Efnb3 Cas9-KO Strategy

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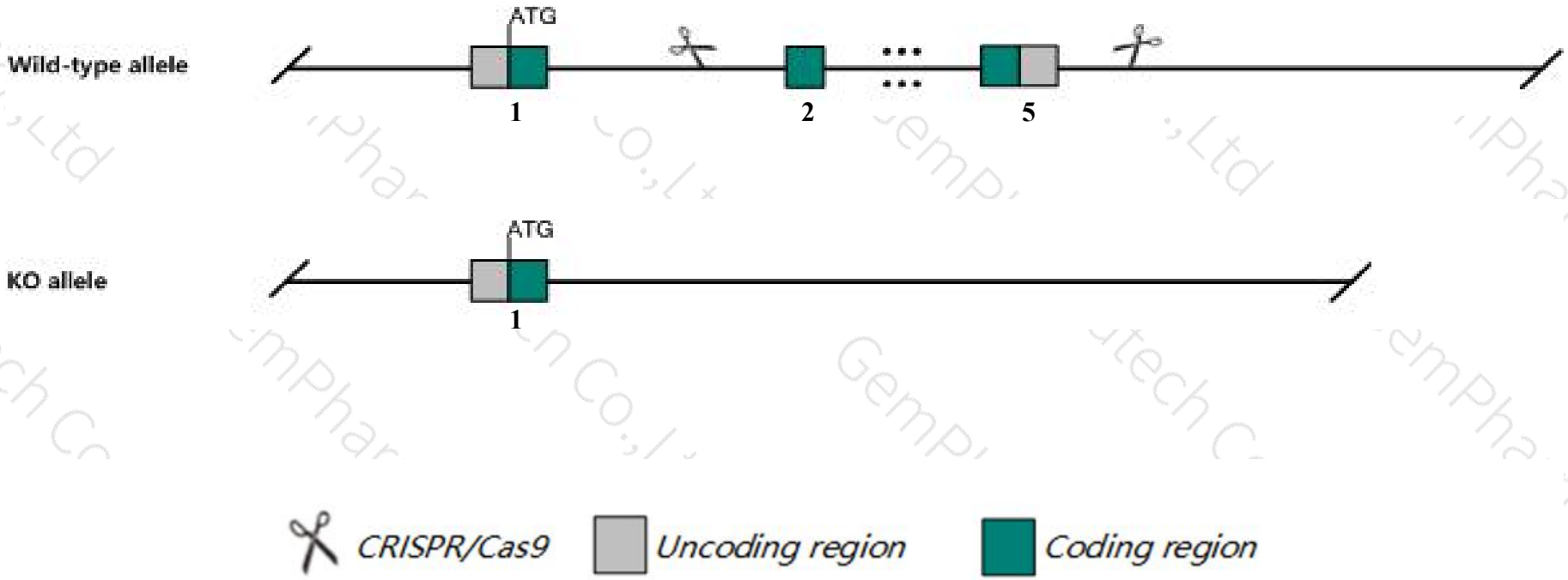
Project Overview

Project Name	***Efnb3***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Efnb3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Efnb3* gene has 1 transcript. According to the structure of *Efnb3* gene, exon2-exon5 of *Efnb3-201* (ENSMUST00000004036.5) 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 *Efnb3* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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.

Notice

- According to the existing MGI data,Mice homozygous for null mutations exhibit a hopping gait due to corticospinal tract defects, mutations that remove only the cytoplasmic domain of the protein do not result in the gait or CNS phenotypes, and a G244E mutation causes ataxia
- The knockout region is near to the N-terminal of *Dnah2* gene,this strategy may influence the regulatory function of the N-terminal of *Dnah2* gene.
- The *Efnb3* 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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Efnb3 ephrin B3 [*Mus musculus* (house mouse)]

Gene ID: 13643, updated on 5-Nov-2019

Summary

Official Symbol	Efnb3 provided by MGI
Official Full Name	ephrin B3 provided by MGI
Primary source	MGI:MGI:109196
See related	Ensembl:ENSMUSG00000003934
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	Epl8; EFL-6; ELF-3; Elk-L3; LERK-8; NLERK-2
Expression	Biased expression in CNS E18 (RPKM 44.0), whole brain E14.5 (RPKM 38.6) and 9 other tissues See more
Orthologs	human all

Genomic context

Location: 11 B3; 11 42.8 cM

See Efnb3 in Genome Data Viewer

Exon count: 6

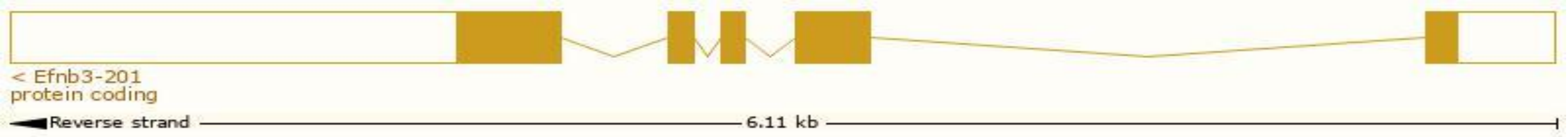
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	11	NC_000077.6 (69554092..69561150, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	11	NC_000077.5 (69367594..69373739, complement)

Transcript information（Ensembl）

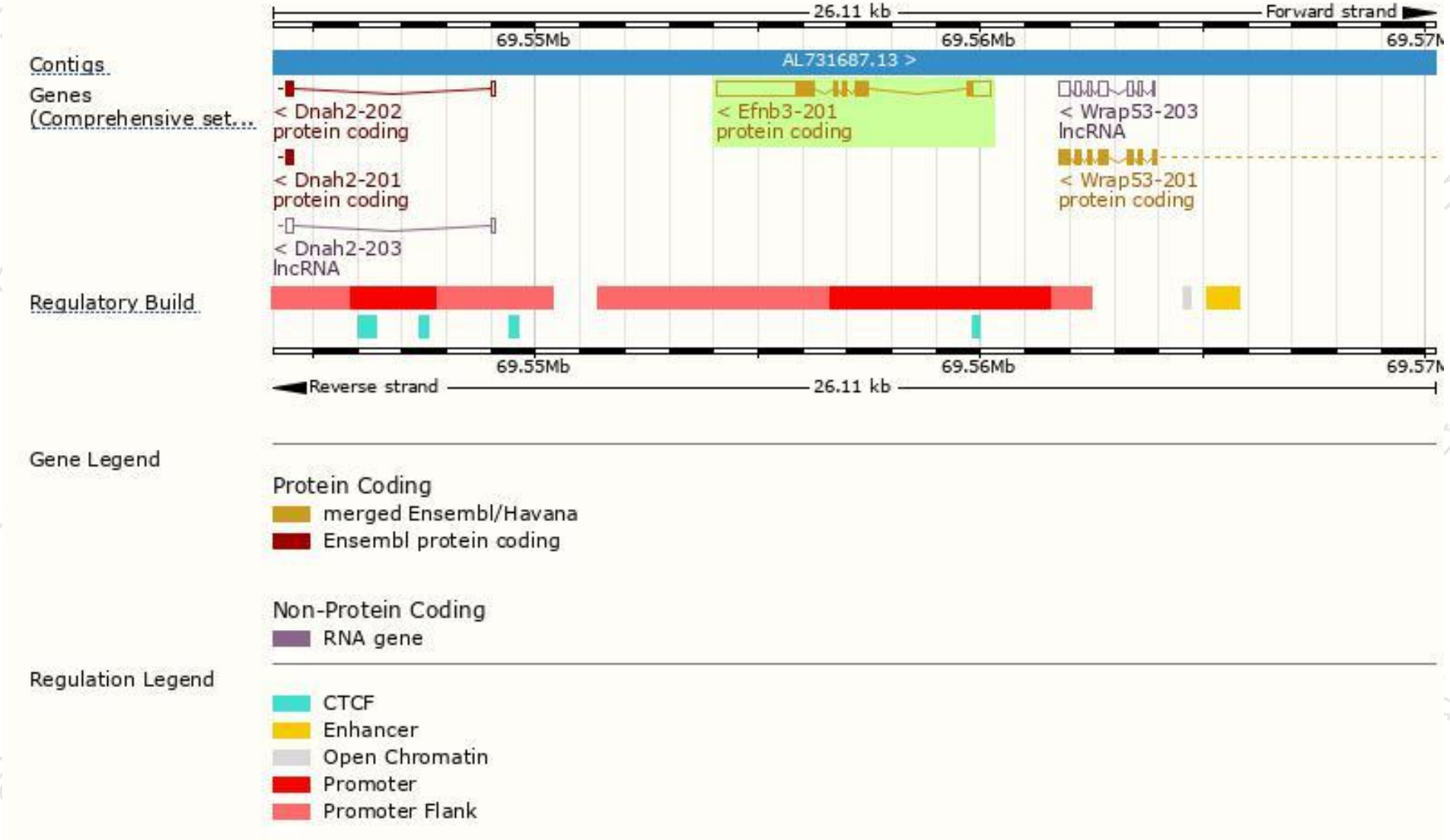
The gene has 1 transcript,and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Efnb3-201	ENSMUST00000004036.5	3183	340aa	Protein coding	CCDS24896	O35393 Q543Q7	TSL:1 GENCODE basic APPRIS P1

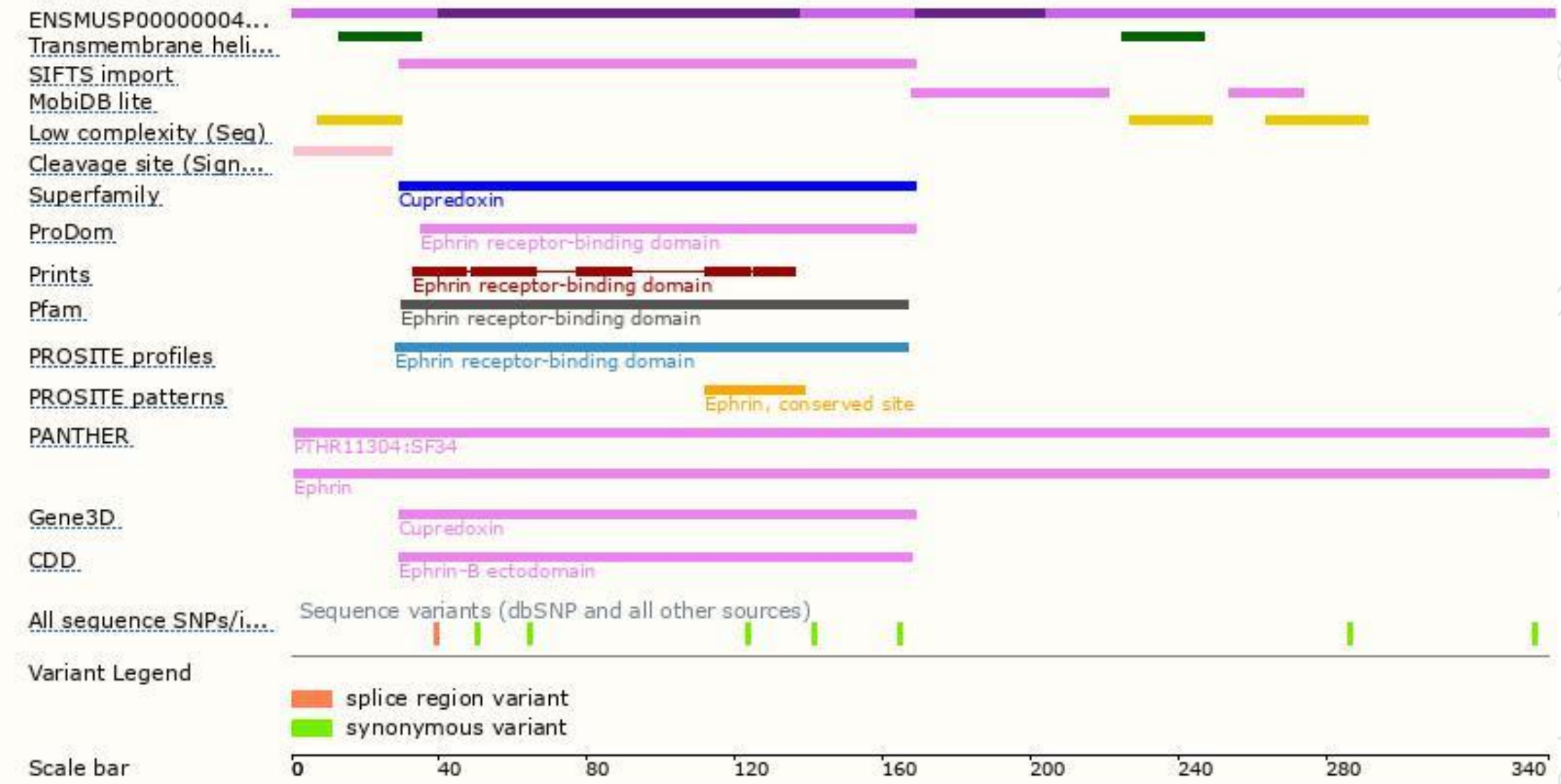
The strategy is based on the design of *Efnb3-201* transcript,The transcription is shown below



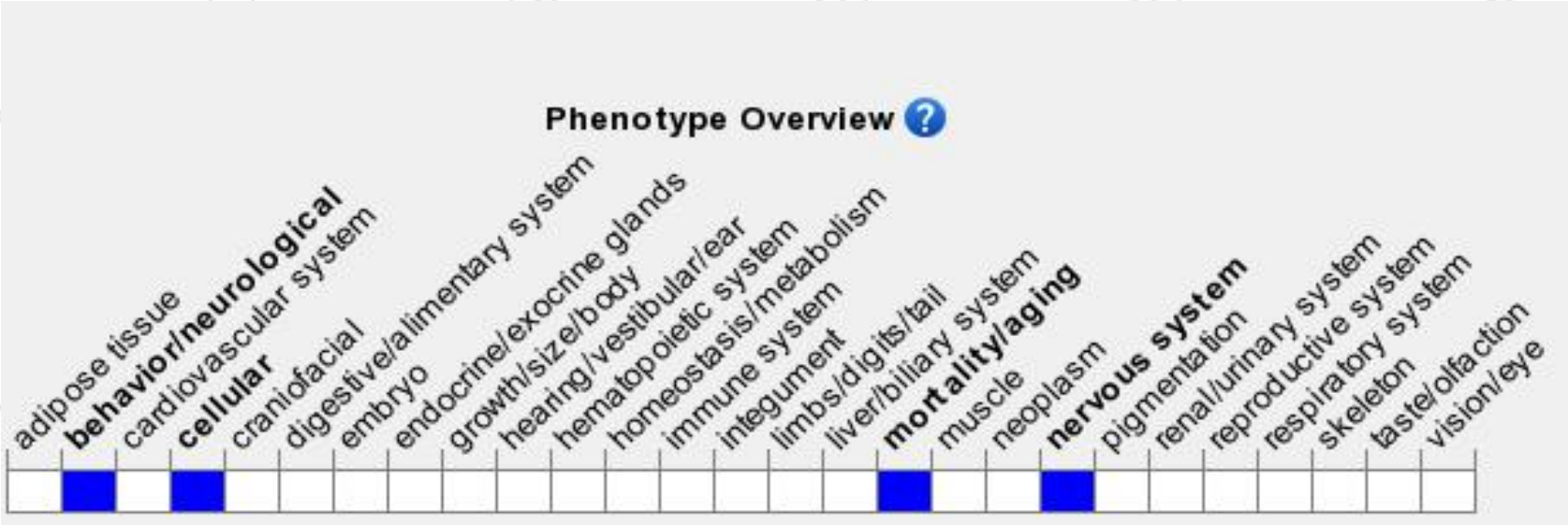
Genomic location distribution



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/).

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

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