

Mthfd1 Cas9-CKO Strategy

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Project Overview

Project Name

Mthfd1

Project type

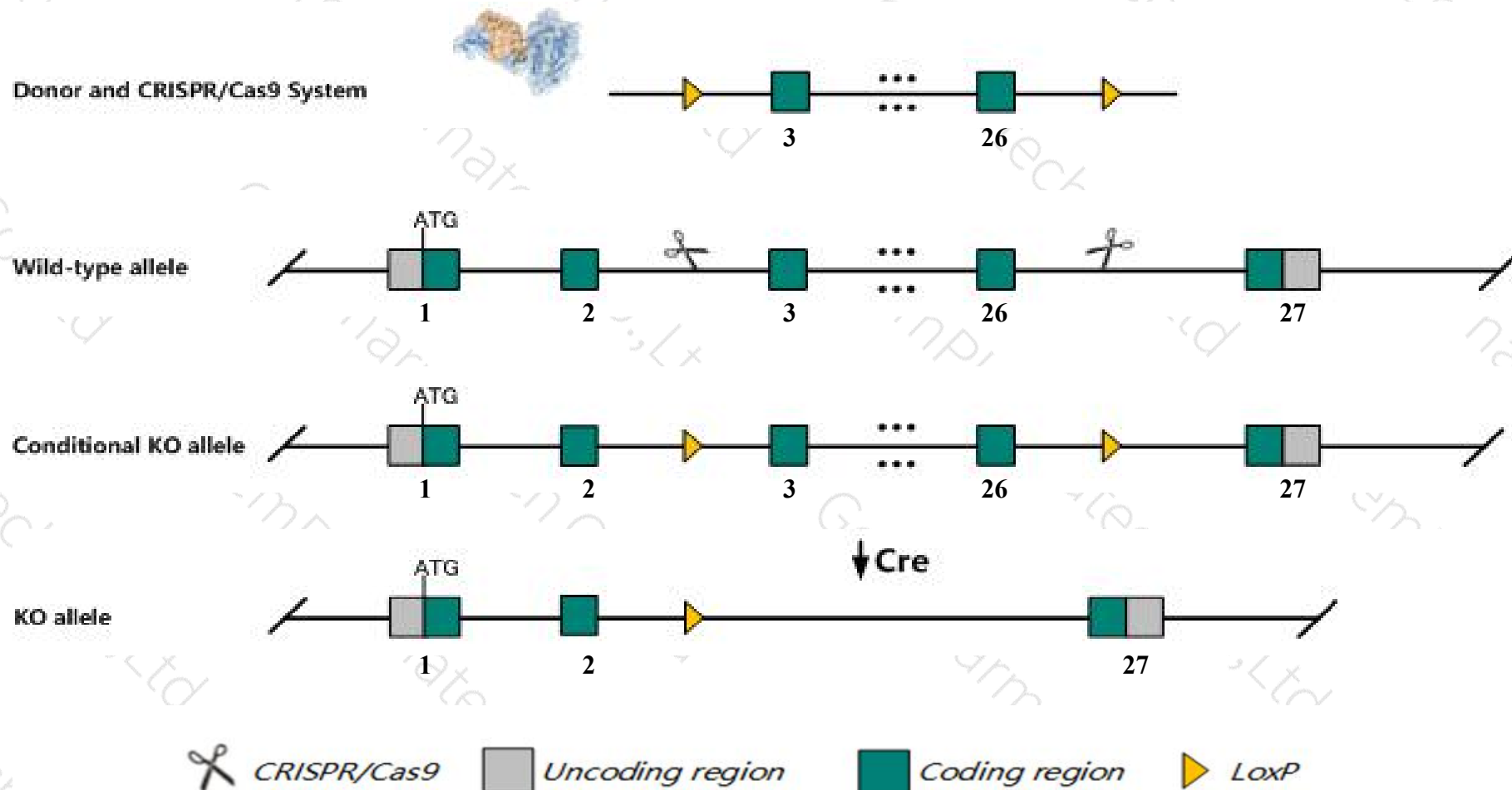
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mthfd1* gene. The schematic diagram is as follows:



- The *Mthfd1* gene has 7 transcripts. According to the structure of *Mthfd1* gene, exon3-exon26 of *Mthfd1-201* (ENSMUST00000021443.6) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Mthfd1* 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.

- According to the existing MGI data, Mice homozygous for a gene trapped allele exhibit embryonic lethality. Mice heterozygous for a gene trap allele exhibit altered amino acid levels and nucleotide metabolism related to dietary folate and choline concentrations.
- The flox region is about 1.6 kb from the 5th end of the *Gm47526-201* gene, and the insertion of loxp may affect the regulation of the 5th end of the gene.
- *Gm34868-201* will be deleted at the same time.
- The *Mthfd1* gene is located on the Chr12. 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)

Mthfd1 methylenetetrahydrofolate dehydrogenase (NADP+ dependent), methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthase [Mus musculus (house mouse)]

Gene ID: 108156, updated on 12-Mar-2019

Summary



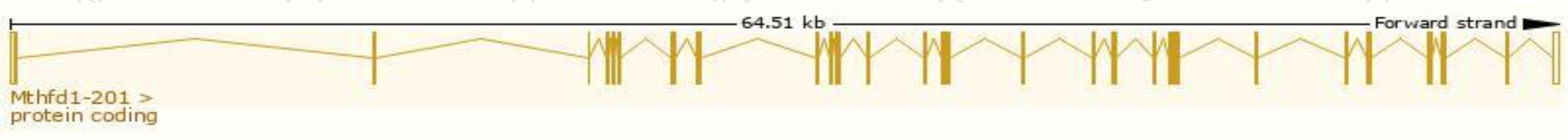
Official Symbol	Mthfd1 provided by MGI
Official Full Name	methylenetetrahydrofolate dehydrogenase (NADP+ dependent), methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthase provided by MGI
Primary source	MGI:MGI:1342005
See related	Ensembl:ENSMUSG000000021048
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Dcs, E430024A07Rik, Mthfd
Summary	This gene encodes a trifunctional cytoplasmic enzyme. The encoded protein functions as a methylenetetrahydrofolate dehydrogenase, a methenyltetrahydrofolate cyclohydrolase, and a formyltetrahydrofolate synthase. The encoded enzyme functions in de novo synthesis of purines and thymidylate and in regeneration of methionine from homocysteine. [provided by RefSeq, Oct 2009]
Expression	Ubiquitous expression in kidney adult (RPKM 44.6), liver adult (RPKM 41.3) and 27 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

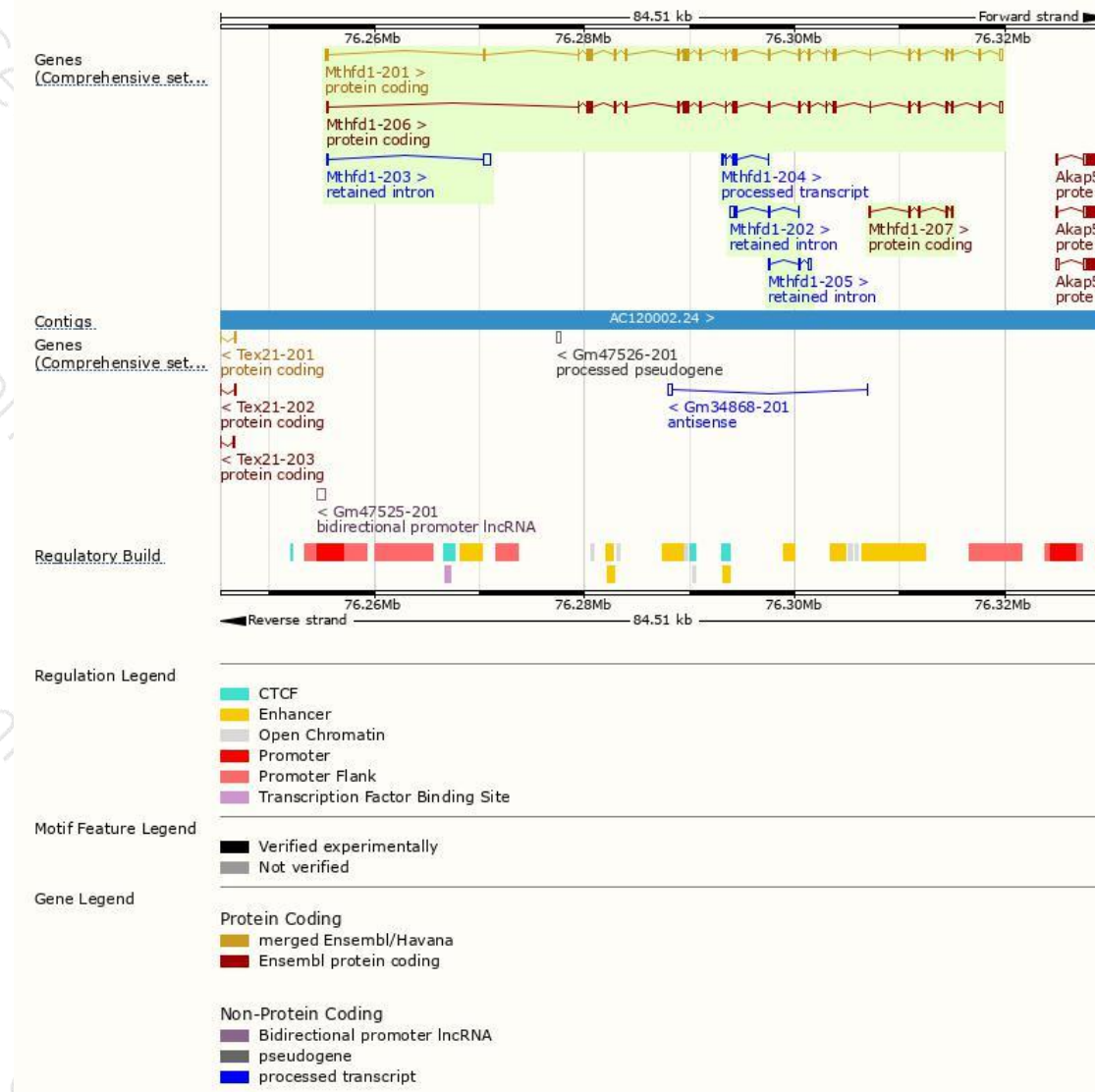
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mthfd1-201	ENSMUST00000021443.6	3241	935aa	Protein coding	CCDS25990	Q922D8	TSL:1 GENCODE basic APPRIS P1
Mthfd1-206	ENSMUST00000220046.1	3040	755aa	Protein coding	-	A0A1W2P733	TSL:1 GENCODE basic
Mthfd1-207	ENSMUST00000220321.1	738	160aa	Protein coding	-	A0A1W2P7L5	CDS 3' incomplete TSL:2
Mthfd1-204	ENSMUST00000218331.1	359	No protein	Processed transcript	-	-	TSL:3
Mthfd1-202	ENSMUST00000218010.1	674	No protein	Retained intron	-	-	TSL:3
Mthfd1-203	ENSMUST00000218110.1	642	No protein	Retained intron	-	-	TSL:2
Mthfd1-205	ENSMUST00000218513.1	406	No protein	Retained intron	-	-	TSL:3

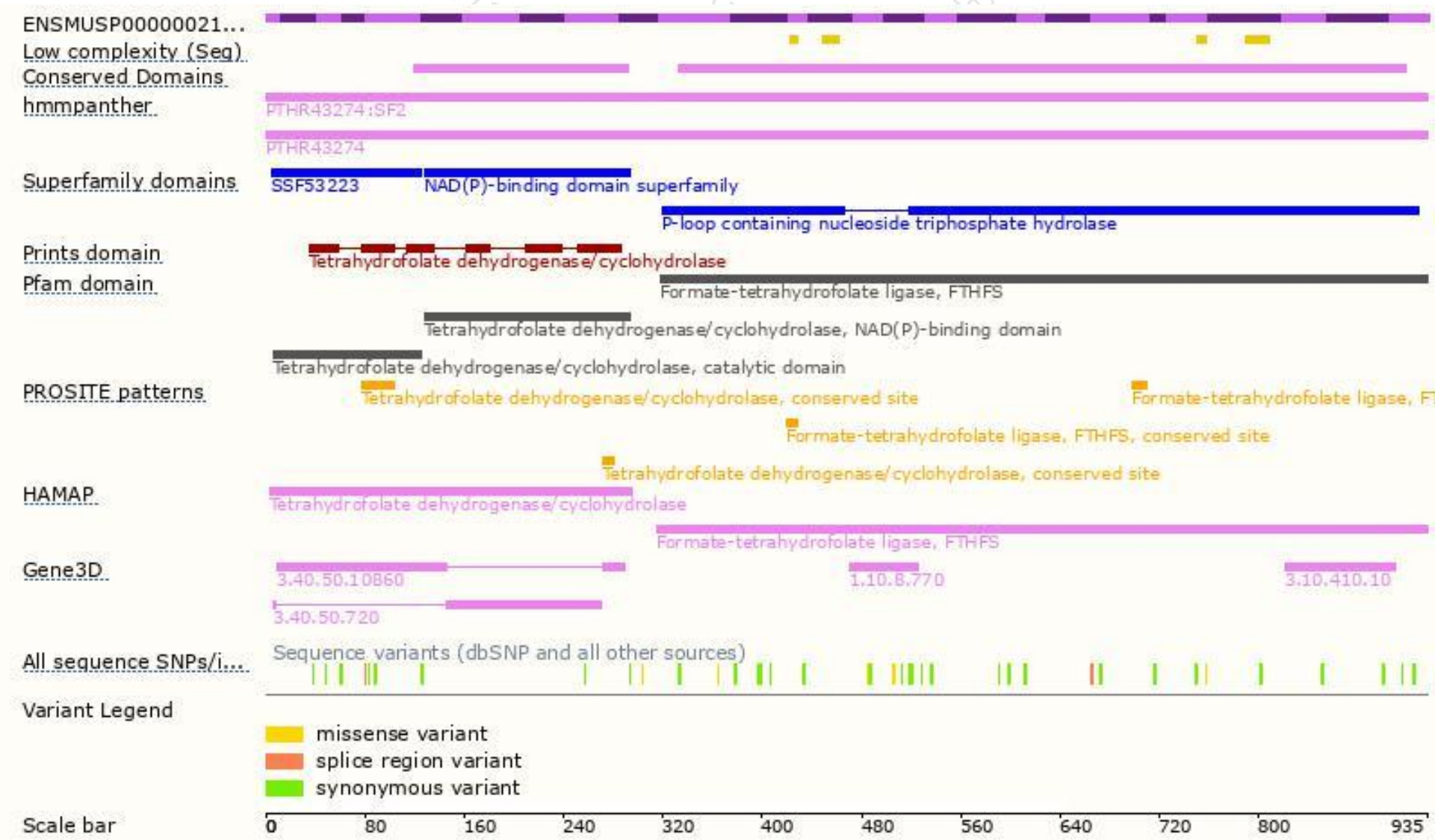
The strategy is based on the design of *Mthfd1-201* transcript,The transcription is shown below



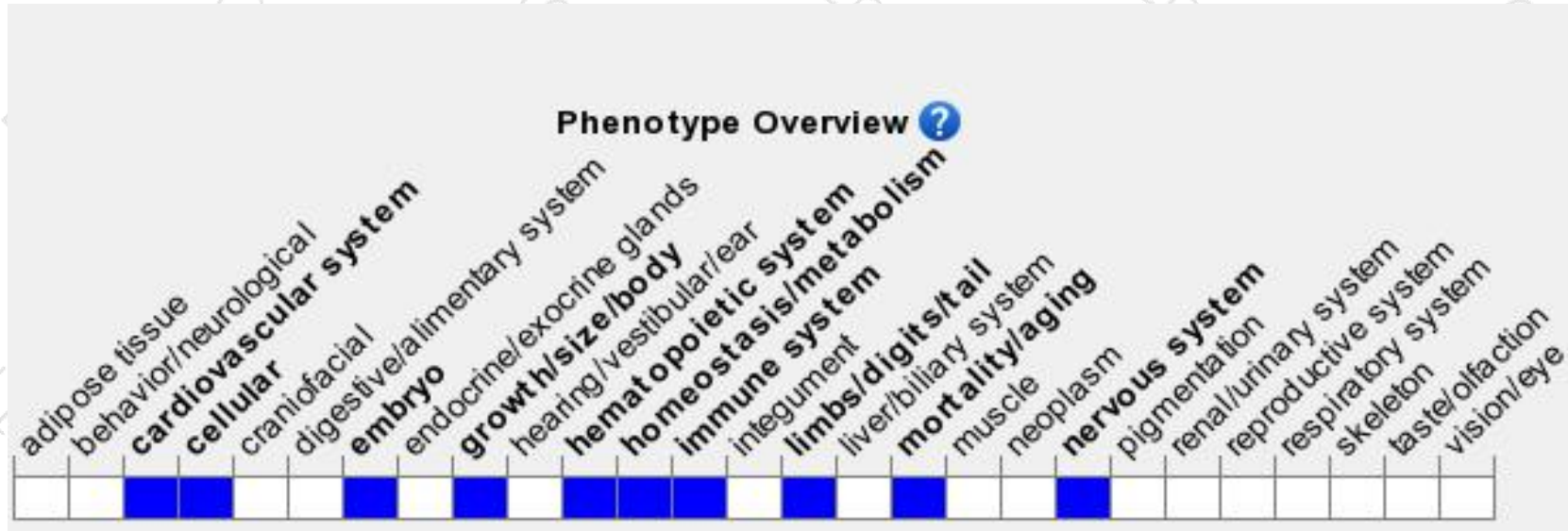
Genomic location distribution



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 gene trapped allele exhibit embryonic lethality. Mice heterozygous for a gene trap allele exhibit altered amino acid levels and nucleotide metabolism related to dietary folate and choline concentrations.

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

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