

Nes Cas9-CKO Strategy

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Project Overview

Project Name

Nes

Project type

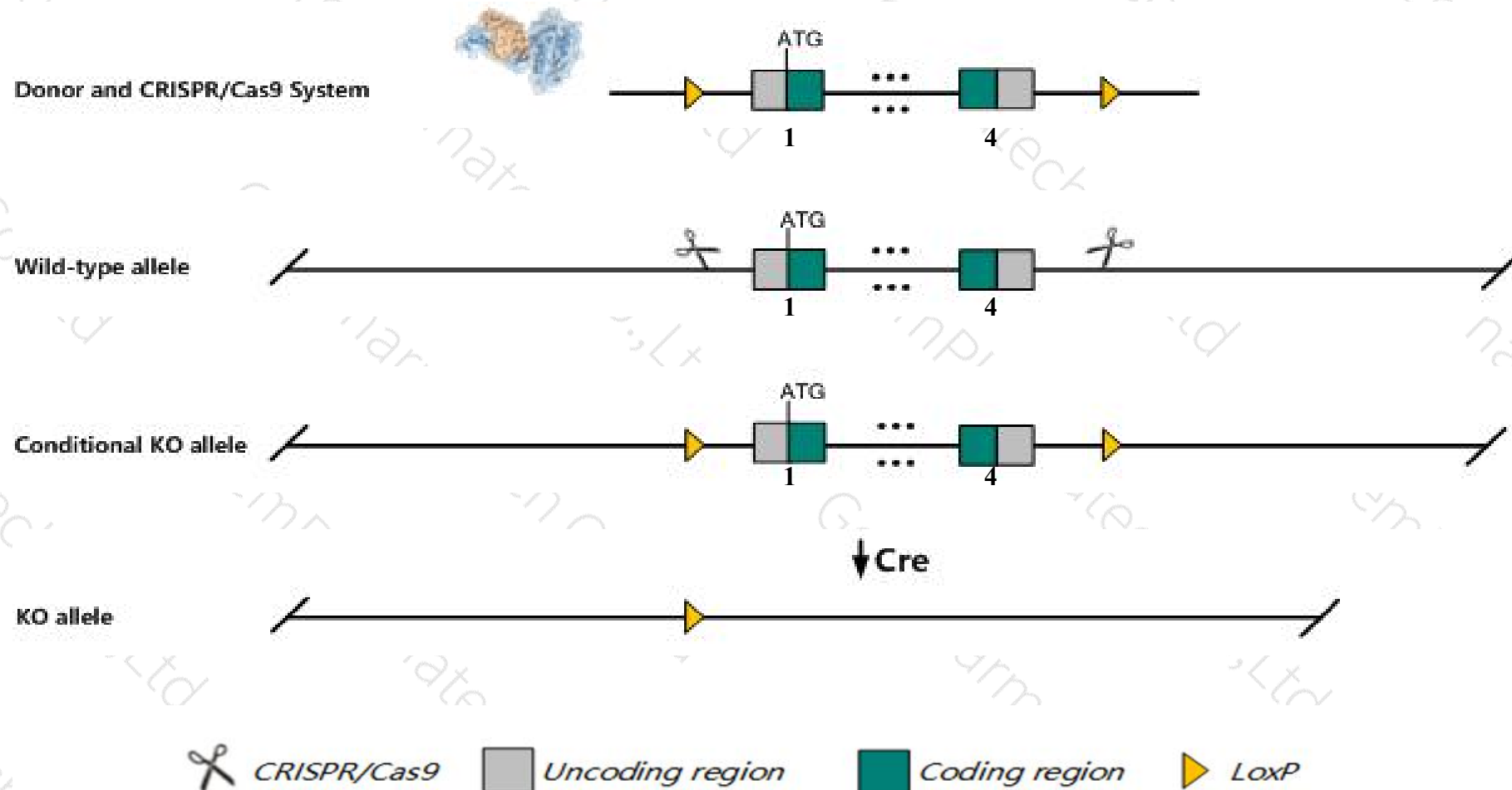
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Nes* gene has 3 transcripts. According to the structure of *Nes* gene, exon1-exon4 of *Nes-201* (ENSMUST00000090973.11) transcript is recommended as the knockout region. The region contains all 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 *Nes* 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 knock-out allele display a high incidence of embryonic lethality, reduced embryo and birth body size, and fewer neural stem cells and increased apoptosis in the neuroepithelium of the developing neural tube.
- The *Gm3745* gene may be deleted at the same time.
- The *Nes* gene is located on the Chr3. 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)

Nes nestin [*Mus musculus* (house mouse)]

Gene ID: 18008, updated on 31-Dec-2019

Summary

Official Symbol	Nes provided by MGI
Official Full Name	nestin provided by MGI
Primary source	MGI:MGI:101784
See related	Ensembl:ENSMUSG00000004891
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	RC2; Marc2; C78523; ESTM46; Ifaprc2; AA166324
Expression	Biased expression in CNS E11.5 (RPKM 68.0), CNS E14 (RPKM 14.9) and 4 other tissues See more
Orthologs	human all

Genomic context

Location: 3 F1; 3 38.78 cM

Exon count: 4

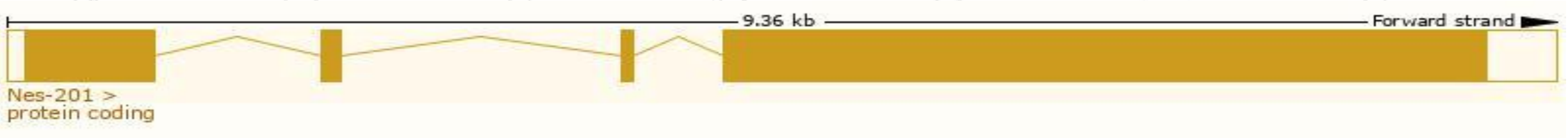
[See Nes in Genome Data Viewer](#)

Transcript information (Ensembl)

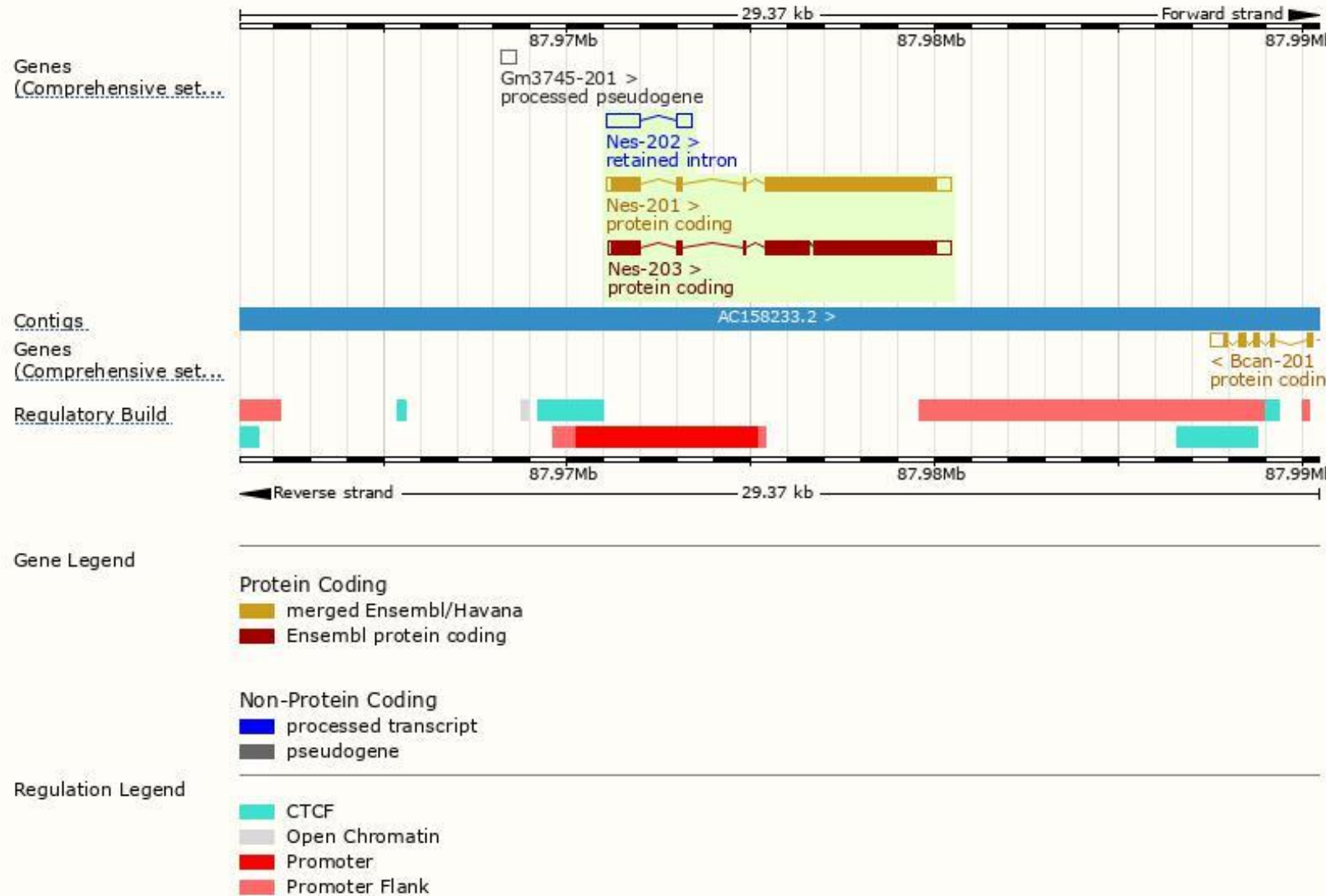
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nes-201	ENSMUST00000090973.11	6126	1864aa	Protein coding	CCDS17461	Q6P5H2	TSL:1 GENCODE basic APPRIS P2
Nes-203	ENSMUST00000160694.1	5958	1820aa	Protein coding	-	Q6P5H2	TSL:1 GENCODE basic APPRIS ALT2
Nes-202	ENSMUST00000159830.1	1311	No protein	Retained intron	-	-	TSL:1

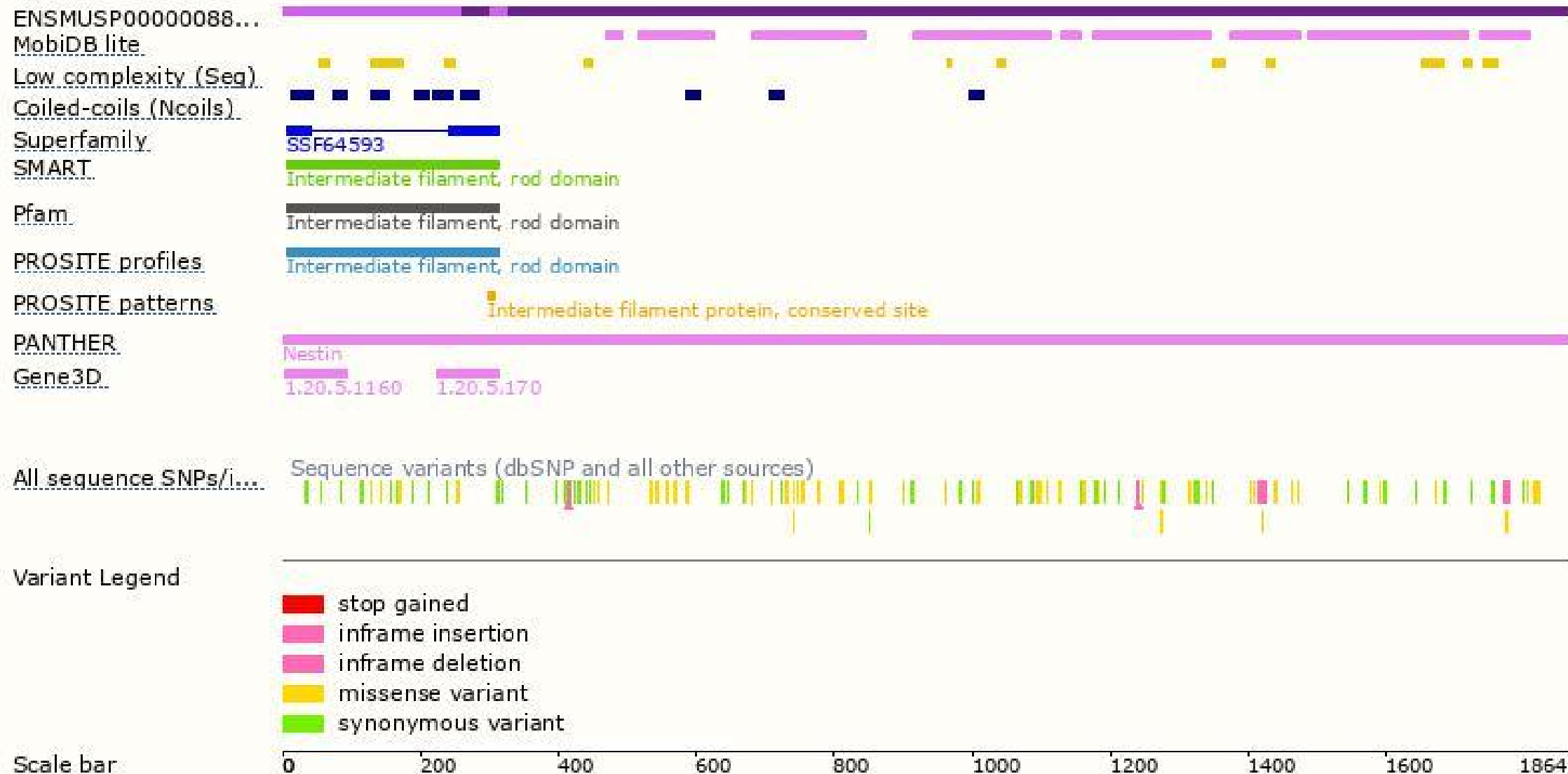
The strategy is based on the design of *Nes-201* transcript,The transcription is shown below



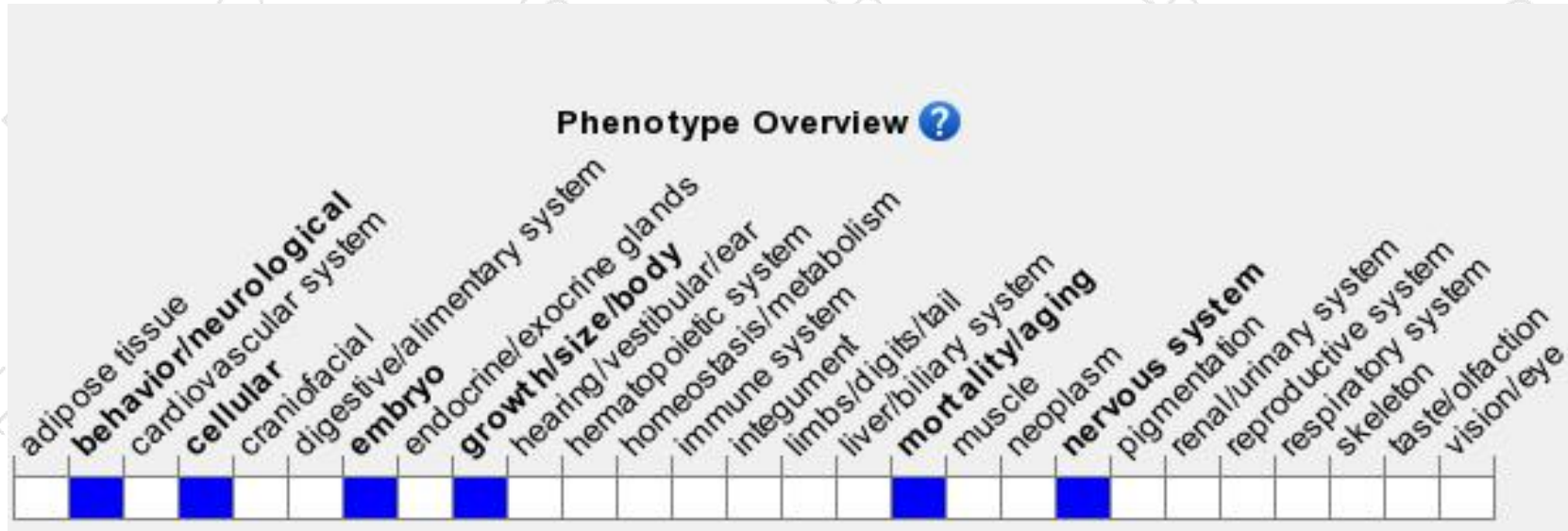
Genomic location distribution



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 knock-out allele display a high incidence of embryonic lethality, reduced embryo and birth body size, and fewer neural stem cells and increased apoptosis in the neuroepithelium of the developing neural tube.

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

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