  
H R D L A A R N I L L T H G R I T K I C E F G L A R D I R N D S N Y  
CAGAGATTTG GCAGCCAGGA ATATCCTCCT CACTCACGGG CGGATCACAA AGATTTCGA ATTTCGGGCTA GCCAGAGACA TCAGGAATGA TTCGAATTAC  
GTCTCTAAAC CGTCGGTCCT TATAGGAGGA GTGAGTGCCC GCCTAGTGTT TCTAAACGCT TAAGCCCGAT CGGTCTCTGT AGTCCTTACT AAGCTTAATG  
V V K G N  
GTGGTCAAAG GAAATGTGAG TACCTTTCTC CATCTCATGA GTCTACCCAG GGTGCTTTGG TATCCAGTCT TGATTCTAAA TTGTTTTCTA TGATCATTAC  
CACCAGTTTC CTTTACACTC ATGGAAAGAG GTAGAGTACT CAGATGGGTC CCACGAAACC ATAGGTCAGA ACTAAGATTT AACAAAAGAT ACTAGTAATG

D808E突变

The yellow region is exon17 of *Kit*-204, the red region represents the mutation site.

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

Donor and CRISPR/Cas9 System





- According to the data of MGI, mutations at this locus affect migration of embryonic stem cell populations, resulting in mild to severe impairments in hematopoiesis, and pigmentation. Some alleles are homozygous lethal, sterile, or result in the formation of gastrointestinal tumors. However, homozygous mice with gene mutations such as Y719F and K641D have a phenotype of infertility.
- One or two synonymous mutations of amino acids will be introduced on exon17 of *Kit*-204.
- Mouse *Kit* gene is located on Chr5. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr5, 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)

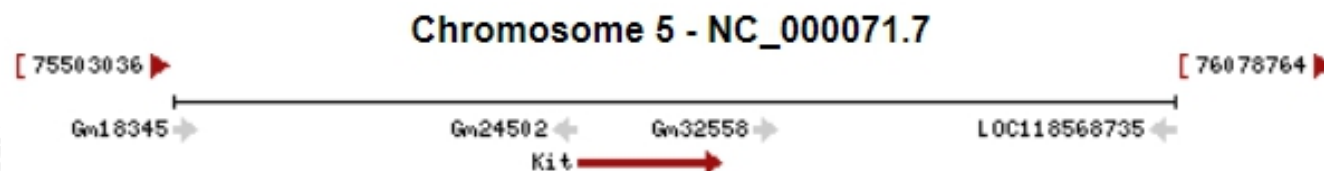
**Kit** KIT proto-oncogene receptor tyrosine kinase [ *Mus musculus* (house mouse) ]

[Download Datasets](#)

Gene ID: 16590, updated on 9-Mar-2021

## Summary

<b>Official Symbol</b>	Kit provided by <a href="#">MGI</a>
<b>Official Full Name</b>	KIT proto-oncogene receptor tyrosine kinase provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:96677</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG000000005672</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	REVIEWED
<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>	W; Bs; SC; SO; Fdc; Ssm; Gsfs; SCO1; SCO5; SOW3; Tr-k; c-KI; CD117; belly; c-KIT; Tr-kit; Gsfsc01; Gsfsc05; Gsfscow3
<b>Summary</b>	The c-Kit proto-oncogene is the cellular homolog of the transforming gene of a feline retrovirus (v-Kit). The c-kit protein includes characteristics of a protein kinase transmembrane receptor. Alternatively spliced transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Jul 2008]
<b>Expression</b>	Broad expression in lung adult (RPKM 39.0), cerebellum adult (RPKM 19.6) and 25 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

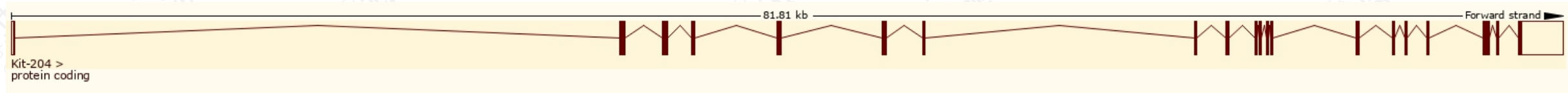


# Transcript information (Ensembl)

The gene has 8 transcripts, and all transcripts are shown below:

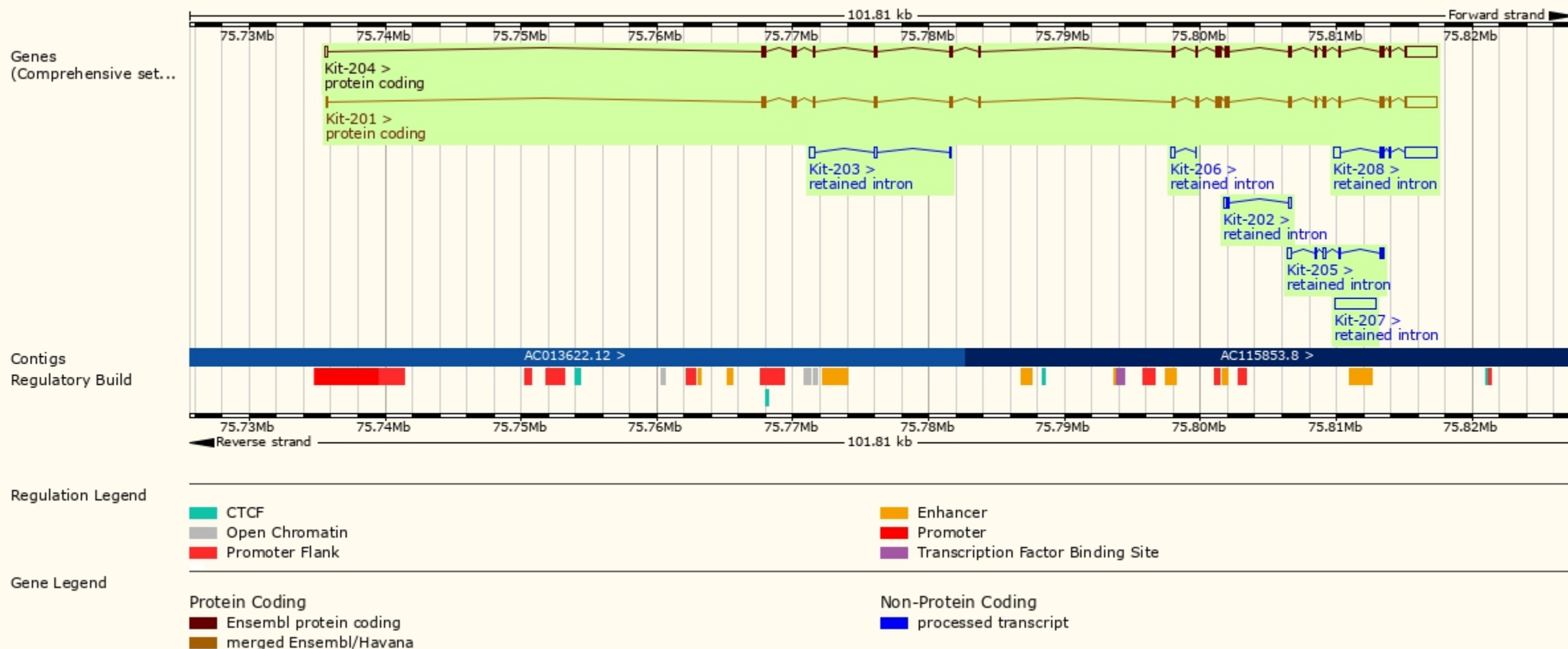
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Kit-201	<a href="#">ENSMUST00000005815.7</a>	5214	<a href="#">979aa</a>	Protein coding	<a href="#">CCDS51525</a>	<a href="#">P05532-1</a>	TSL:1 GENCODE basic APPRIS P3
Kit-204	<a href="#">ENSMUST00000144270.8</a>	5241	<a href="#">975aa</a>	Protein coding	<a href="#">CCDS80302</a>	<a href="#">P05532-2</a>	TSL:1 GENCODE basic APPRIS ALT2
Kit-208	<a href="#">ENSMUST00000202167.2</a>	3175	No protein	Retained intron	-	-	TSL:1
Kit-207	<a href="#">ENSMUST00000201240.2</a>	3104	No protein	Retained intron	-	-	TSL:NA
Kit-205	<a href="#">ENSMUST00000148993.5</a>	842	No protein	Retained intron	-	-	TSL:5
Kit-203	<a href="#">ENSMUST00000143221.2</a>	648	No protein	Retained intron	-	-	TSL:2
Kit-202	<a href="#">ENSMUST00000136002.2</a>	392	No protein	Retained intron	-	-	TSL:5
Kit-206	<a href="#">ENSMUST00000151357.2</a>	354	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Kit-204* transcript, the transcription is shown below:

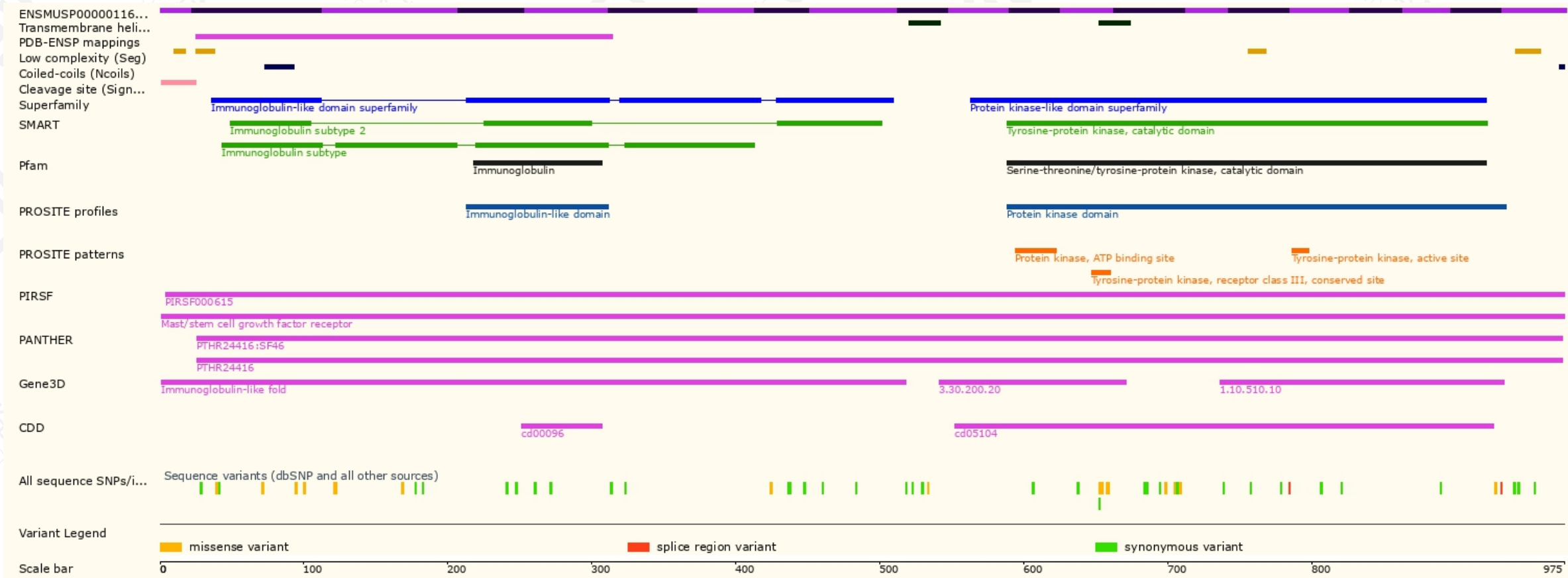




# Genomic location distribution



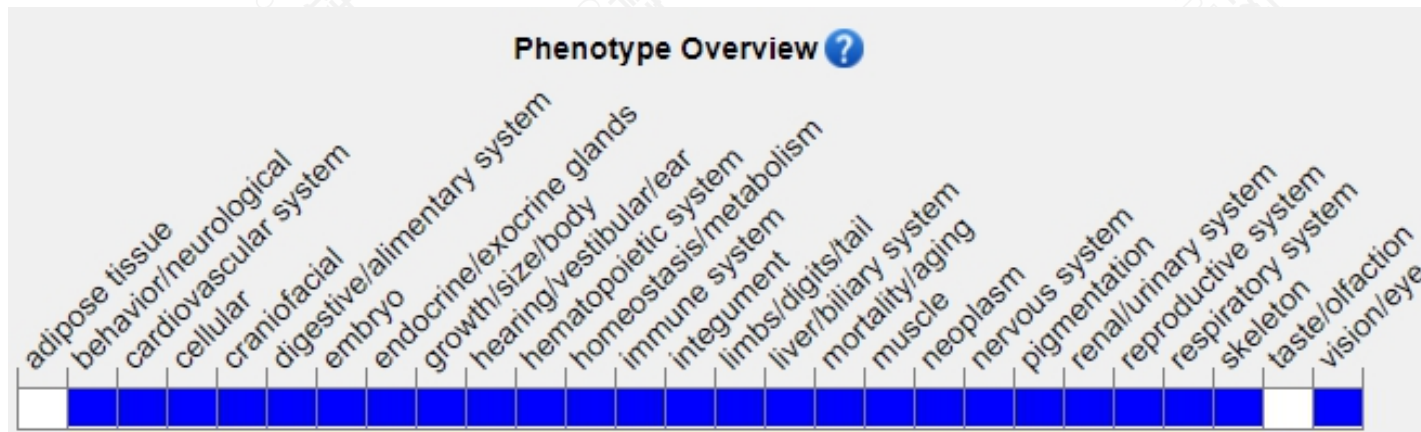
## Protein domains for ENSMUSP00000116465.3



# Mouse phenotype description(MGI)

URL link is as follows:

<http://www.informatics.jax.org/marker/MGI:96677>



Mutations at this locus affect migration of embryonic stem cell populations, resulting in mild to severe impairments in hematopoiesis, and pigmentation. Some alleles are homozygous lethal, sterile, or result in the formation of gastrointestinal tumors.

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