

Nedd4 Cas9-CKO Strategy

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Project Overview

Project Name

Nedd4

Project type

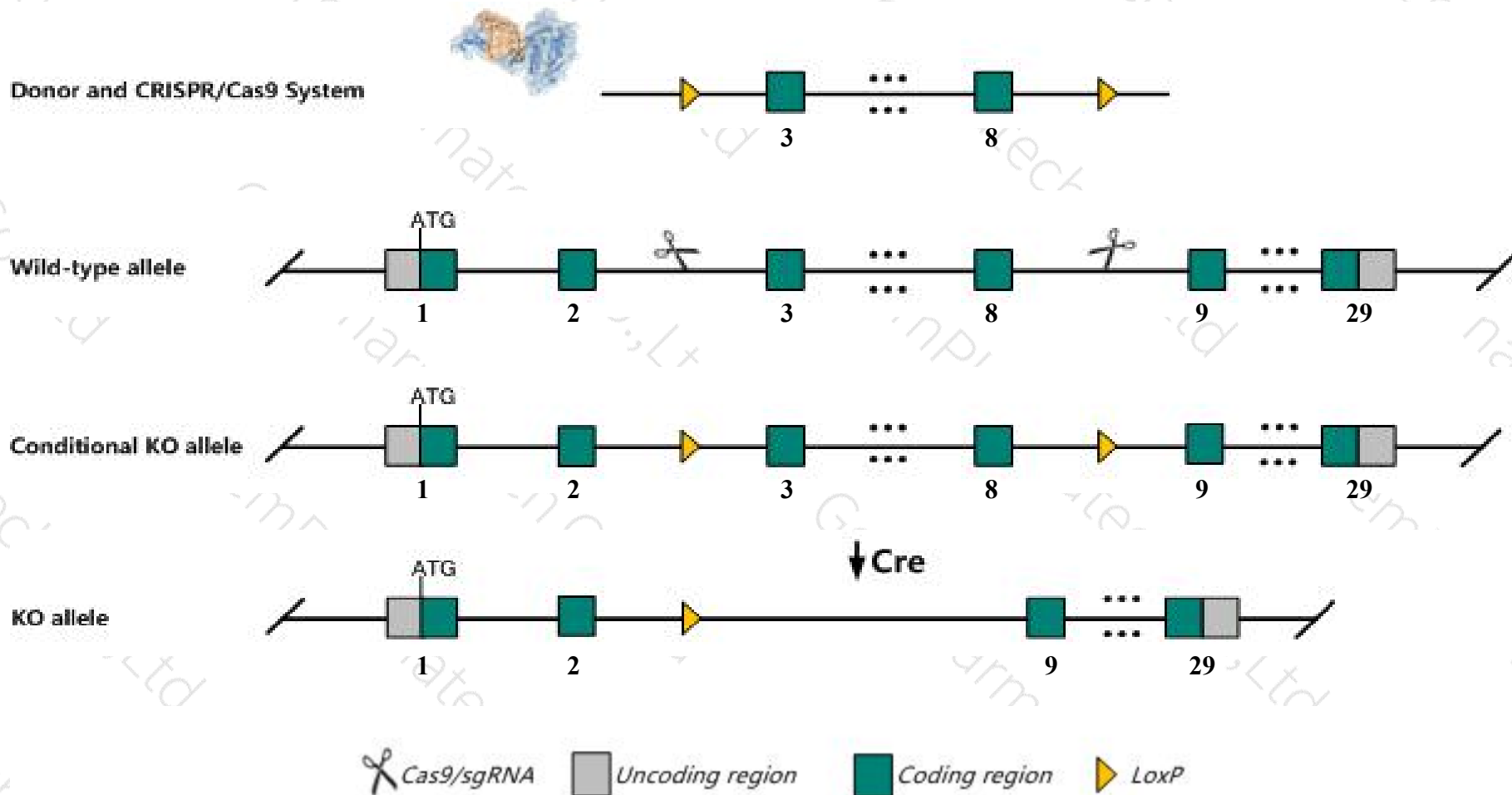
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nedd4* gene. The schematic diagram is as follows:



- The *Nedd4* gene has 10 transcripts. According to the structure of *Nedd4* gene, exon3-exon8 of *Nedd4-201* (ENSMUST00000034740.14) transcript is recommended as the knockout region. The region contains 359bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Nedd4* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector 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, Homozygous mutation of this gene results in neonatal lethality and heterozygous mice have decreased body weights. Mice homozygous for a knockout allele exhibit impaired neurite development.
- Because the incompleteness of the transcript *Nedd4*-202&211, the influence on these transcripts is unknown in this strategy.
- Transcript *Nedd4*-203&204&206&208 may not be affected.
- *A530064N14Rik* gene and *MGI:3648991* gene will be deleted together in this strategy.
- The floxed region is near to the N-terminal of *Gm37955* gene, this strategy may influence the regulatory function of the N-terminal of *Gm37955* gene.
- The *Nedd4* gene is located on the Chr9. 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)

Nedd4 neural precursor cell expressed, developmentally down-regulated 4 [Mus musculus (house mouse)]

Gene ID: 17999, updated on 31-Jan-2019

Summary



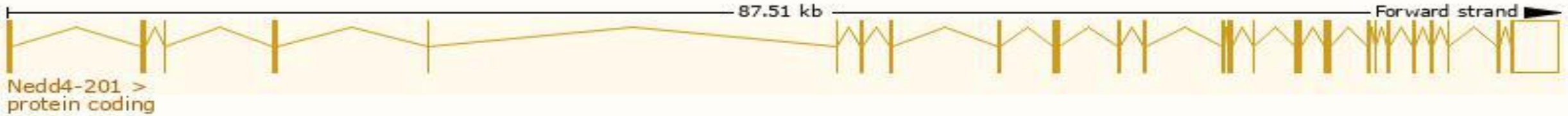
Official Symbol	Nedd4 provided by MGI
Official Full Name	neural precursor cell expressed, developmentally down-regulated 4 provided by MGI
Primary source	MGI:MGI:97297
See related	Ensembl:ENSMUSG00000032216
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AA959633, AL023035, AU019897, E430025J12Rik, EG639396, Gm7265, KIAA0093, Nedd4-1, Nedd4a
Expression	Ubiquitous expression in limb E14.5 (RPKM 241.1), CNS E11.5 (RPKM 217.5) and 25 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

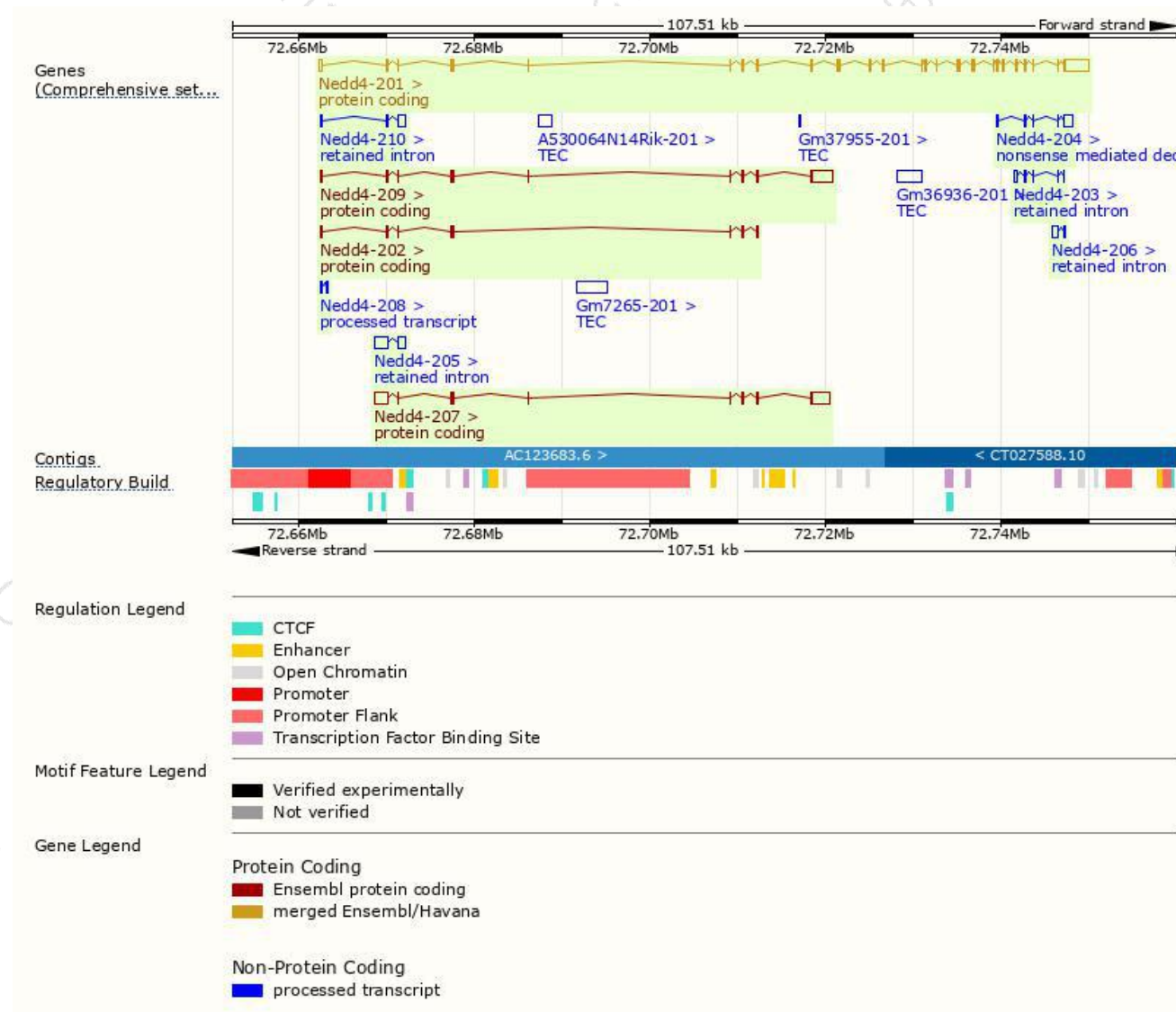
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nedd4-201	ENSMUST00000034740.14	5499	887aa	Protein coding	CCDS72275	B2RSC8 P46935	TSL:1 GENCODE basic APPRIS P1
Nedd4-207	ENSMUST00000184333.1	4034	195aa	Protein coding	-	V9G XK3	TSL:1 GENCODE basic
Nedd4-209	ENSMUST00000184450.7	3329	305aa	Protein coding	-	Q3V335	TSL:1 GENCODE basic
Nedd4-202	ENSMUST00000183375.7	660	196aa	Protein coding	-	V9G WV8	CDS 3' incomplete TSL:3
Nedd4-204	ENSMUST00000184020.7	1368	30aa	Nonsense mediated decay	-	V9G XS6	CDS 5' incomplete TSL:3
Nedd4-208	ENSMUST00000184386.1	312	No protein	Processed transcript	-	-	TSL:5
Nedd4-205	ENSMUST00000184180.1	2348	No protein	Retained intron	-	-	TSL:1
Nedd4-210	ENSMUST00000184737.1	1260	No protein	Retained intron	-	-	TSL:1
Nedd4-206	ENSMUST00000184287.1	779	No protein	Retained intron	-	-	TSL:2
Nedd4-203	ENSMUST00000183451.1	550	No protein	Retained intron	-	-	TSL:2

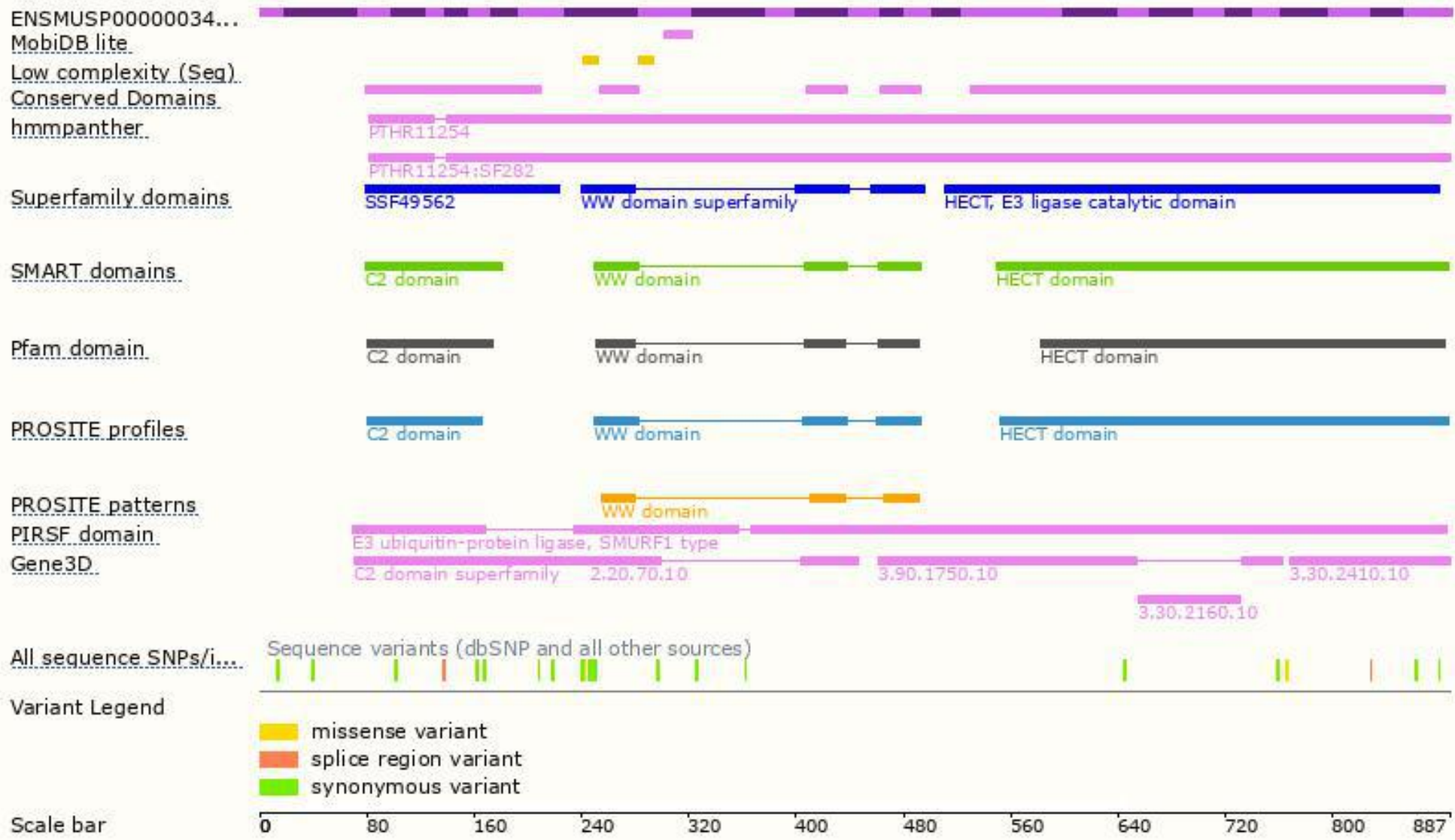
The strategy is based on the design of *Nedd4-201* transcript,The transcription is shown below



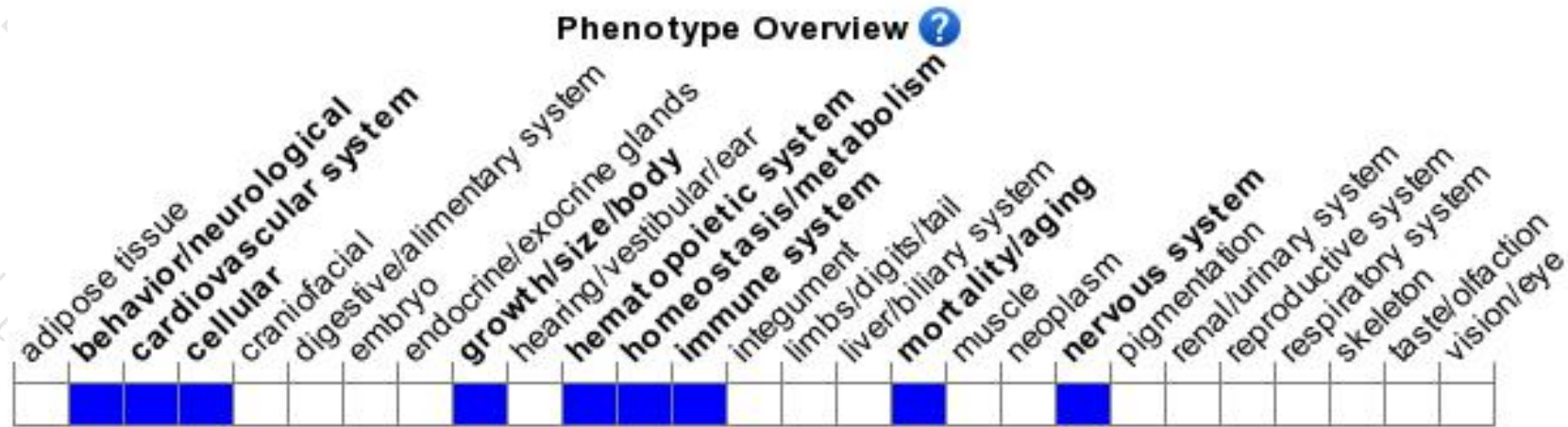
Genomic location distribution



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, Homozygous mutation of this gene results in neonatal lethality and heterozygous mice have decreased body weights. Mice homozygous for a knockout allele exhibit impaired neurite development.

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

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