

Mthfd2 Cas9-CKO Strategy

Designer:

Project Overview

Project Name

Mthfd2

Project type

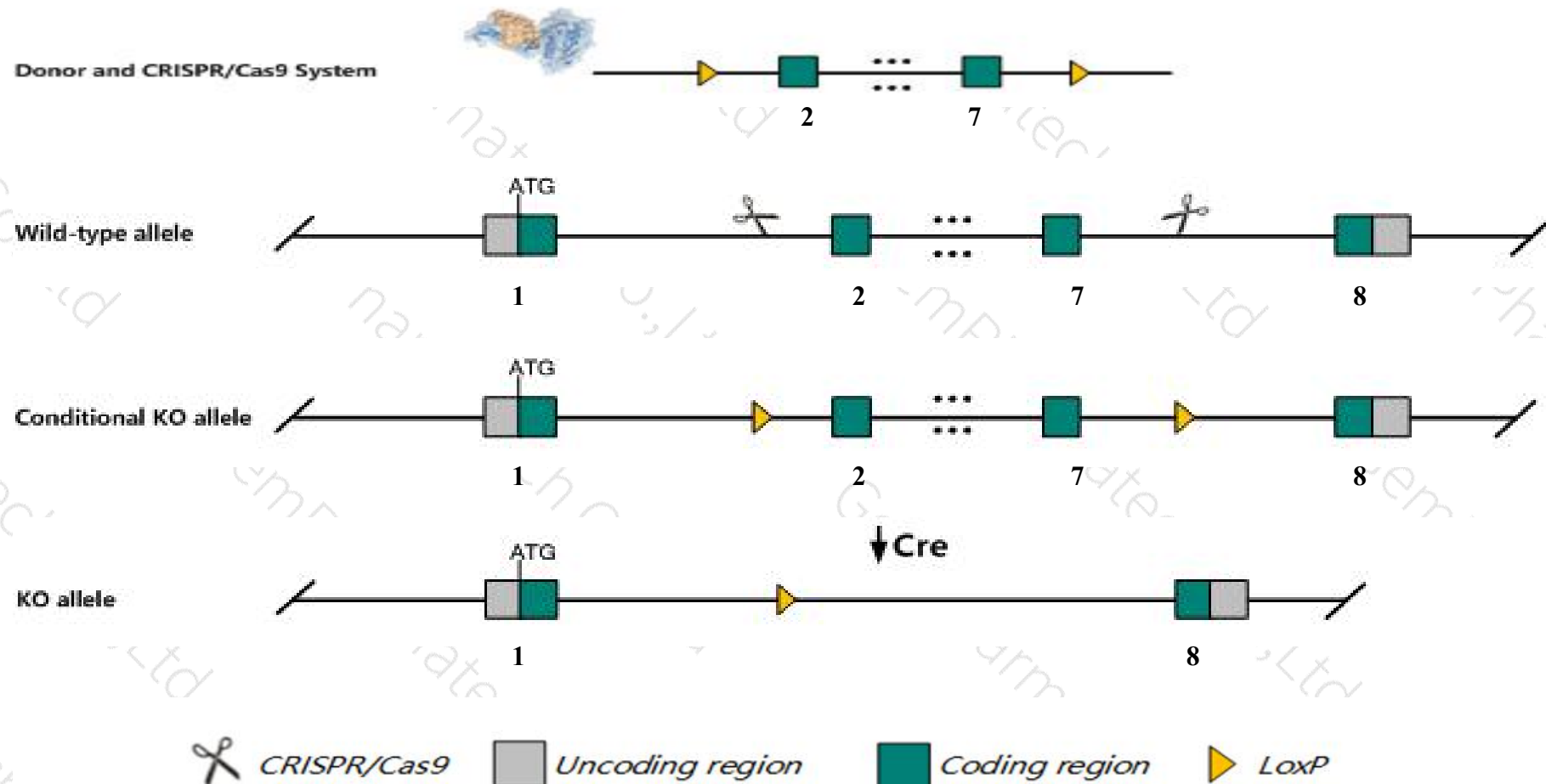
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mthfd2* gene. The schematic diagram is as follows:



- The *Mthfd2* gene has 7 transcripts. According to the structure of *Mthfd2* gene, exon2-exon7 of *Mthfd2*-201 (ENSMUST00000005810.8) transcript is recommended as the knockout region. The region contains 788bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Mthfd2* 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 disruptions in this gene die as embryos before E15.5.
- The *Mthfd2* gene is located on the Chr6. 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

Mthfd2 methylenetetrahydrofolate dehydrogenase (NAD⁺ dependent), methenyltetrahydrofolate cyclohydrolase [Mus musculus (house mouse)]

Gene ID: 17768, updated on 31-Jan-2019

Summary



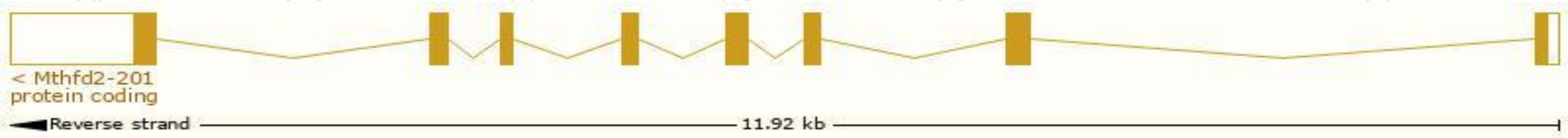
Official Symbol	Mthfd2 provided by MGI
Official Full Name	methylenetetrahydrofolate dehydrogenase (NAD ⁺ dependent), methenyltetrahydrofolate cyclohydrolase provided by MGI
Primary source	MGI:MGI:1338850
See related	Ensembl:ENSMUSG00000005667
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW558851, NMDMC
Expression	Broad expression in liver E14 (RPKM 25.7), liver E14.5 (RPKM 21.2) and 17 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

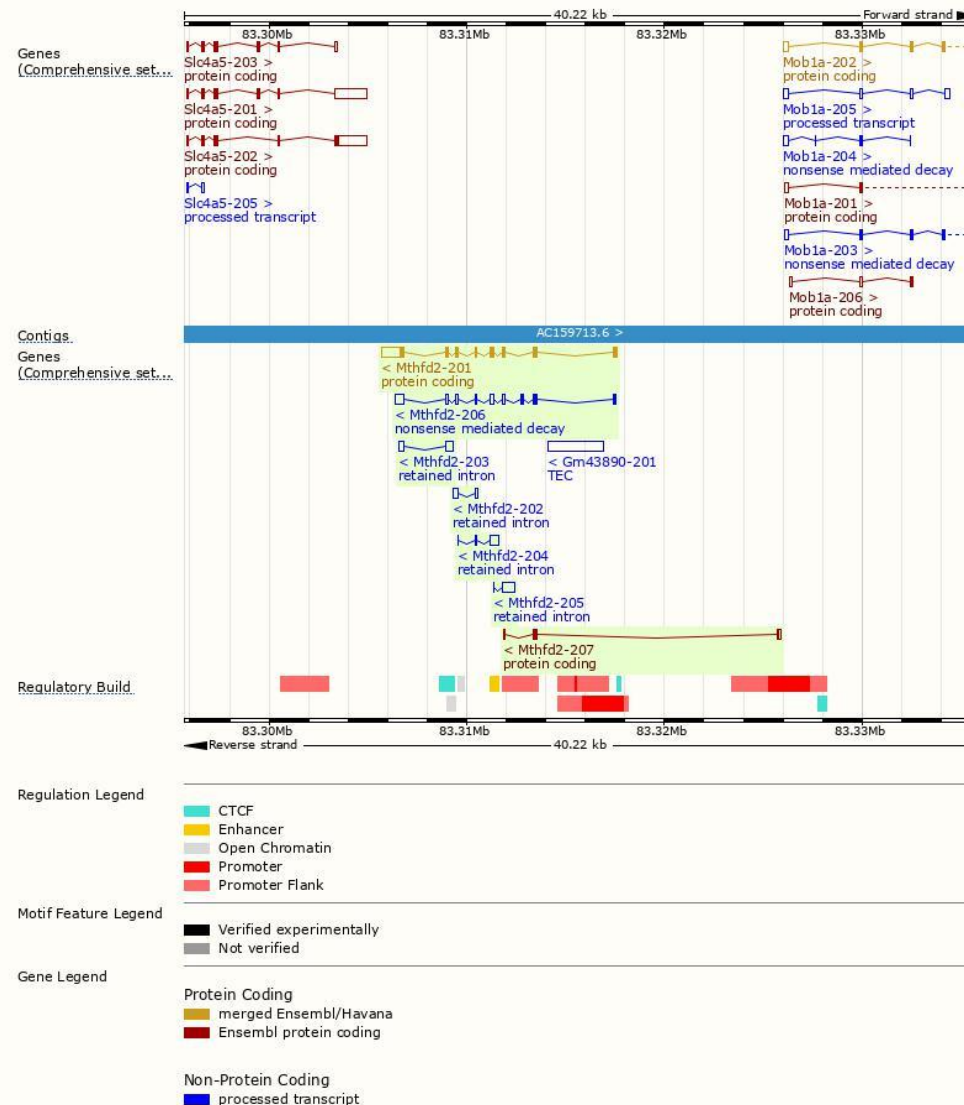
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mthfd2-201	ENSMUST00000005810.8	2085	350aa	Protein coding	CCDS20277	P18155	TSL:1 GENCODE basic APPRIS P1
Mthfd2-207	ENSMUST00000204472.1	443	97aa	Protein coding	-	A0A0N4SVS2	CDS 3' incomplete TSL:3
Mthfd2-206	ENSMUST00000203847.2	1421	123aa	Nonsense mediated decay	-	A0A0N4SVW2	TSL:5
Mthfd2-203	ENSMUST00000141044.1	627	No protein	Retained intron	-	-	TSL:1
Mthfd2-205	ENSMUST00000203375.1	612	No protein	Retained intron	-	-	TSL:3
Mthfd2-204	ENSMUST00000141193.1	495	No protein	Retained intron	-	-	TSL:1
Mthfd2-202	ENSMUST00000139802.1	358	No protein	Retained intron	-	-	TSL:3

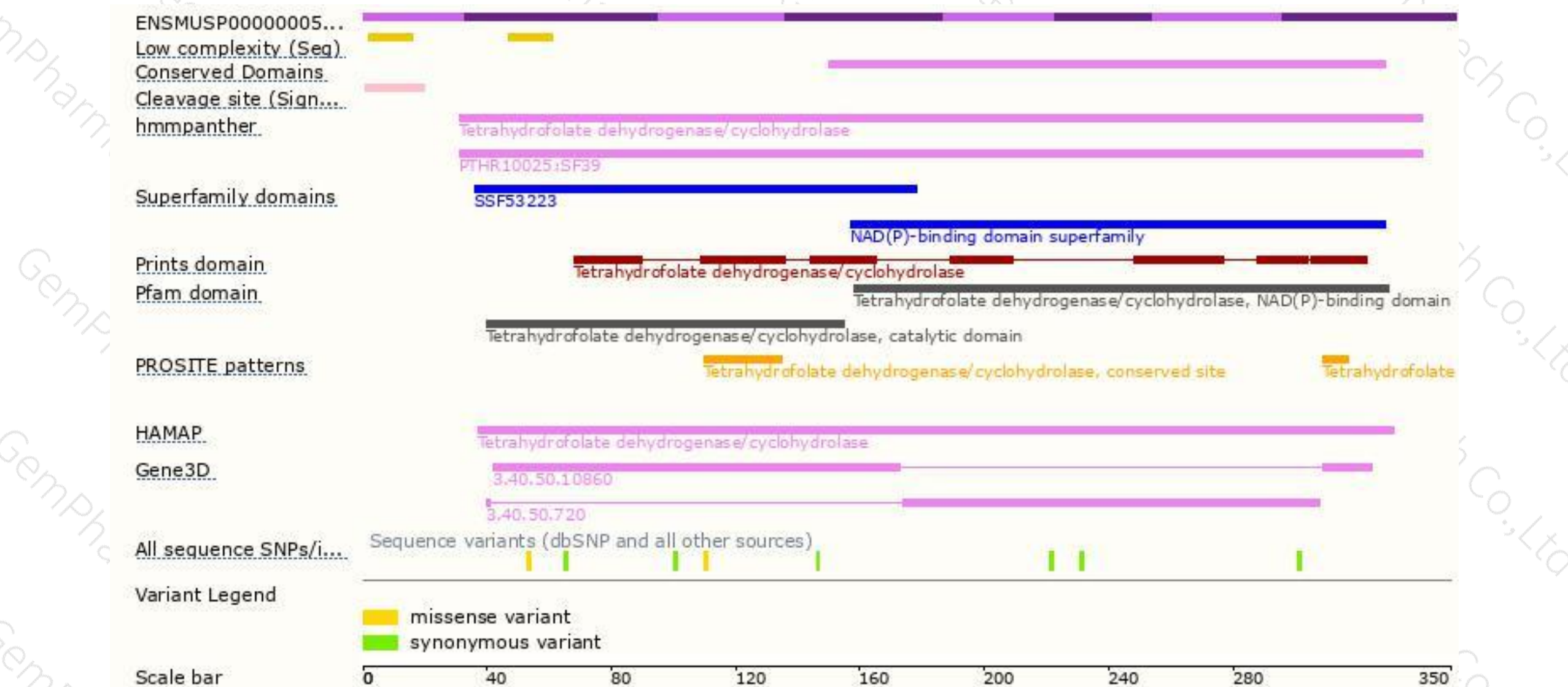
The strategy is based on the design of *Mthfd2-201* transcript,The transcription is shown below



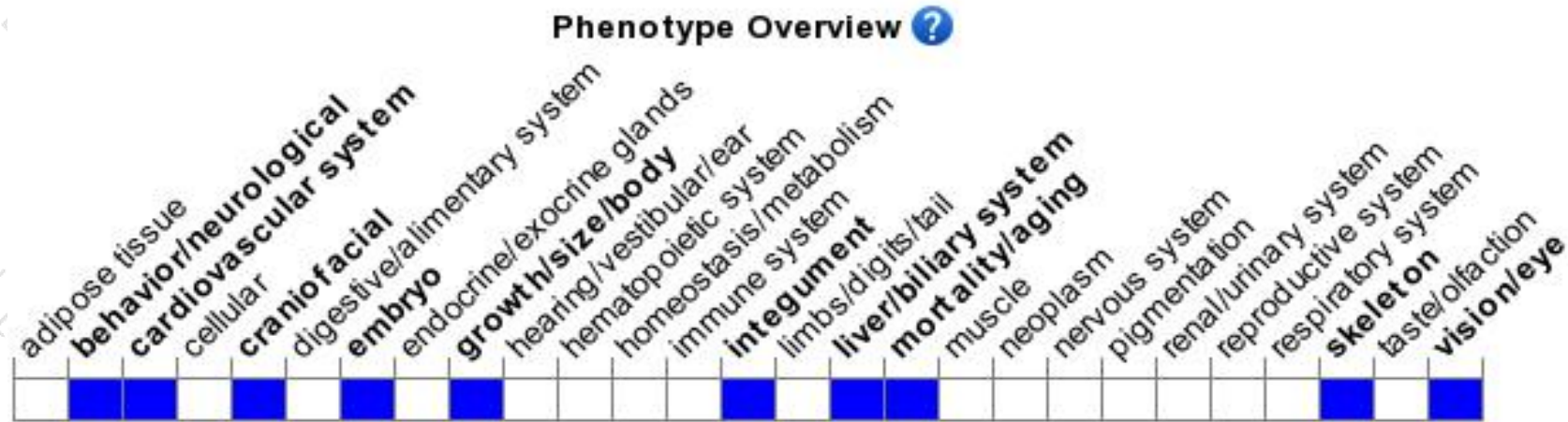
Genomic location distribution



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 disruptions in this gene die as embryos before E15.5.

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

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