Nfasc Cas9-CKO Strategy

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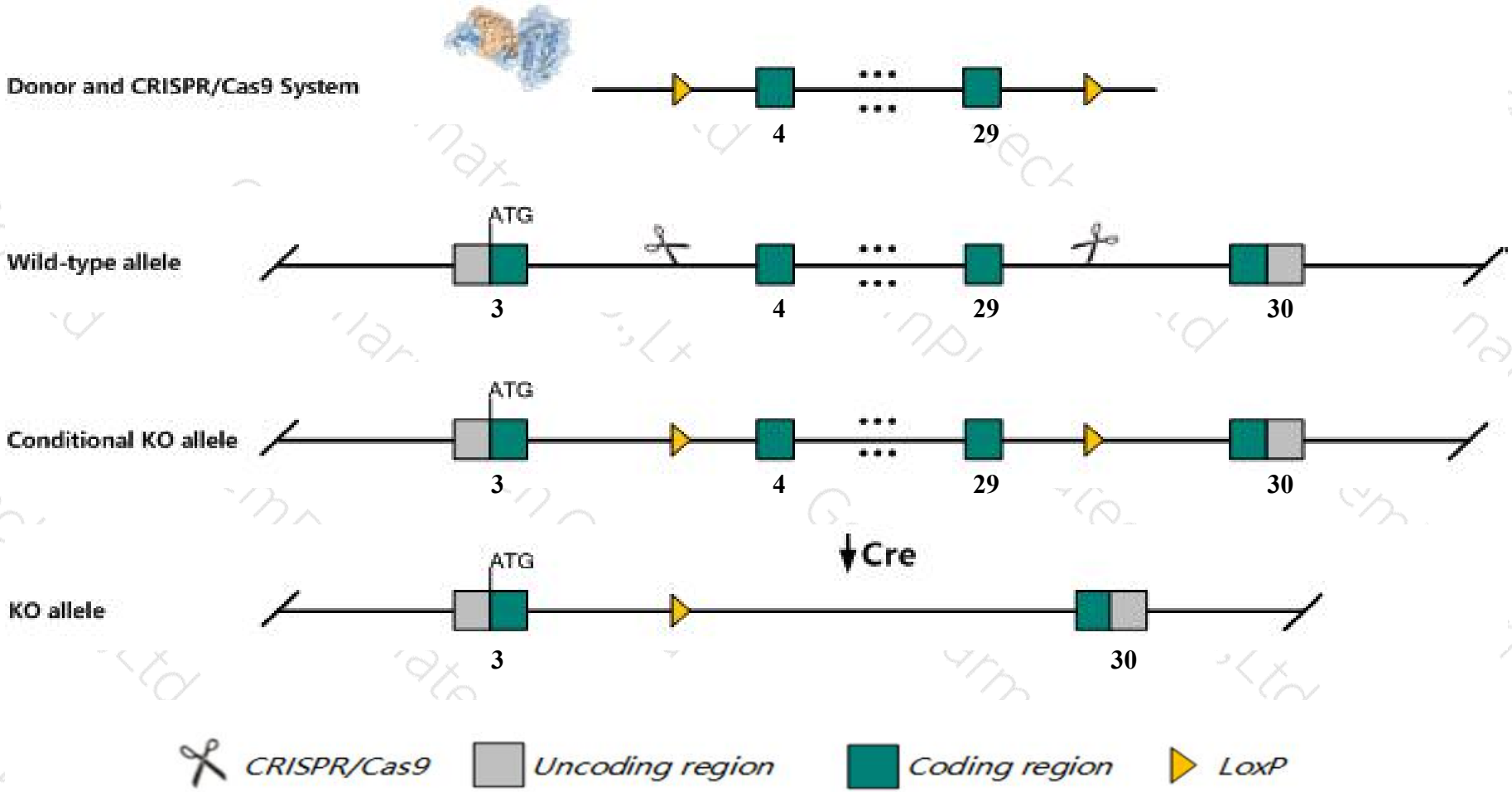
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Nfasc* gene has 10 transcripts. According to the structure of *Nfasc* gene, exon4-exon29 of *Nfasc-202* (ENSMUST00000094569.10) transcript is recommended as the knockout region.The region contains 3400bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Nfasc* 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 null allele die within 6 to 7 days of birth, exhibit reduced nerve conduction velocity and abnormal paranodal junction formation.
- Transcript 209 may not be affected.
- The *Nfasc* gene is located on the Chr1. 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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Nfasc neurofascin [*Mus musculus* (house mouse)]

Gene ID: 269116, updated on 10-Oct-2019

Summary

Official Symbol	Nfasc provided by MGI
Official Full Name	neurofascin provided by MGI
Primary source	MGI:MGI:104753
See related	Ensembl:ENSMUSG00000026442
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	NF; AA387016; mKIAA0756; D430023G06Rik
Summary	This gene encodes an L1 family immunoglobulin cell adhesion molecule with multiple IGcam and fibronectin domains. The protein functions in neurite outgrowth, neurite fasciculation, and organization of the axon initial segment (AIS) and nodes of Ranvier on axons during early development. Both the AIS and nodes of Ranvier contain high densities of voltage-gated Na+ (Nav) channels which are clustered by interactions with cytoskeletal and scaffolding proteins including this protein, gliomedin, ankyrin 3 (ankyrin-G), and betaIV spectrin. This protein links the AIS extracellular matrix to the intracellular cytoskeleton. This gene undergoes extensive alternative splicing, and the full-length nature of some variants has not been determined. [provided by RefSeq, May 2009]
Expression	Biased expression in cerebellum adult (RPKM 16.4), cortex adult (RPKM 13.2) and 6 other tissues See more
Orthologs	human all

Genomic context

Location: 1 E4; 1 57.42 cM

See Nfasc in Genome Data Viewer

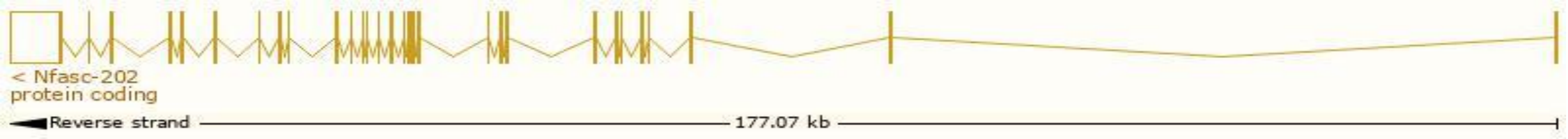
Exon count: 39

Transcript information（Ensembl）

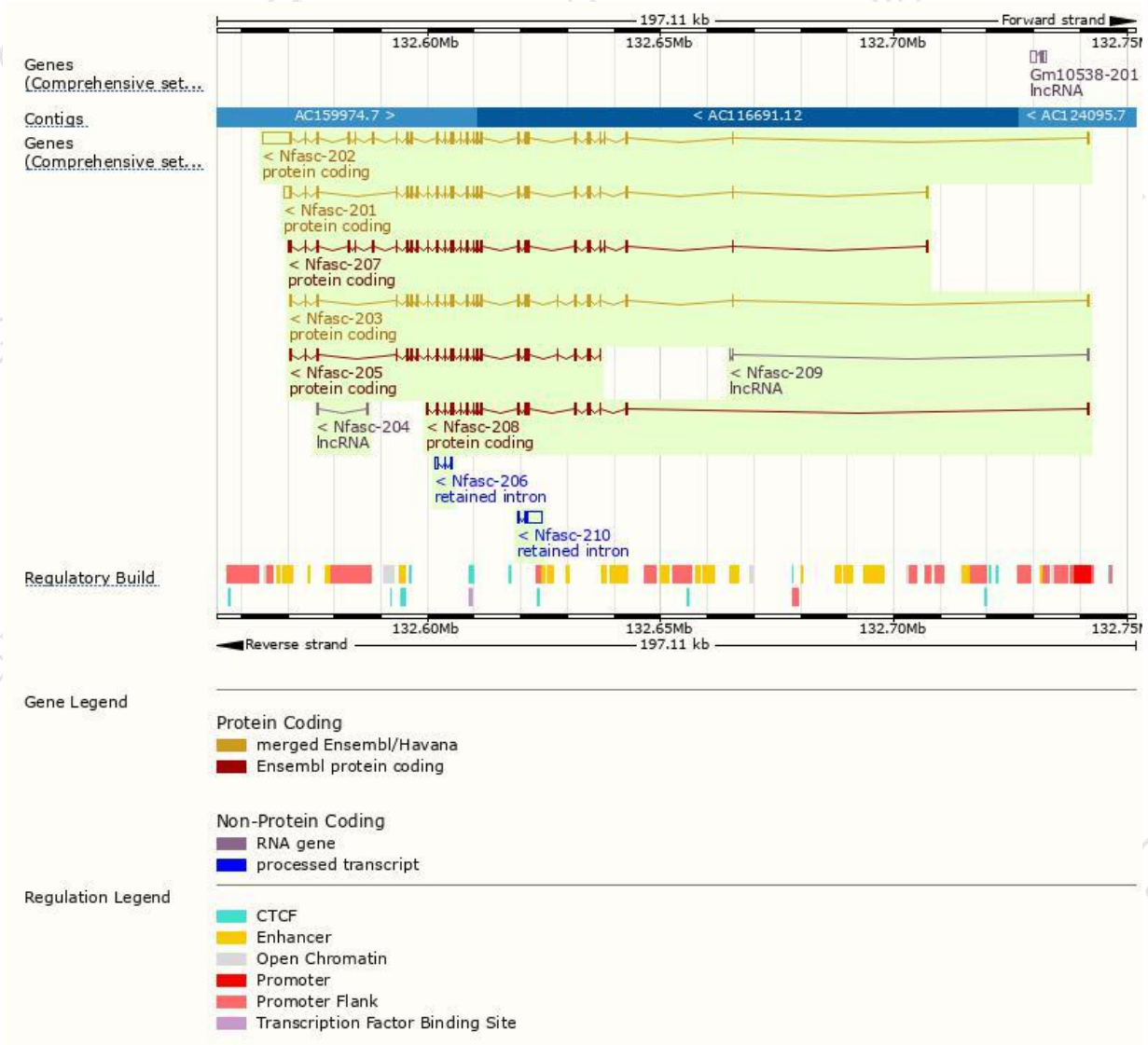
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nfasc-202	ENSMUST00000094569.10	9739	1240aa	Protein coding	CCDS35707	Q810U3	TSL:1 GENCODE basic APPRIS P3
Nfasc-201	ENSMUST00000043189.13	5072	1157aa	Protein coding	CCDS48358	E9Q171	TSL:1 GENCODE basic APPRIS ALT2
Nfasc-203	ENSMUST00000163770.7	3903	1174aa	Protein coding	CCDS48359	E9PW06	TSL:5 GENCODE basic APPRIS ALT2
Nfasc-207	ENSMUST00000187861.6	4927	1347aa	Protein coding	-	A0A087WPX3	TSL:5 GENCODE basic APPRIS ALT2
Nfasc-205	ENSMUST00000186389.6	3476	1159aa	Protein coding	-	A0A087WR56	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Nfasc-208	ENSMUST00000188307.1	3049	899aa	Protein coding	-	A0A087WNW2	TSL:1 GENCODE basic
Nfasc-210	ENSMUST00000191294.1	3314	No protein	Retained intron	-	-	TSL:5
Nfasc-206	ENSMUST00000186539.1	750	No protein	Retained intron	-	-	TSL:3
Nfasc-209	ENSMUST00000189219.1	326	No protein	lncRNA	-	-	TSL:2
Nfasc-204	ENSMUST00000186330.1	172	No protein	lncRNA	-	-	TSL:5

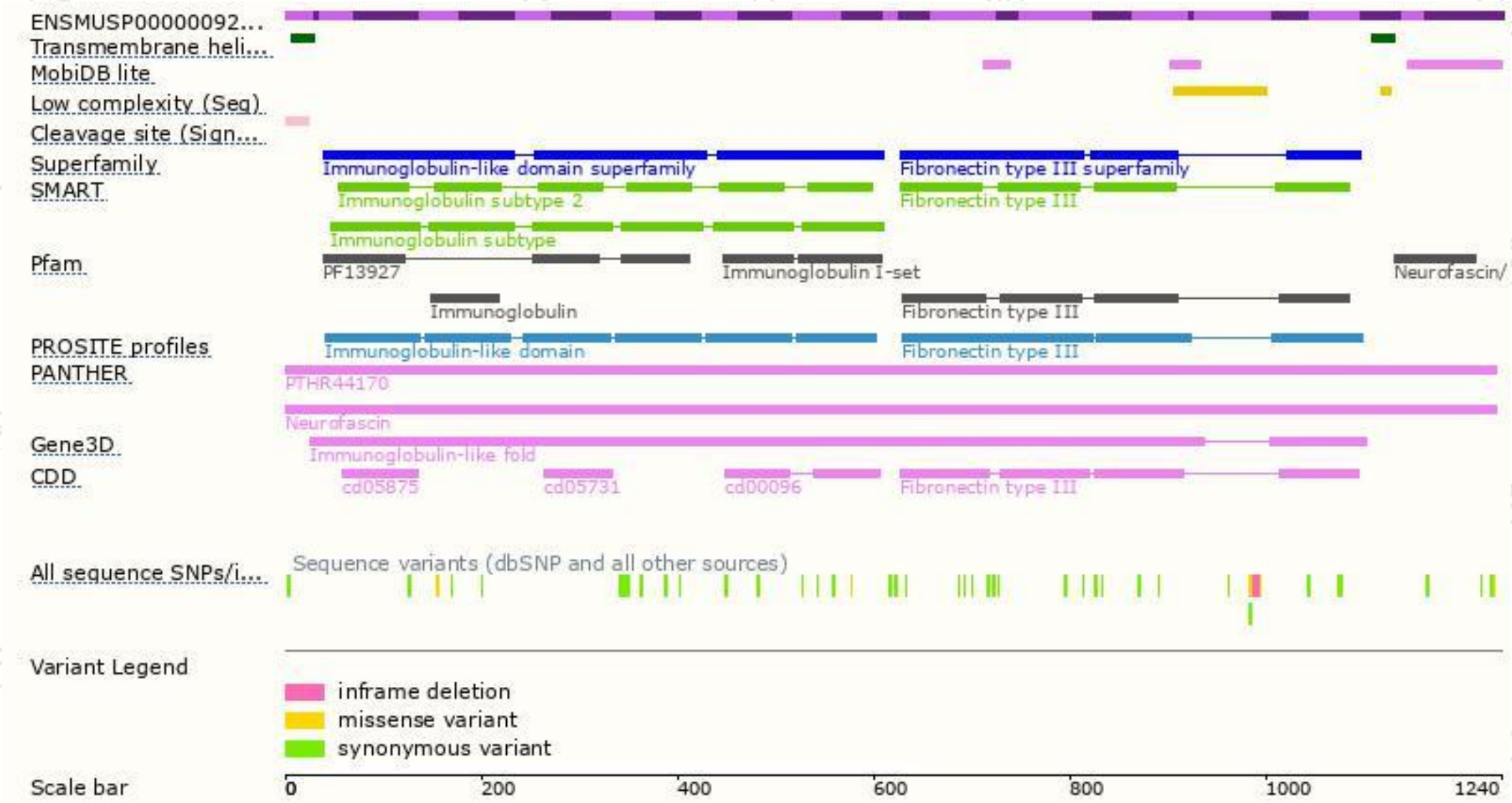
The strategy is based on the design of *Nfasc-202* transcript,The transcription is shown below



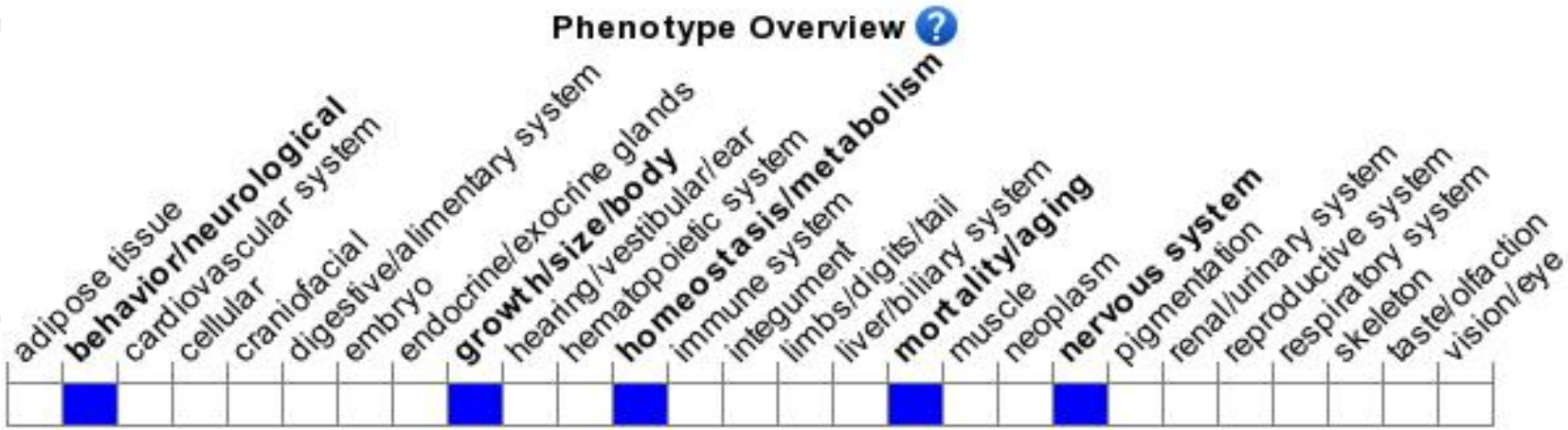
Genomic location distribution



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 null allele die within 6 to 7 days of birth, exhibit reduced nerve conduction velocity and abnormal paranodal junction formation.

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

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