

Mettl2 Cas9-CKO Strategy

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Project Overview

Project Name

Mettl2

Project type

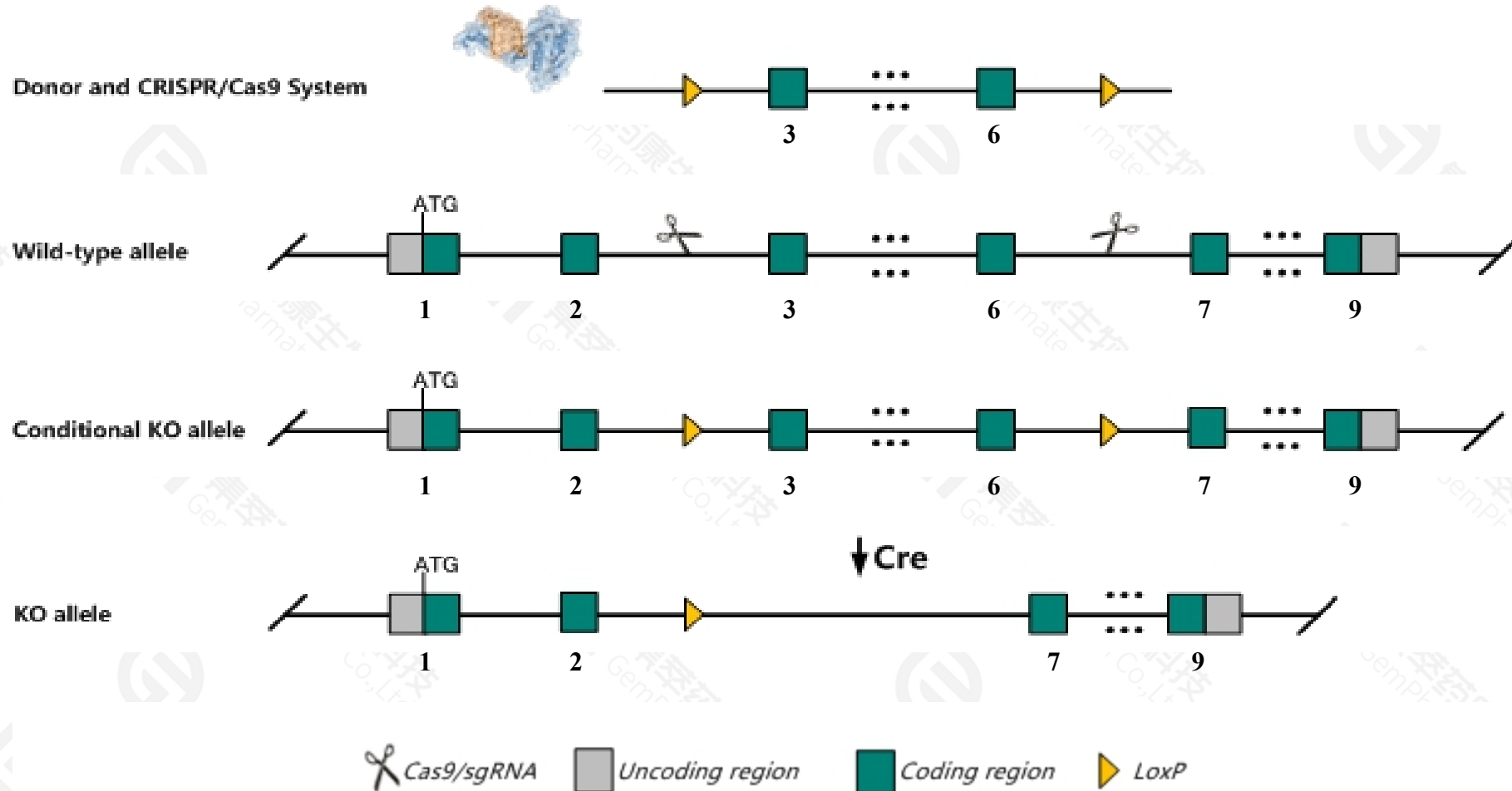
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mettl2* gene. The schematic diagram is as follows:



Technical routes

- The *Mettl2* gene has 5 transcripts. According to the structure of *Mettl2* gene, exon3-exon6 of *Mettl2*-201(ENSMUST00000021030.8) transcript is recommended as the knockout region. The region contains 586bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Mettl2* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor 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 exhibit reduced 3-methylcytidine (m3C) methyltransferases modification of tRNA.
- The *Mettl2* gene is located on the Chr11. 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)

Mettl2 methyltransferase like 2 [Mus musculus (house mouse)]

Gene ID: 52686, updated on 17-Dec-2020

Summary



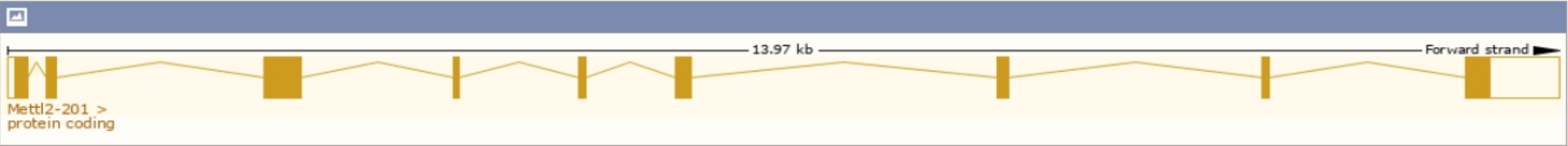
Official Symbol	Mettl2 provided by MGI
Official Full Name	methyltransferase like 2 provided by MGI
Primary source	MGI:MGI:1289171
See related	Ensembl:ENSMUSG00000020691
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2810438F06Rik, AW493404, C130031G21Rik, D11Ert768, D11Ert768e, METL, PS, PSENIP1
Expression	Ubiquitous expression in CNS E11.5 (RPKM 6.6), limb E14.5 (RPKM 6.5) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

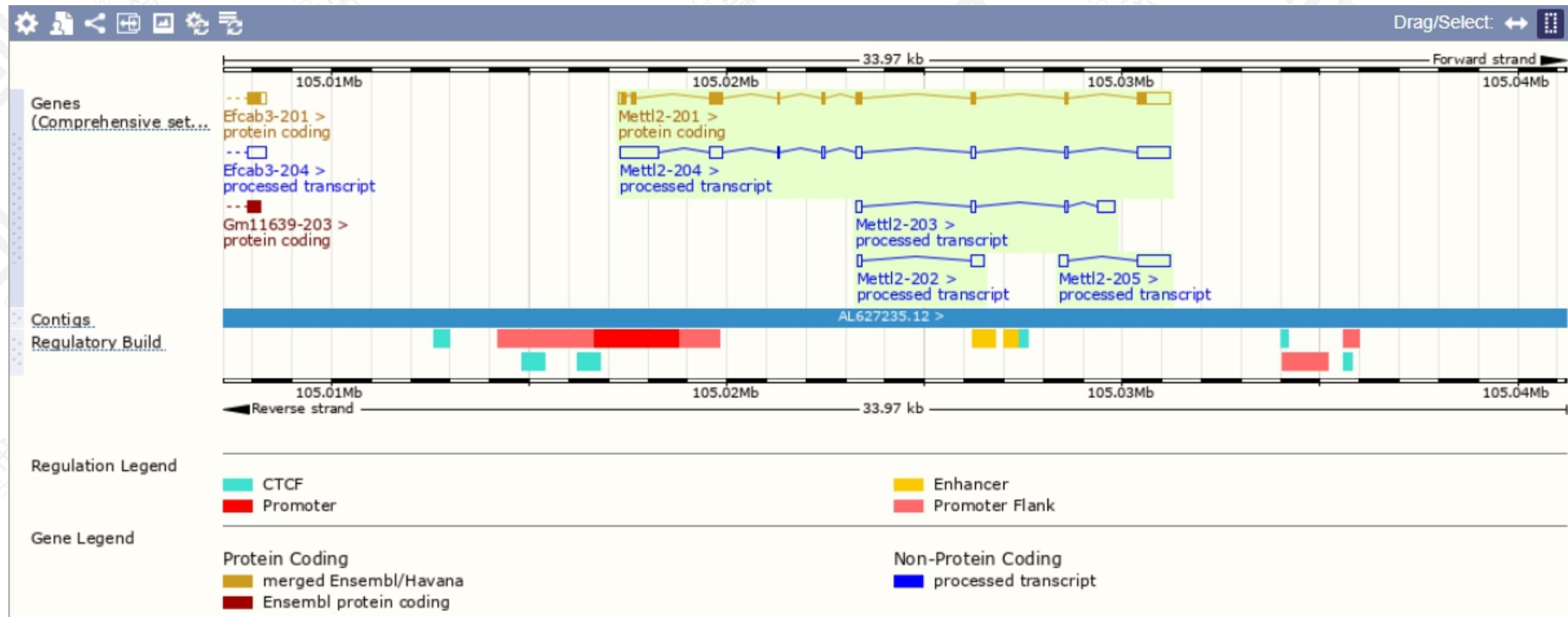
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mettl2-201	ENSMUST00000021030.8	1867	389aa	Protein coding	CCDS25538		TSL:1 , GENCODE basic , APPRIS P1 ,
Mettl2-204	ENSMUST00000136214.8	2556	No protein	Processed transcript	-		TSL:1 ,
Mettl2-205	ENSMUST00000155460.2	1056	No protein	Processed transcript	-		TSL:2 ,
Mettl2-203	ENSMUST00000132185.2	726	No protein	Processed transcript	-		TSL:3 ,
Mettl2-202	ENSMUST00000129012.2	440	No protein	Processed transcript	-		TSL:5 ,

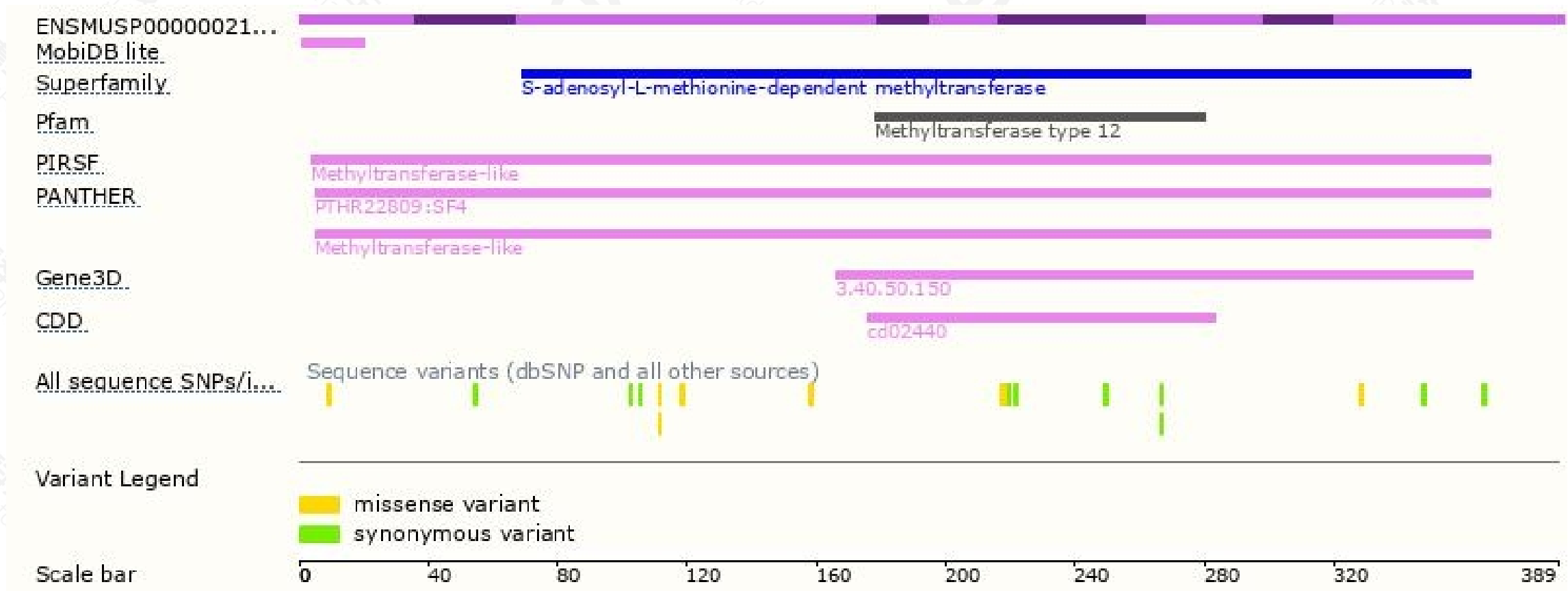
The strategy is based on the design of *Mettl2-201* transcript,the transcription is shown below:



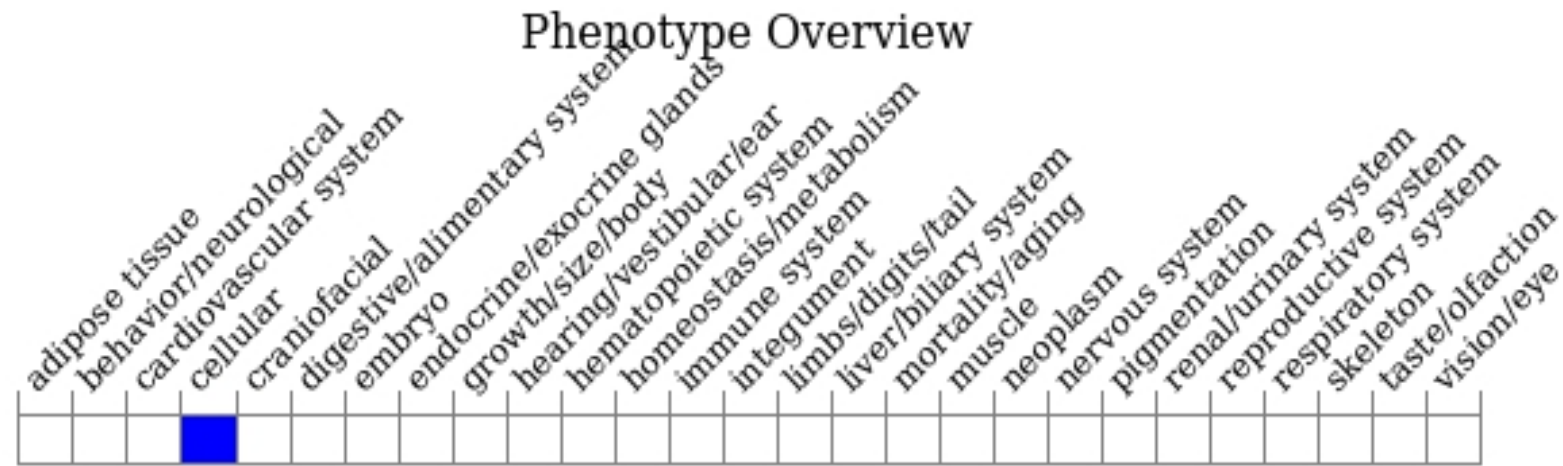
Genomic location distribution



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/>).

According to the existing MGI data, mice homozygous for a knock-out allele exhibit reduced 3-methylcytidine (m3C) methyltransferases modification of tRNA.

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

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