Dot1l Cas9-CKO Strategy

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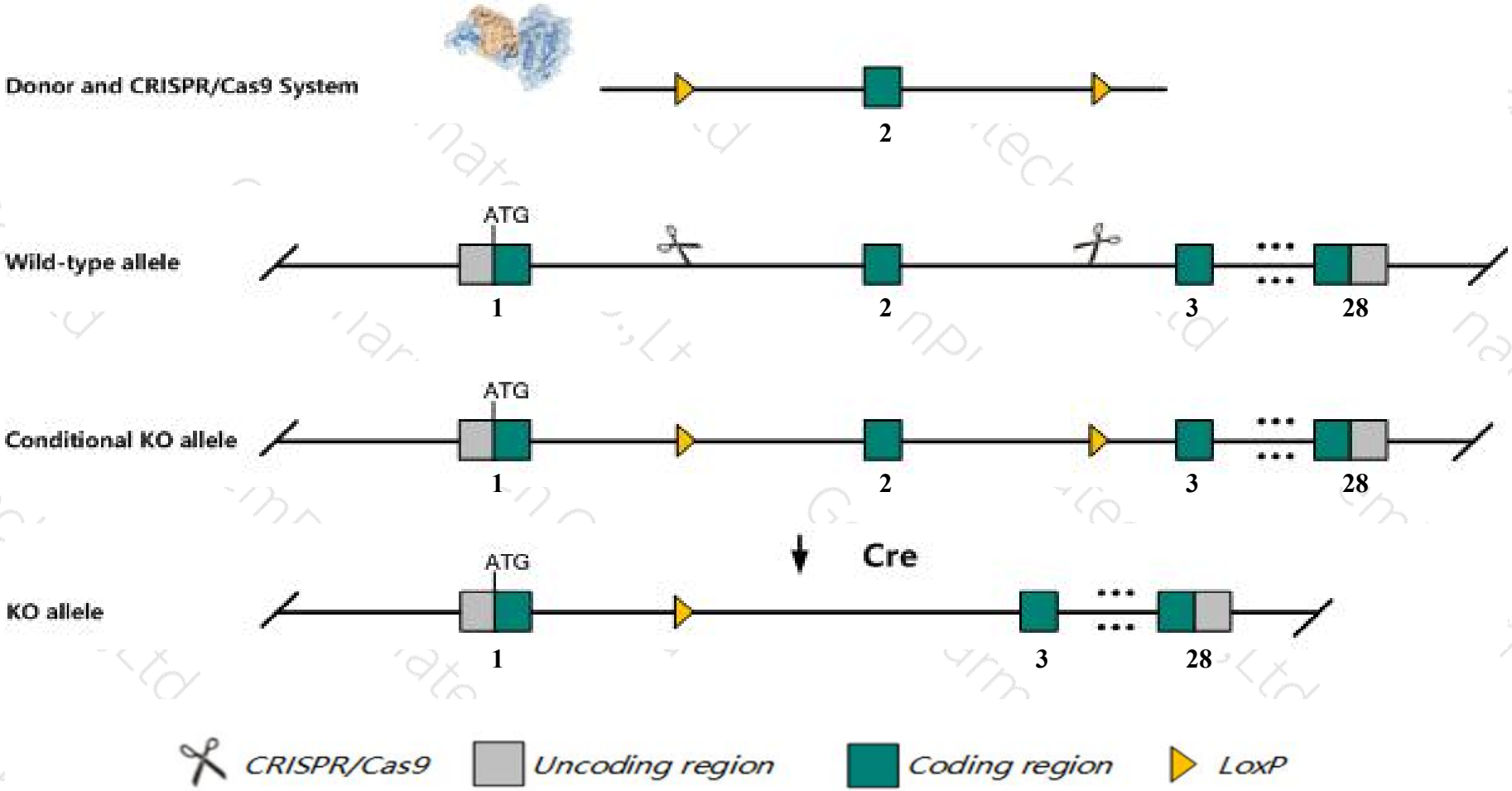
Project Overview

Project Name	***Dot1l***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Dot1l* gene. The schematic diagram is as follows:



Technical routes

- The *Dot1l* gene has 7 transcripts. According to the structure of *Dot1l* gene, exon2 of *Dot1l-201* (ENSMUST00000105336.8) transcript is recommended as the knockout region.The region contains 44bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Dot1l* 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.

Notice

- According to the existing MGI data,Mice homozygous for a gene trap allele show late embryonic lethality. Mice homozygous for a null allele die by E10.5 displaying a growth arrest, abnormal yolk sac angiogenesis and heart dilation while mutant ES cells show elevated apoptosis, G2 cell cycle arrest, telomere elongation and aneuploidy.
- Transcript *Dot1l-203,206* may not be affected.
- The *Dot1l* 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）

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Dot1l DOT1-like, histone H3 methyltransferase (S. cerevisiae) [Mus musculus (house mouse)]

Gene ID: 208266, updated on 12-Feb-2019

Summary

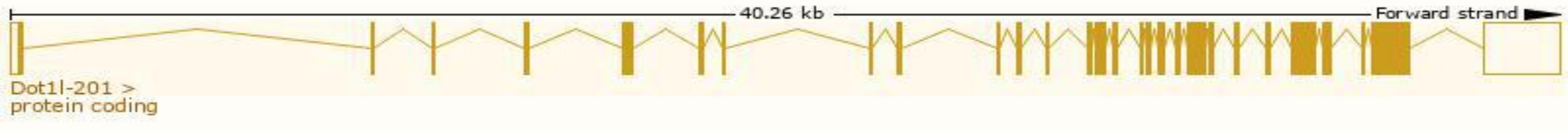
Official Symbol	Dot1l provided by MGI
Official Full Name	DOT1-like, histone H3 methyltransferase (S. cerevisiae) provided by MGI
Primary source	MGI:MGI:2143886
See related	Ensembl:ENSMUSG00000061589
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	A630076O07, AW907654, Dot1, KMT4, mDot1
Expression	Ubiquitous expression in testis adult (RPKM 34.5), adrenal adult (RPKM 27.1) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

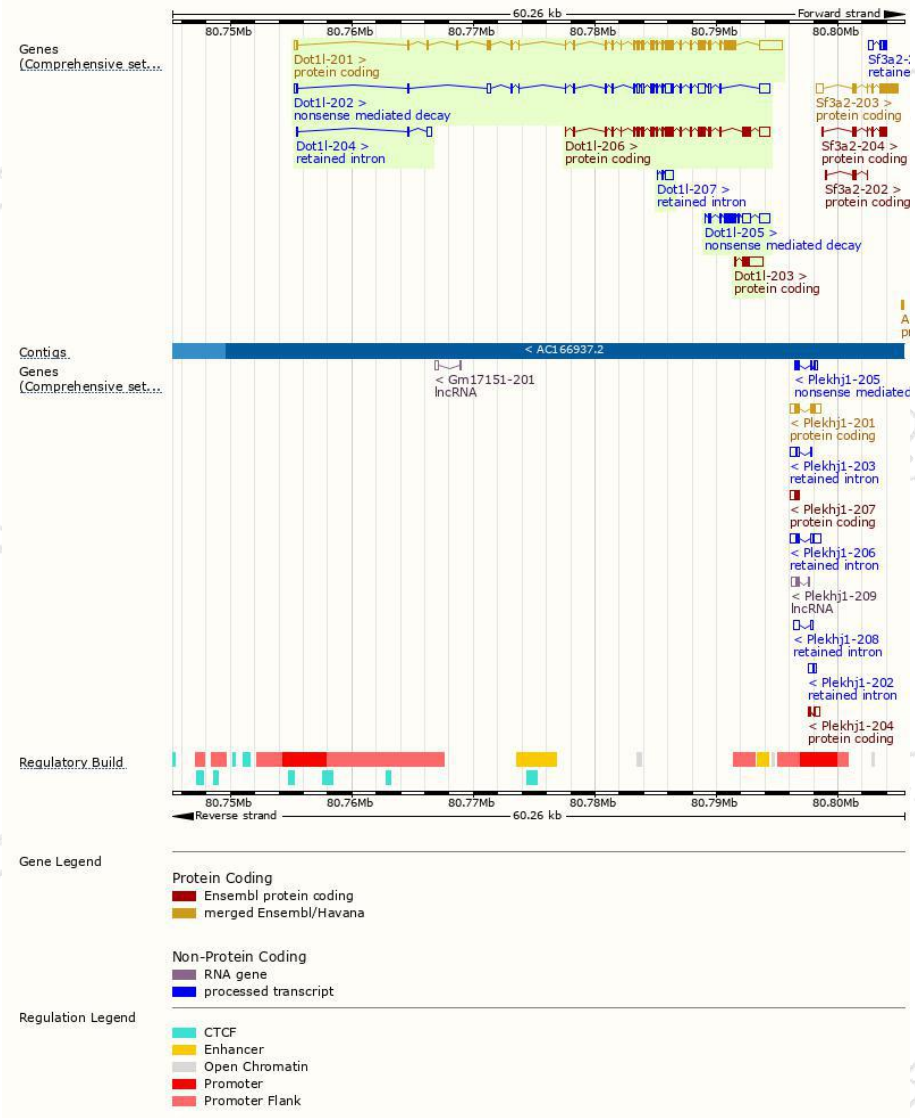
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dot1l-201	ENSMUST00000105336.8	6808	1540aa	Protein coding	CCDS35985	Q6XZL8	TSL:1 GENCODE basic APPRIS P1
Dot1l-206	ENSMUST00000150338.7	4529	1202aa	Protein coding	-	F7CVL0	CDS 5' incomplete TSL:1
Dot1l-203	ENSMUST00000138505.1	1784	234aa	Protein coding	-	F6S070	CDS 5' incomplete TSL:1
Dot1l-202	ENSMUST00000127740.7	4600	49aa	Nonsense mediated decay	-	E9Q6I8	TSL:1
Dot1l-205	ENSMUST00000149394.1	2820	424aa	Nonsense mediated decay	-	F6SJX8	CDS 5' incomplete TSL:1
Dot1l-207	ENSMUST00000163526.1	790	No protein	Retained intron	-	-	TSL:3
Dot1l-204	ENSMUST00000147579.1	531	No protein	Retained intron	-	-	TSL:2

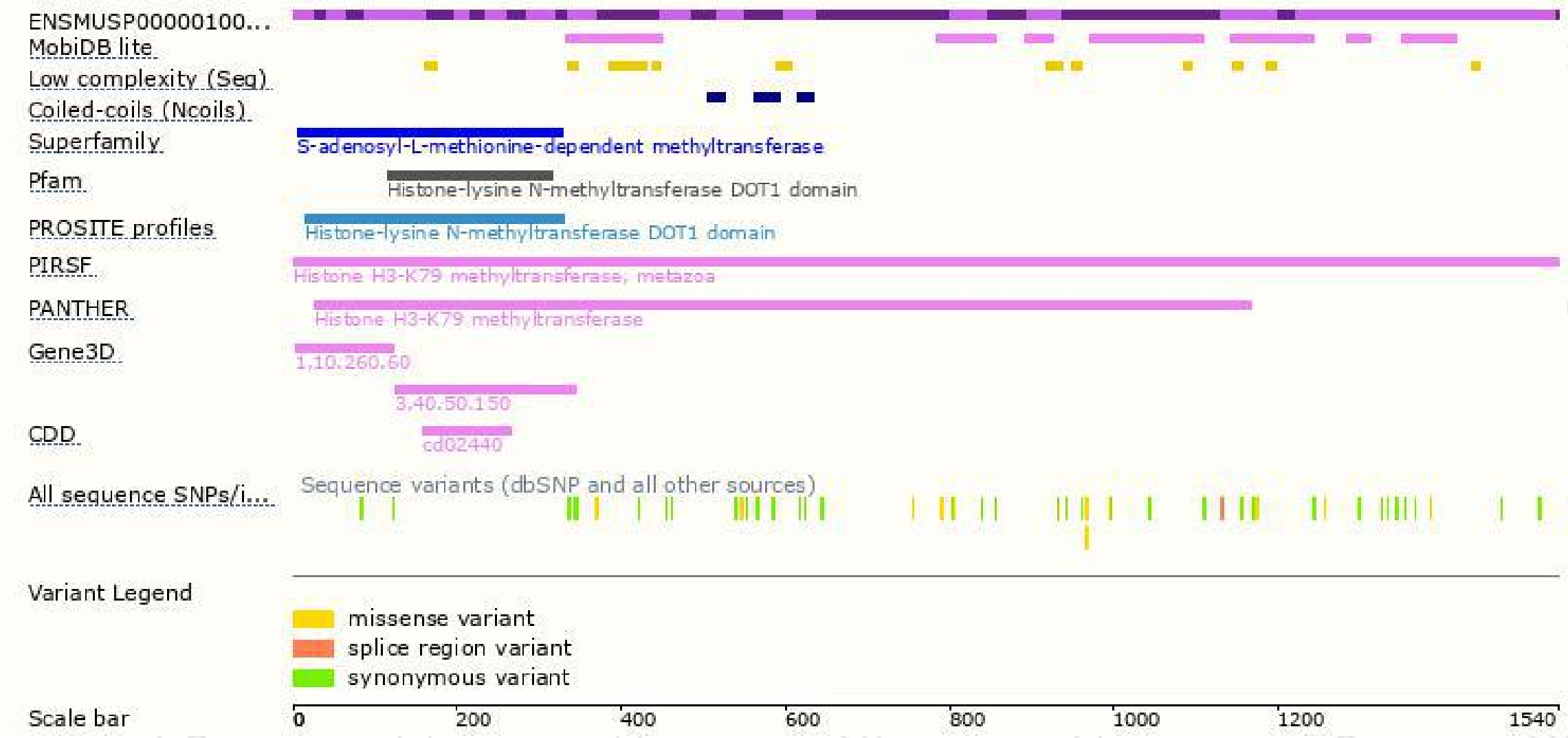
The strategy is based on the design of *Dot1l-201* transcript,The transcription is shown below



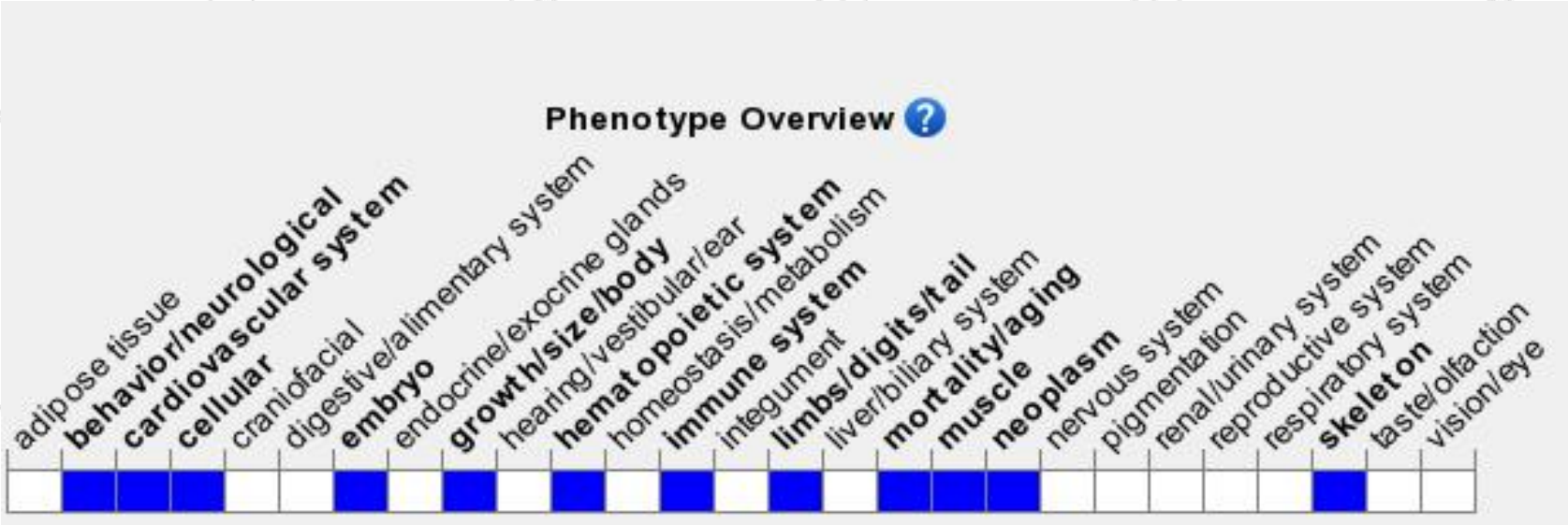
Genomic location distribution



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/).

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

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