

# ***Kmt5b* Cas9-CKO Strategy**

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# Project Overview

**Project Name**

***Kmt5b***

**Project type**

**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

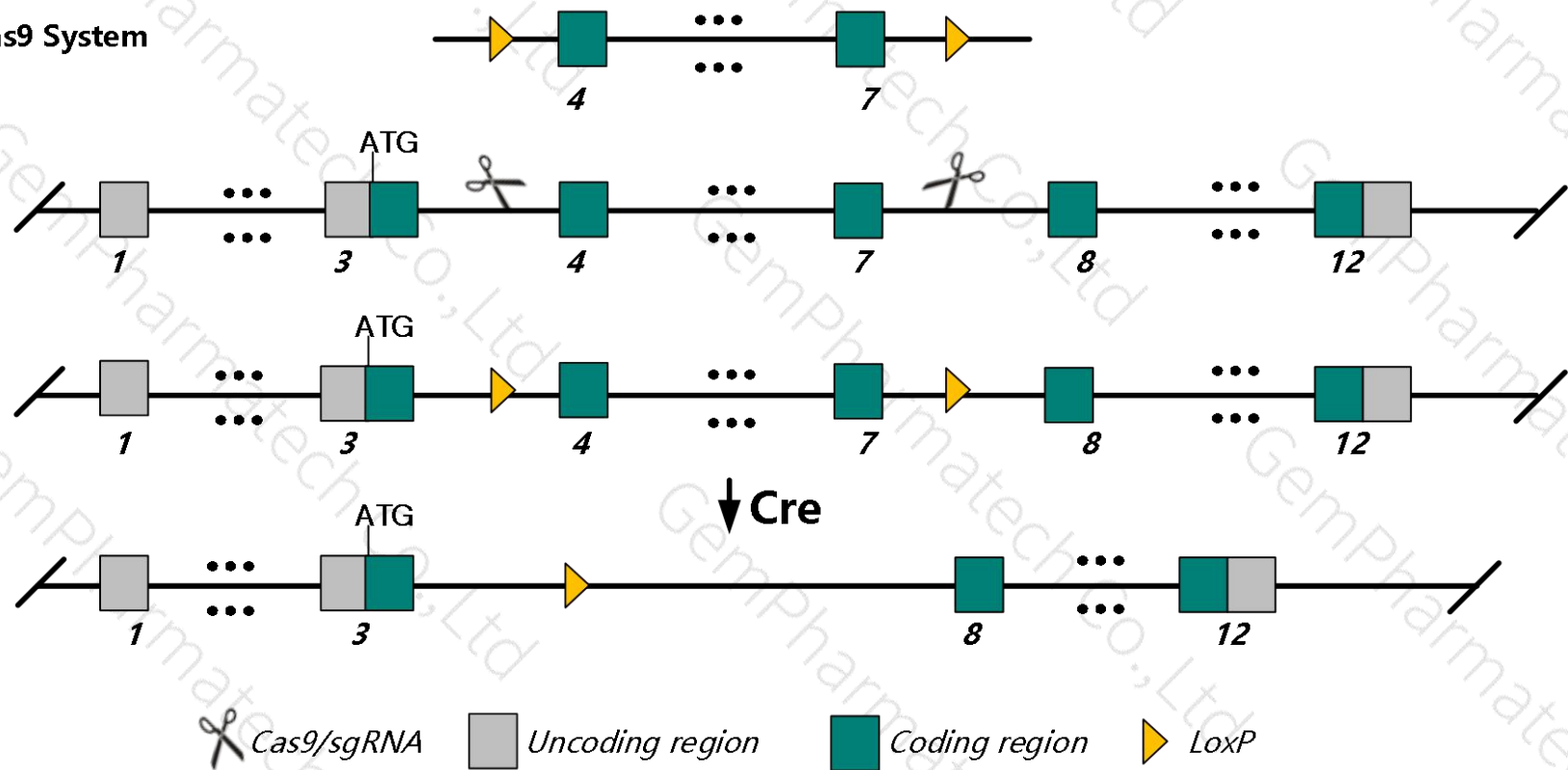
This model will use CRISPR/Cas9 technology to edit the *Kmt5b* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Wild-type allele

Conditional KO allele

KO allele



- The *Kmt5b* gene has 21 transcripts. According to the structure of *Kmt5b* gene, exon4-exon7 of *Kmt5b*-205 (ENSMUST00000113972.8) transcript is recommended as the knockout region. The region contains 493bp coding region. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Kmt5b* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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, Mice homozygous for a knock-out allele are born at sub-Mendelian ratios, are smaller than control littermates, and die within a few hours of birth, probably due to alveolar defects.
- The KO region contains functional region of the *Gm16066* gene. Knockout the region may affect the function of *Gm16066* gene.
- The *Kmt5b* gene is located on the Chr19. 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)

## Kmt5b lysine methyltransferase 5B [ *Mus musculus* (house mouse) ]

Gene ID: 225888, updated on 12-Aug-2019

### Summary



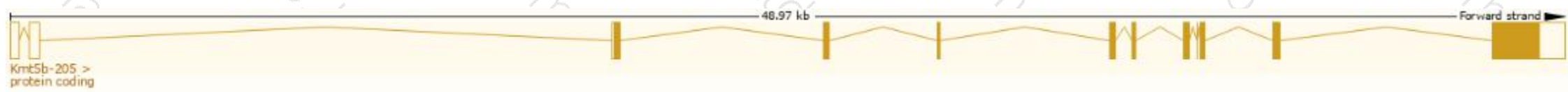
<b>Official Symbol</b>	Kmt5b provided by <a href="#">MGI</a>
<b>Official Full Name</b>	lysine methyltransferase 5B provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:2444557</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000045098</a>
<b>Gene type</b>	protein coding
<b>RefSeq 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>	AA117471; Suv420h1; Suv4-20h1; C630029K18Rik
<b>Expression</b>	Ubiquitous expression in whole brain E14.5 (RPKM 7.3), CNS E14 (RPKM 7.0) 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 21 transcripts,all transcripts are shown below:

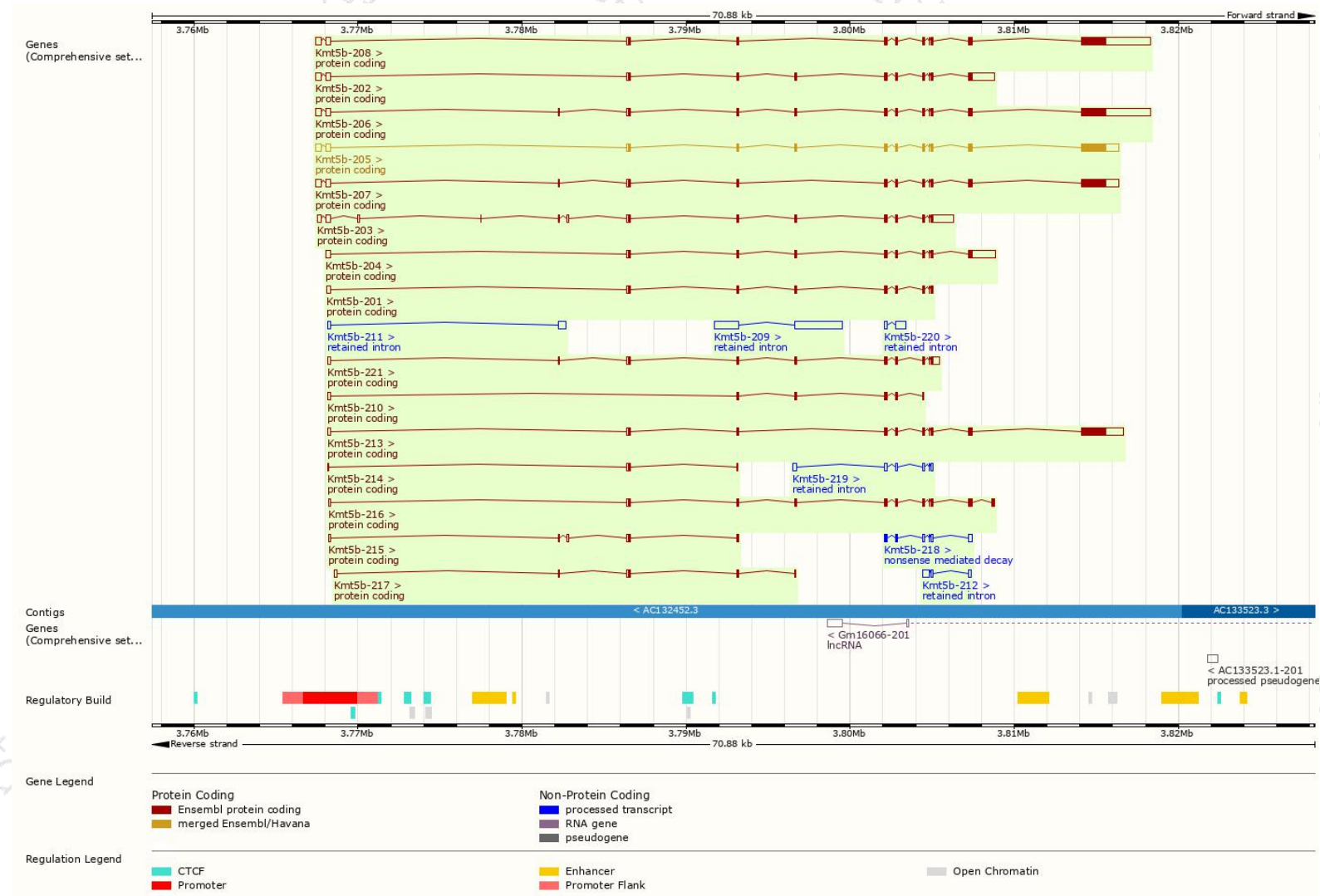
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Kmt5b-206	<a href="#">ENSMUST00000113973.7</a>	6130	<a href="#">883aa</a>	Protein coding	<a href="#">CCDS50343.4</a>	<a href="#">Q3U8K7.4</a>	TSL:5 GENCODE basic
Kmt5b-208	<a href="#">ENSMUST00000113977.8</a>	6002	<a href="#">860aa</a>	Protein coding	<a href="#">CCDS29399.4</a>	<a href="#">Q3U8K7.4</a>	TSL:5 GENCODE basic
Kmt5b-205	<a href="#">ENSMUST00000113972.8</a>	4159	<a href="#">883aa</a>	Protein coding	<a href="#">CCDS50343.4</a>	<a href="#">Q3U8K7.4</a>	TSL:5 GENCODE basic
Kmt5b-207	<a href="#">ENSMUST00000113974.10</a>	4150	<a href="#">860aa</a>	Protein coding	<a href="#">CCDS29399.4</a>	<a href="#">Q3U8K7.4</a>	TSL:5 GENCODE basic
Kmt5b-213	<a href="#">ENSMUST00000176262.7</a>	3933	<a href="#">860aa</a>	Protein coding	<a href="#">CCDS29399.4</a>	<a href="#">Q3U8K7.4</a>	TSL:5 GENCODE basic
Kmt5b-202	<a href="#">ENSMUST00000052699.12</a>	3290	<a href="#">394aa</a>	Protein coding	<a href="#">CCDS50344.4</a>	<a href="#">Q3U8K7.4</a>	TSL:5 GENCODE basic APPRIS P1
Kmt5b-203	<a href="#">ENSMUST00000113968.8</a>	3216	<a href="#">327aa</a>	Protein coding	<a href="#">CCDS50345.4</a>	<a href="#">Q3U8K7.4</a>	TSL:1 GENCODE basic
Kmt5b-204	<a href="#">ENSMUST00000113970.7</a>	2975	<a href="#">394aa</a>	Protein coding	<a href="#">CCDS50344.4</a>	<a href="#">Q3U8K7.4</a>	TSL:1 GENCODE basic APPRIS P1
Kmt5b-221	<a href="#">ENSMUST00000237440.1</a>	1721	<a href="#">327aa</a>	Protein coding	<a href="#">CCDS50345.4</a>	-	GENCODE basic
Kmt5b-201	<a href="#">ENSMUST00000005518.15</a>	1283	<a href="#">327aa</a>	Protein coding	<a href="#">CCDS50345.4</a>	<a href="#">Q3U8K7.4</a>	TSL:5 GENCODE basic
Kmt5b-216	<a href="#">ENSMUST00000176926.7</a>	1551	<a href="#">423aa</a>	Protein coding	-	<a href="#">H3BLE5.4</a>	TSL:5 GENCODE basic
Kmt5b-210	<a href="#">ENSMUST00000152935.7</a>	793	<a href="#">212aa</a>	Protein coding	-	<a href="#">D3YXL6.4</a>	CDS 3' incomplete TSL:3
Kmt5b-217	<a href="#">ENSMUST00000177355.1</a>	624	<a href="#">110aa</a>	Protein coding	-	<a href="#">H3BKZ5.4</a>	CDS 3' incomplete TSL:5
Kmt5b-215	<a href="#">ENSMUST00000176512.7</a>	622	<a href="#">93aa</a>	Protein coding	-	<a href="#">H3BJI9.4</a>	CDS 3' incomplete TSL:3
Kmt5b-214	<a href="#">ENSMUST00000176407.7</a>	398	<a href="#">87aa</a>	Protein coding	-	<a href="#">H3BJ94.4</a>	CDS 3' incomplete TSL:3
Kmt5b-218	<a href="#">ENSMUST00000235768.1</a>	723	<a href="#">85aa</a>	Nonsense mediated decay	-	-	CDS 5' incomplete
Kmt5b-209	<a href="#">ENSMUST00000135586.1</a>	4406	No protein	Retained intron	-	-	TSL:1
Kmt5b-220	<a href="#">ENSMUST00000236861.1</a>	802	No protein	Retained intron	-	-	-
Kmt5b-219	<a href="#">ENSMUST00000236012.1</a>	772	No protein	Retained intron	-	-	-
Kmt5b-212	<a href="#">ENSMUST00000176125.1</a>	755	No protein	Retained intron	-	-	TSL:3
Kmt5b-211	<a href="#">ENSMUST00000155618.1</a>	667	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Kmt5b-205* transcript,The transcription is shown below



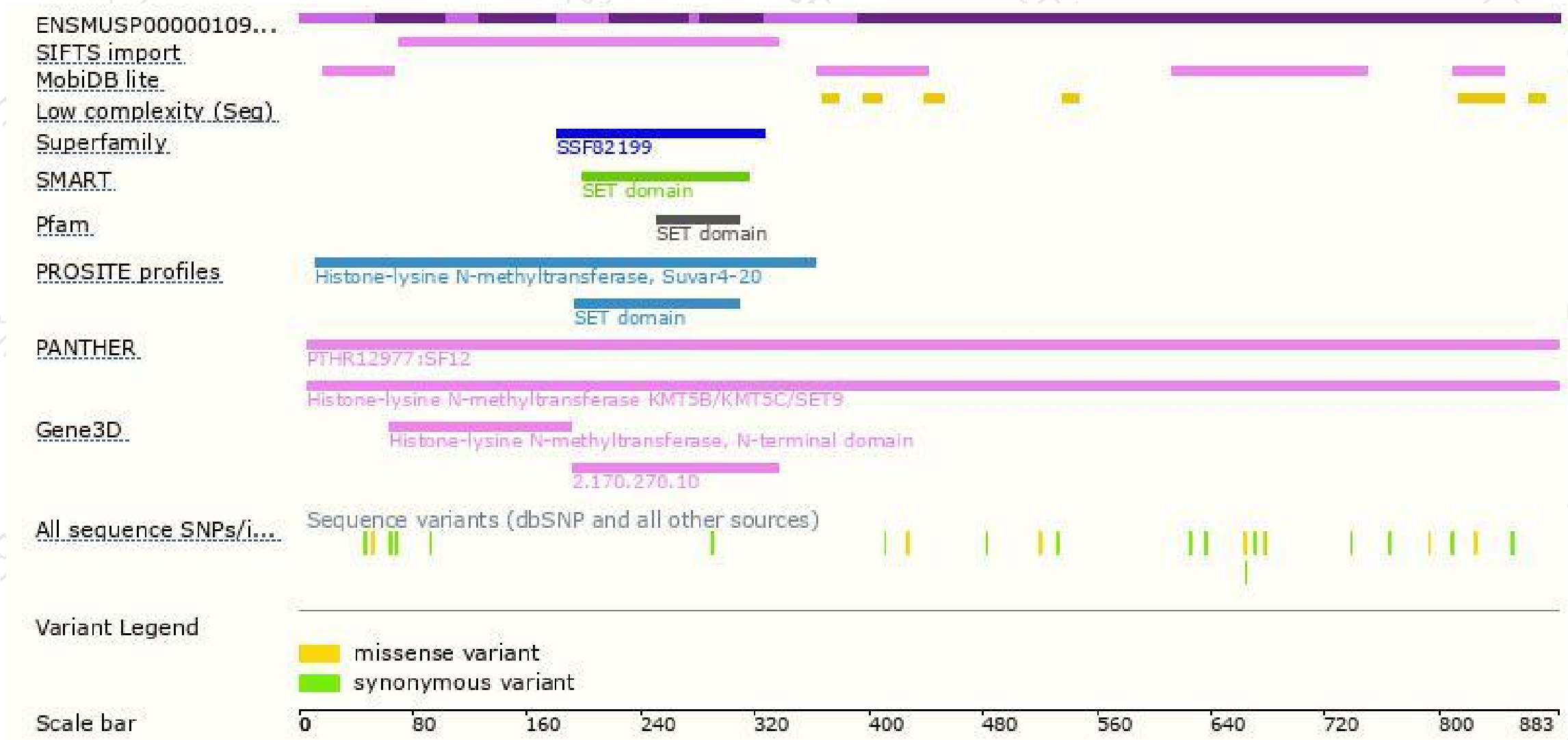


# Genomic location distribution

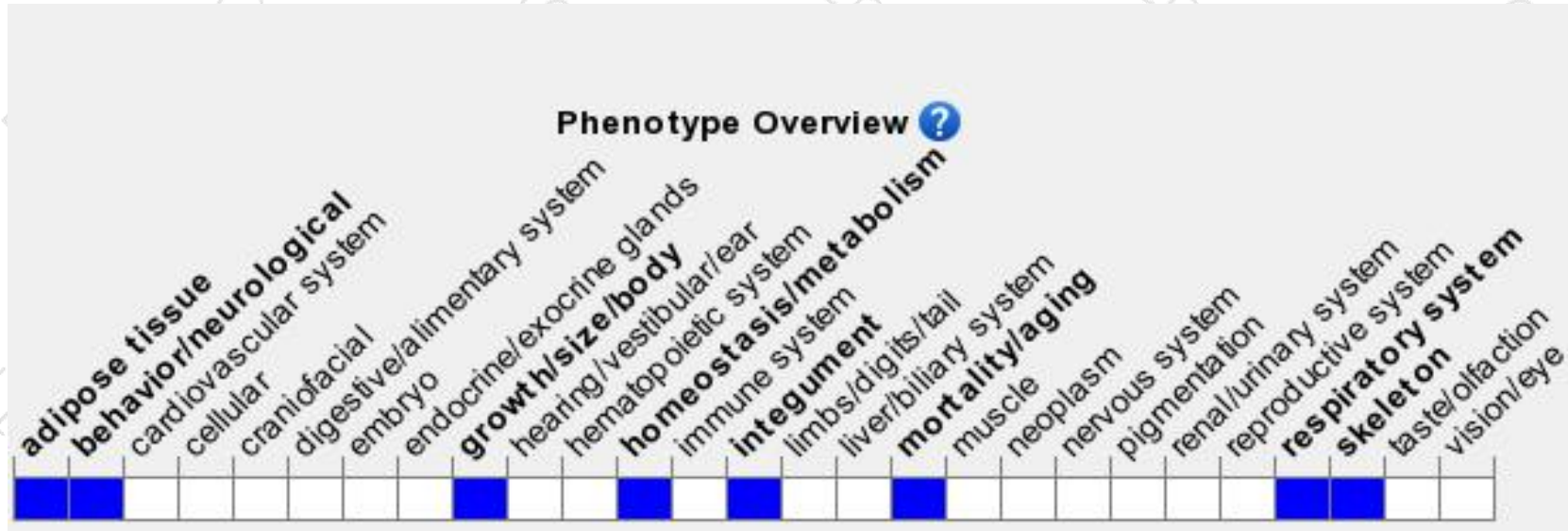




# Protein domain



# 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 are born at sub-Mendelian ratios, are smaller than control littermates, and die within a few hours of birth, probably due to alveolar defects.

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

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