Nanog Cas9-CKO Strategy

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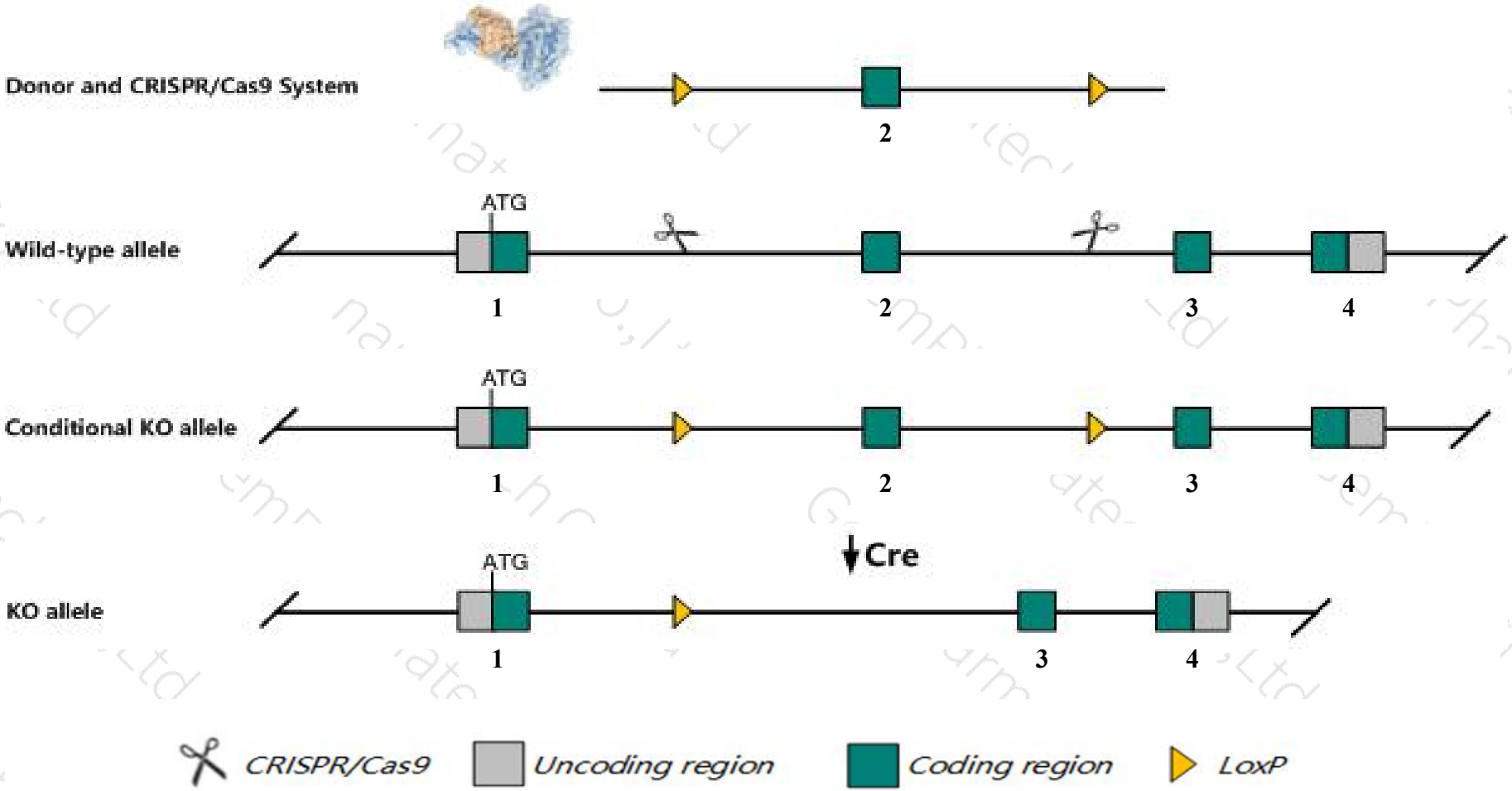
Design Date: 2020-4-22

Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Nanog* gene has 3 transcripts. According to the structure of *Nanog* gene, exon2 of *Nanog-201* (ENSMUST00000012540.4) transcript is recommended as the knockout region.The region contains 266bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Nanog* 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 disruption in this gene die between e3.5 and e5.5 with abnormal embryonic and extraembryonic tissue development.
- The *Nanog* gene is located on the Chr6. 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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Nanog Nanog homeobox [Mus musculus (house mouse)]

Gene ID: 71950, updated on 13-Mar-2020

Summary

Official Symbol	Nanog provided by MGI
Official Full Name	Nanog homeobox provided by MGI
Primary source	MGI:MGI:1919200
See related	Ensembl:ENSMUSG00000012396
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	2410002E02Rik, ENK, ecat4
Summary	The protein encoded by this gene is a DNA binding homeobox transcription factor involved in embryonic stem (ES) cell proliferation, renewal, and pluripotency. The encoded protein can block ES cell differentiation and can also autorepress its own expression in differentiating cells. Several transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Sep 2015]
Expression	Low expression observed in reference datasetSee more
Orthologs	human all

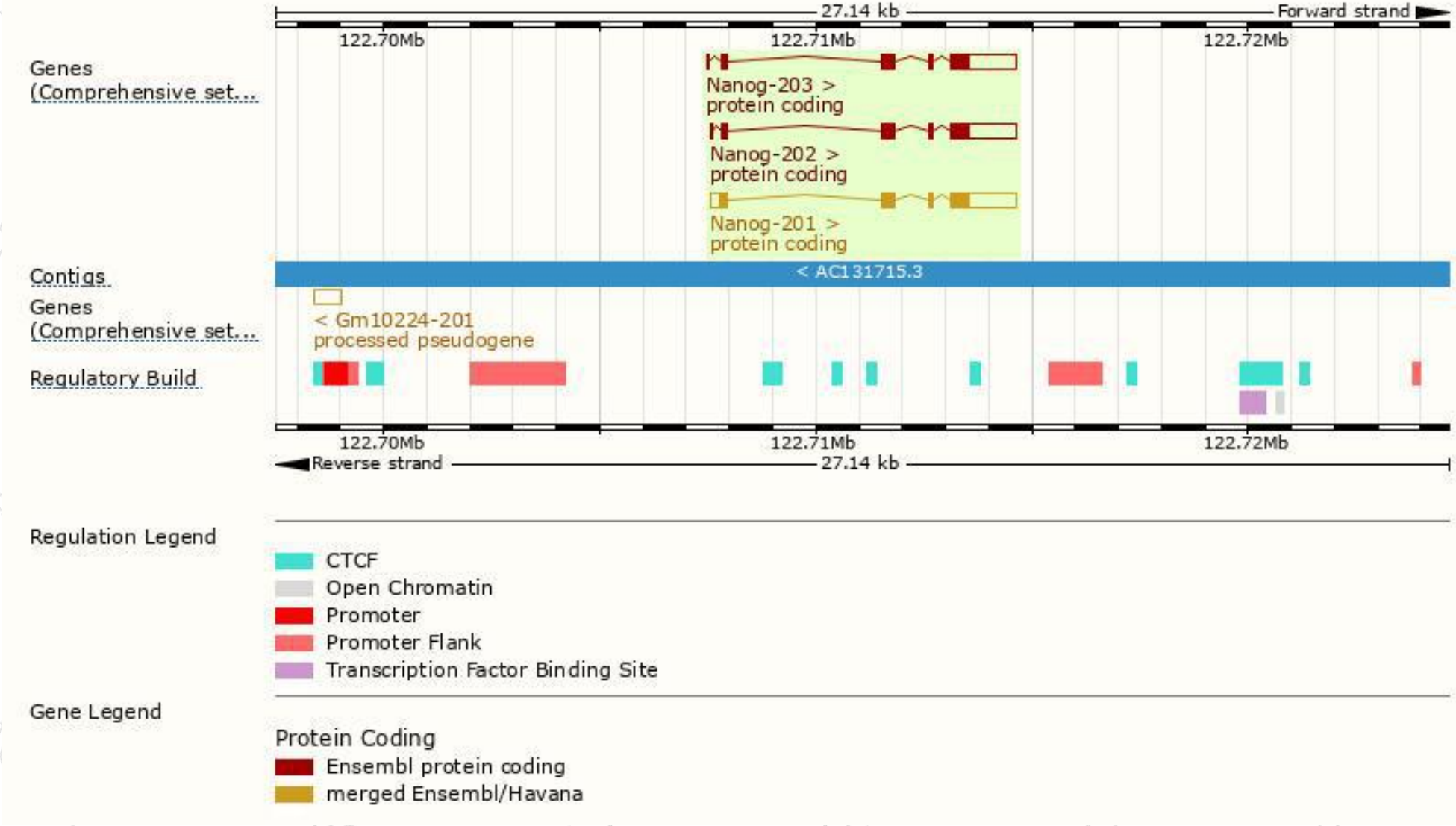
Transcript information（Ensembl）

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

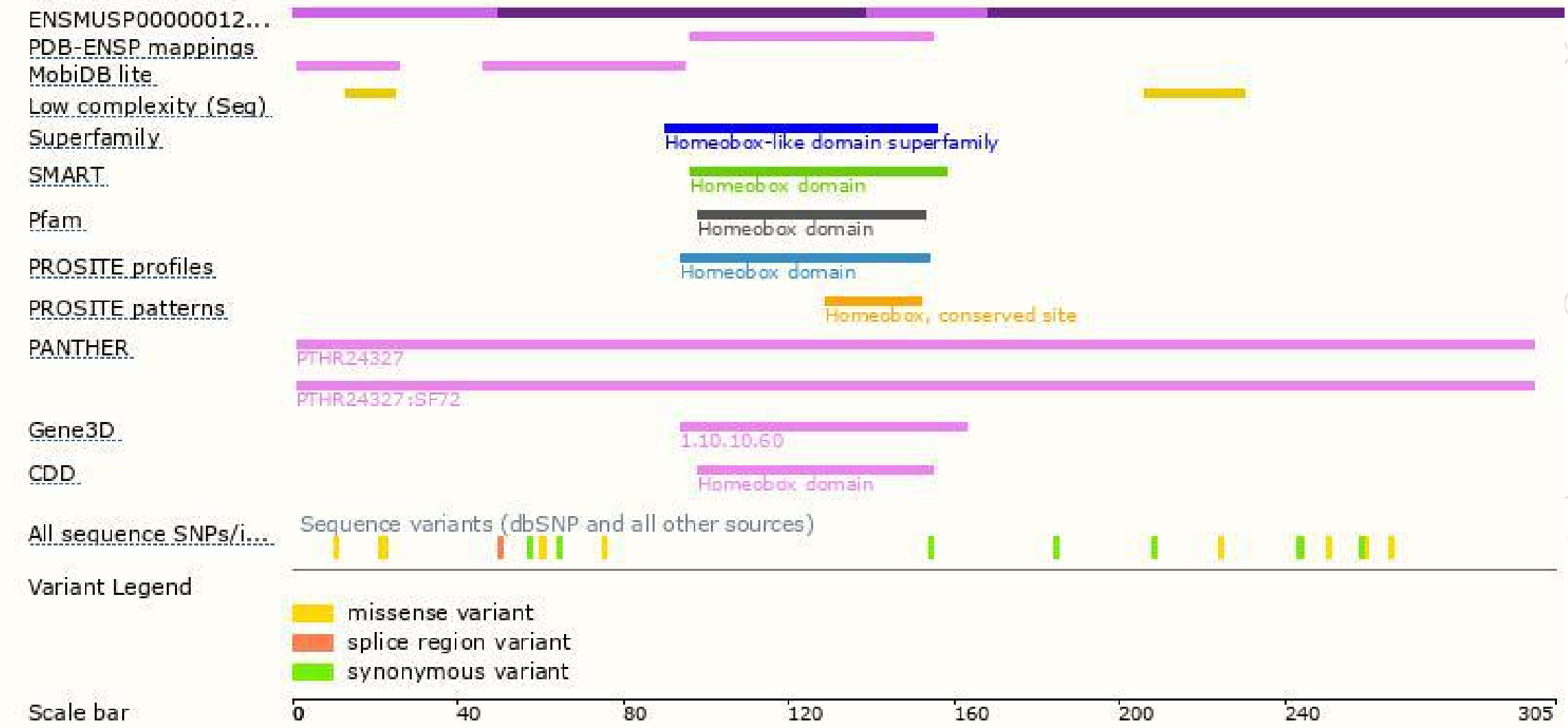
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nanog-201	ENSMUST00000012540.4	2211	305aa	Protein coding	CCDS39623	A2RS90 Q80Z64	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Nanog-202	ENSMUST00000112580.7	2011	280aa	Protein coding	CCDS80607	Q80Z64	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Nanog-203	ENSMUST00000112581.7	1995	280aa	Protein coding	CCDS80607	Q80Z64	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2

The strategy is based on the design of *Nanog-201* transcriptt,he transcription is shown below:

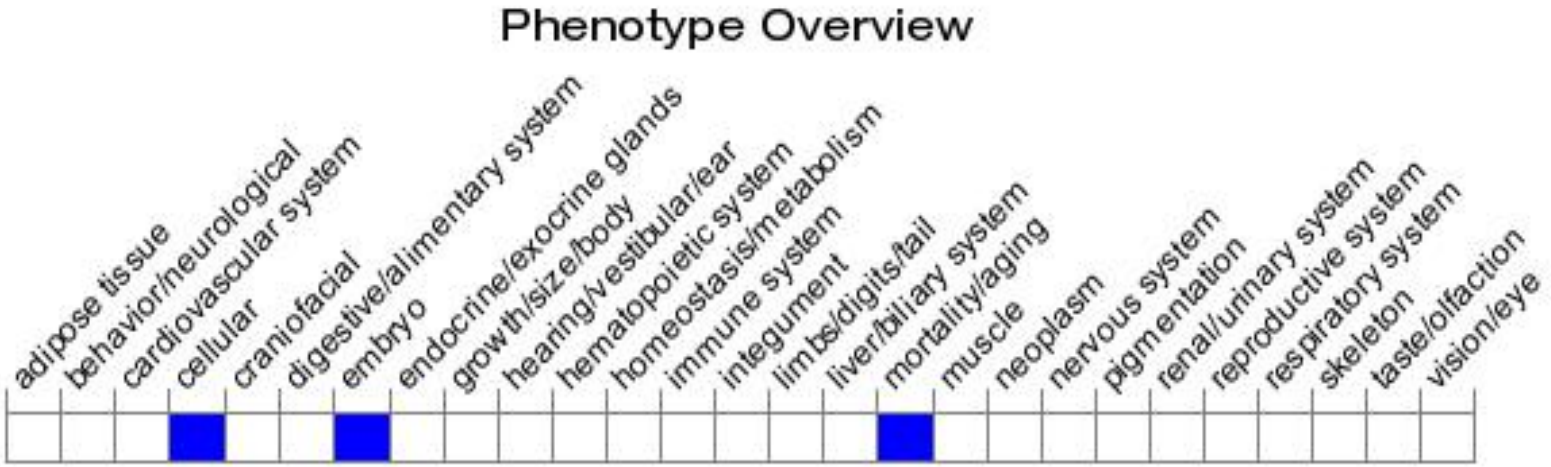
Genomic location distribution



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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