

***Nes-P2A-iCre* Cas9-KI Strategy**

Designer:

Lingyan Wu

Reviewer:

Jinlong Zhao

Design Date:

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Project Overview

Project Name

Nes-P2A-iCre

Project type

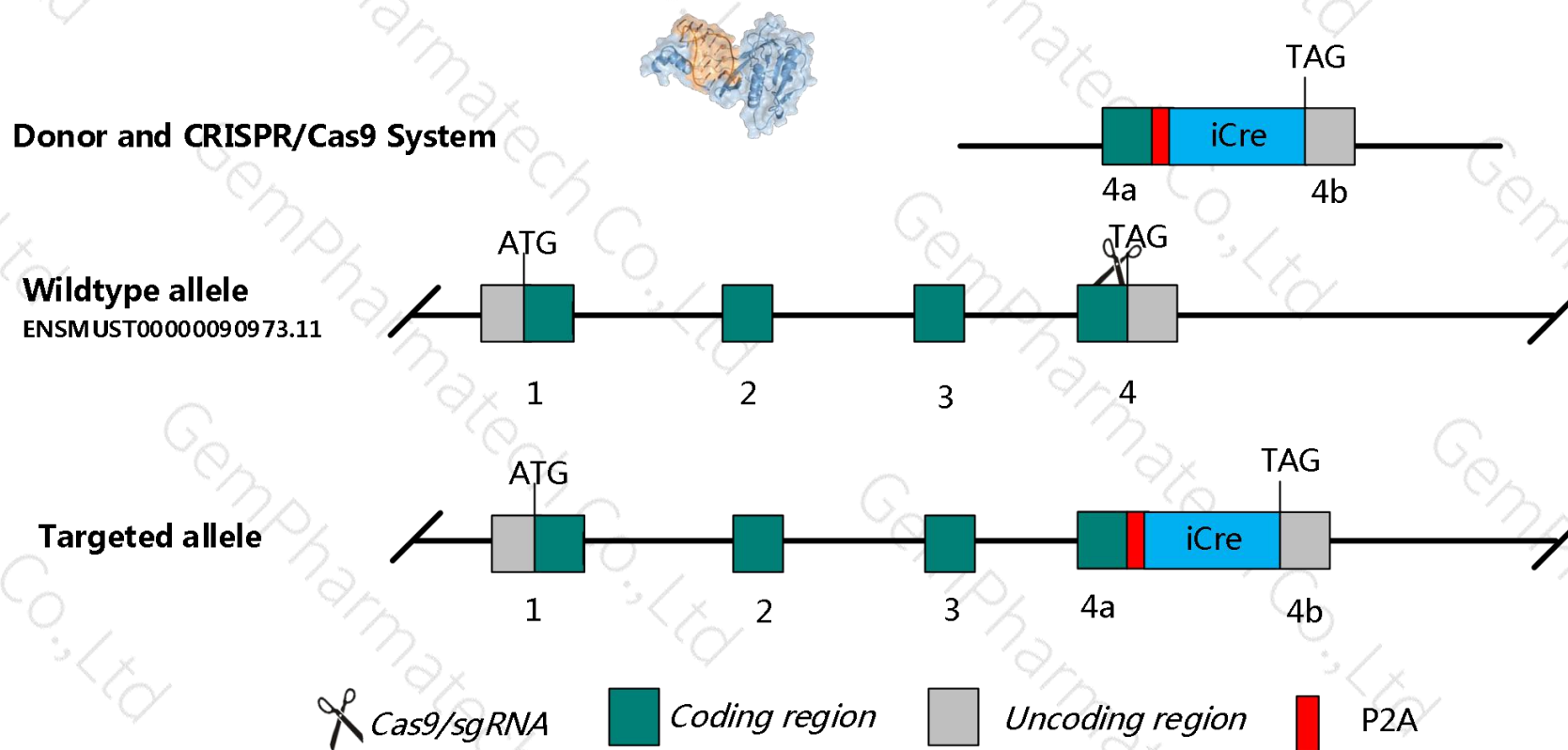
Cas9-KI

Strain background

C57BL/6JGpt

Knockin strategy

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



Technical routes

- The mouse *Nes* gene has 3 transcripts.
- According to the structure of *Nes* gene, the element P2A-iCre will be inserted at the translation stop code of *Nes-201* (ENSMUST00000090973.11), the length of inserted fragment is about 1.1kb.
- In this project, *Nes* gene will be modified by CRISPR/Cas9 technology. 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.

- Mouse *Nes* gene is located on Chr3. Please take the loci in consideration when breeding this knockin mice with other gene modified (e.g., Tg, iCre) strains, if the other gene is also on Chr3, it may be extremely hard to get double gene positive homozygotes.
- It is necessary to introduce 1-2 synonymous mutation in exon4.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene between the 3'UTR and gene coding region may affect the expression of endogenous and foreign genes. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology 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

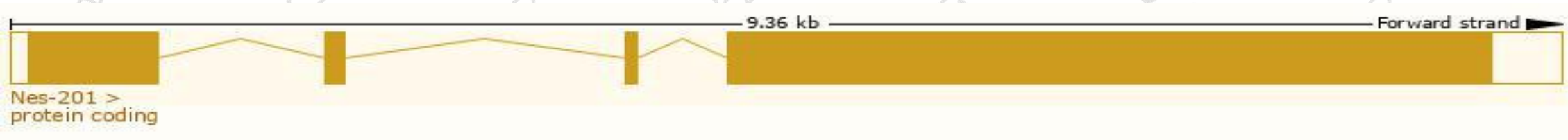
[See Nes in Genome Data Viewer](#)

Transcript information (Ensembl)

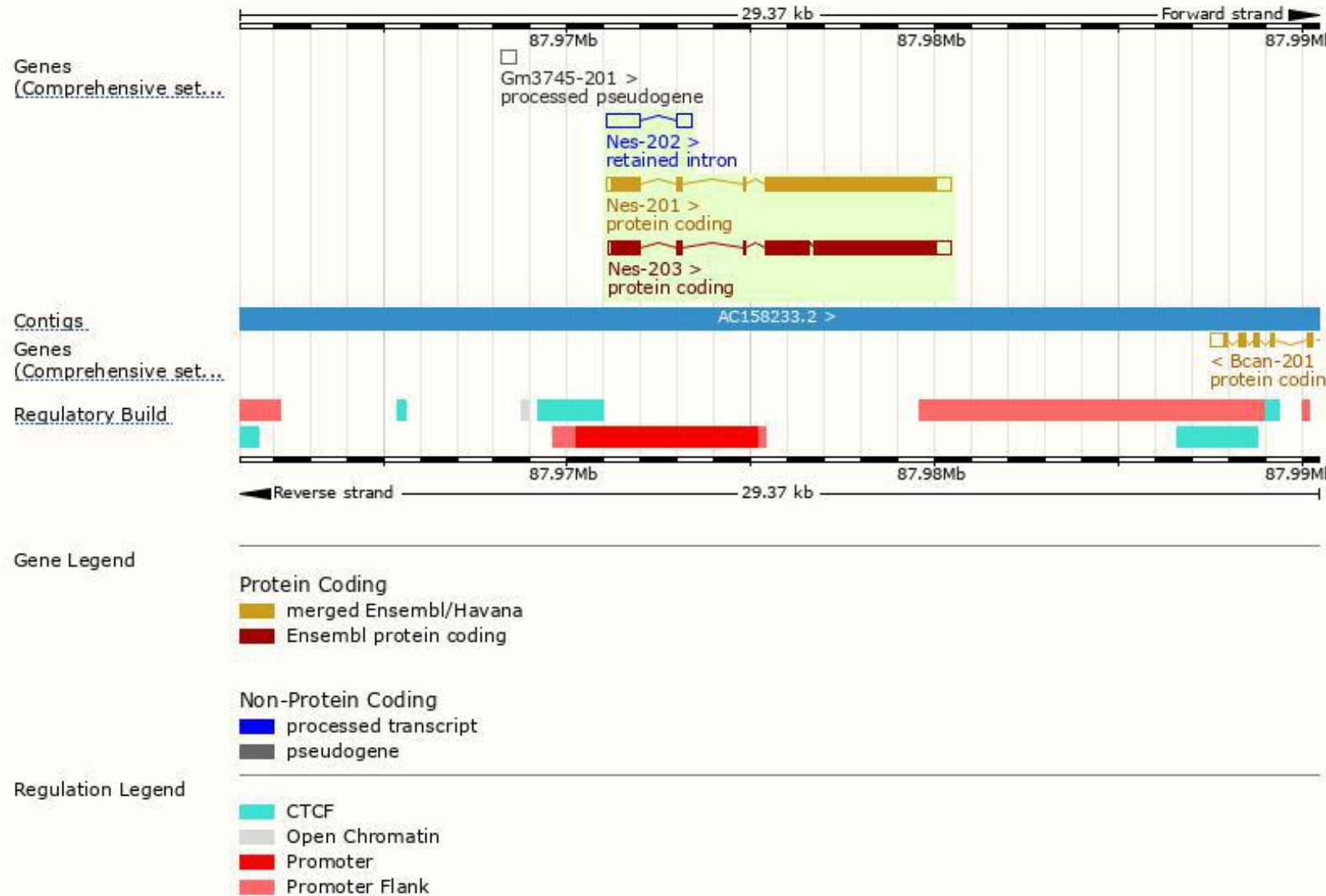
The gene has 3 transcripts,all transcripts are shown below:

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

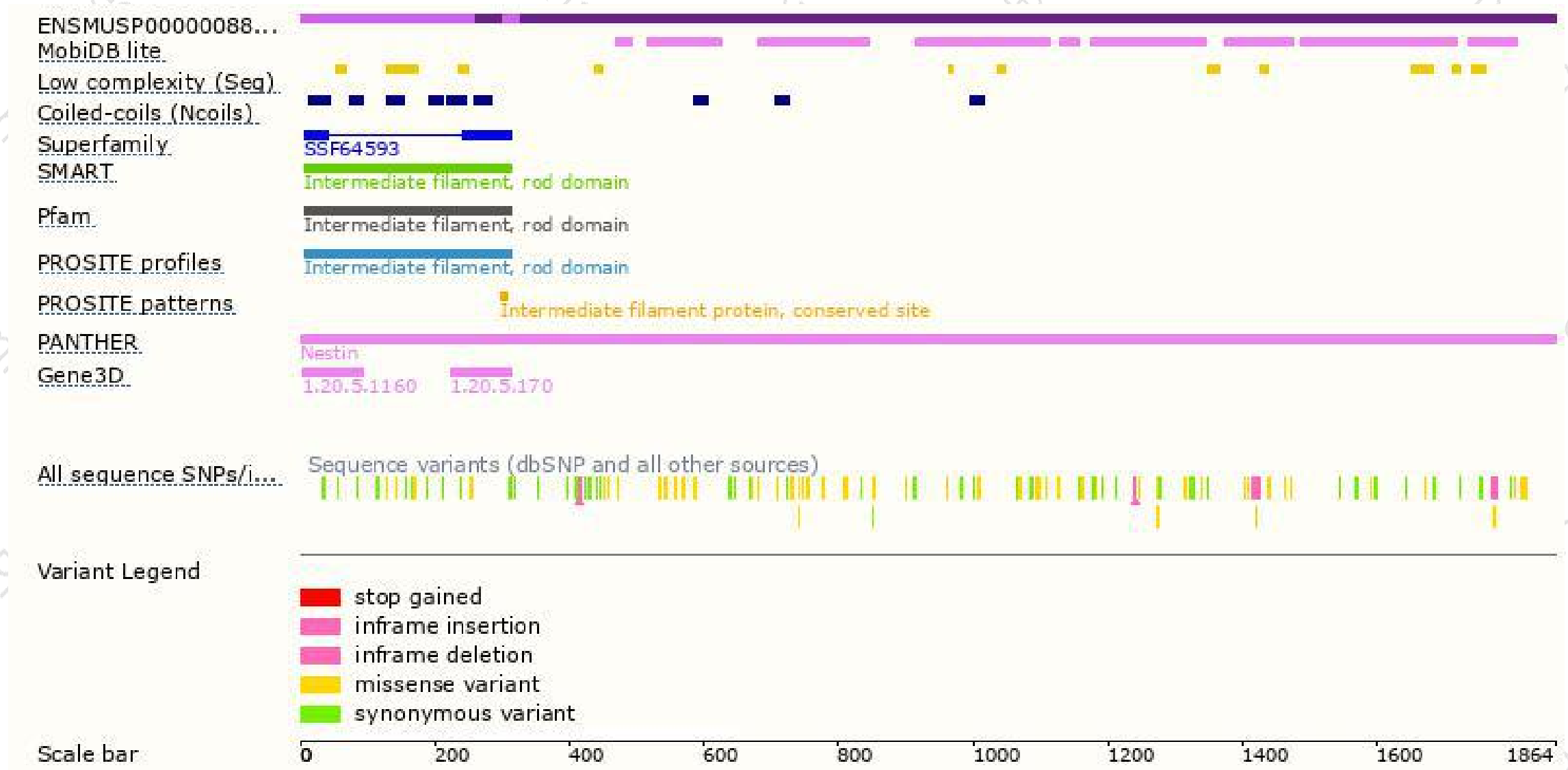
The strategy is based on the design of *Nes-201* transcript,The transcription is shown below



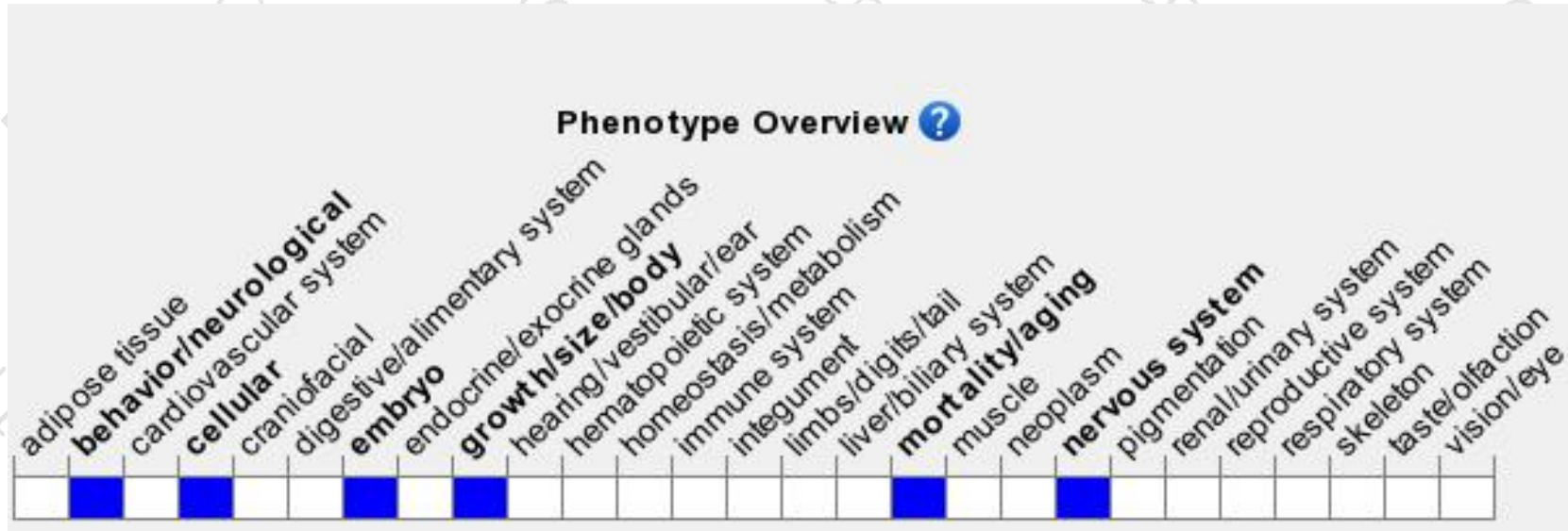
Genomic location distribution



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.
Tel: 400-9660890



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