Edn3 Cas9-CKO Strategy

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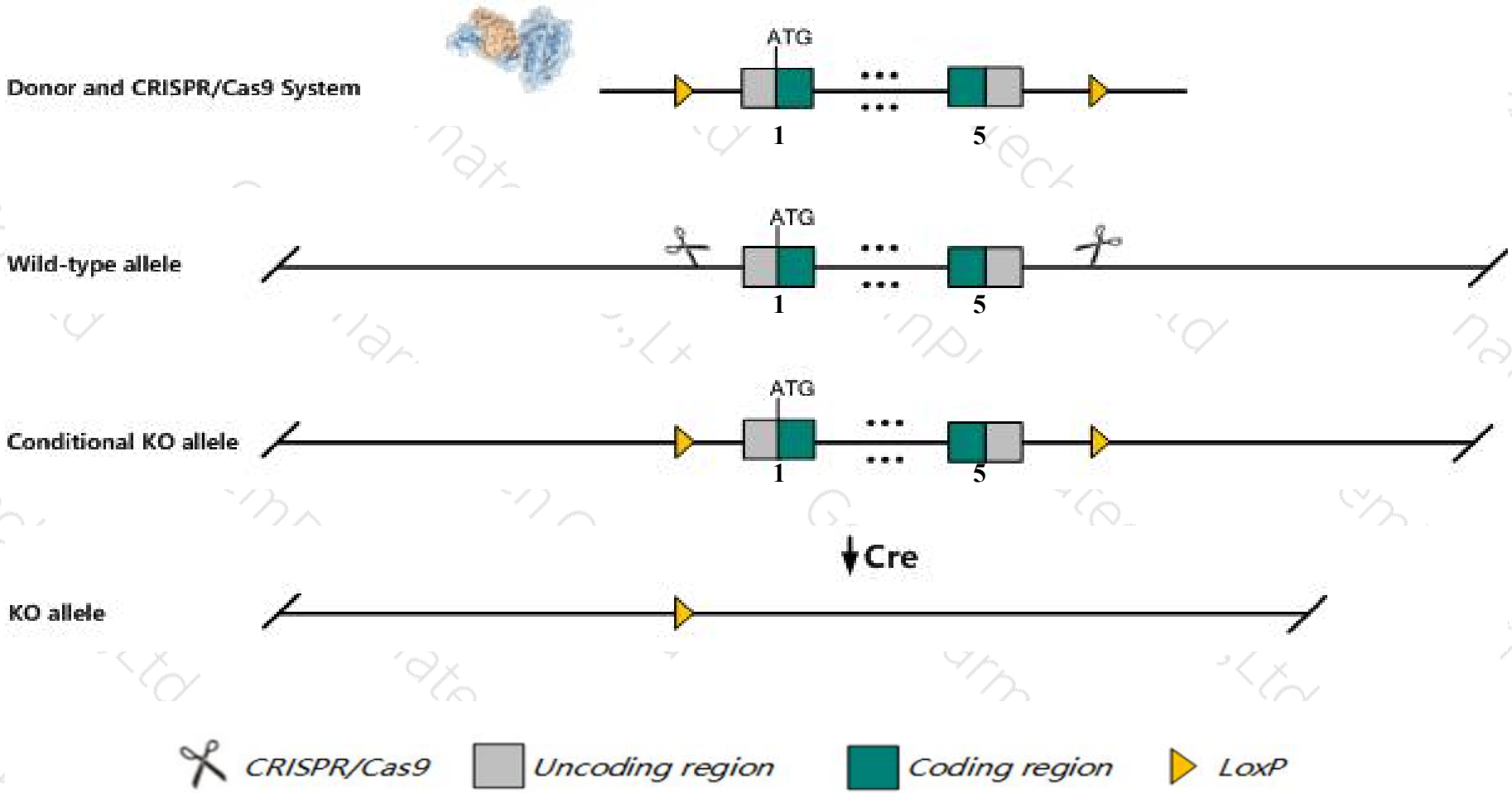
Project Overview

Project Name	***Edn3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Edn3* gene. The schematic diagram is as follows:



Technical routes

- The *Edn3* gene has 4 transcripts. According to the structure of *Edn3* gene, exon1-exon5 of *Edn3-201* (ENSMUST00000029030.8) 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 *Edn3* 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,Homozygotes for mutations at this locus exhibit aganglionic megacolon with white spotting of the hair coat due to impaired expansion and differentiation of epidermal melanoblasts. Mutants die around weaning with impacted colons.

- The *Edn3* gene is located on the Chr2. 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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Edn3 endothelin 3 [Mus musculus (house mouse)]

Gene ID: 13616, updated on 31-Jan-2019

Summary

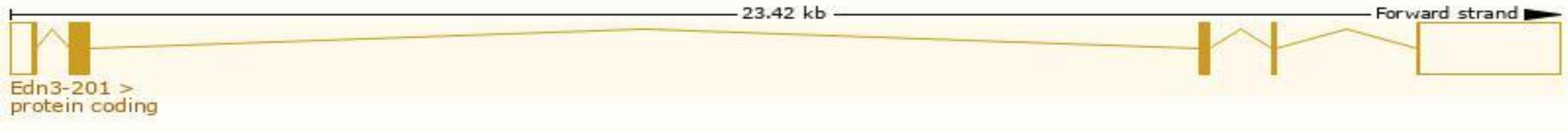
Official Symbol	Edn3 provided by MGI
Official Full Name	endothelin 3 provided by MGI
Primary source	MGI:MGI:95285
See related	Ensembl:ENSMUSG00000027524
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	ET-3, PPET3, ls, tmgc48
Summary	This gene is a member of the endothelin family whose members encode proteins that act on G protein-coupled receptors. Endothelins are produced as large prepropolypeptide precursors that undergo a first cleavage by a subtilisin serine protease to form an inactive intermediate, which in turn is cleaved again by endothelin-converting enzyme 1 (ECE-1) to yield the active 21 amino acid peptide. This gene encodes a protein which is expressed in neural crest cells (NCC), binds to endothelin receptor b (Ednrb) and plays an essential role in the development of NCC-derived cell lineages including melanocytes and enteric neurons. Mutations in this gene are associated with terminal aganglionosis and white spotted coat in mice and Hirschsprung's disease and Waardenburg syndrome in humans. [provided by RefSeq, Apr 2013]
Expression	Broad expression in large intestine adult (RPKM 6.3), small intestine adult (RPKM 5.0) and 18 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

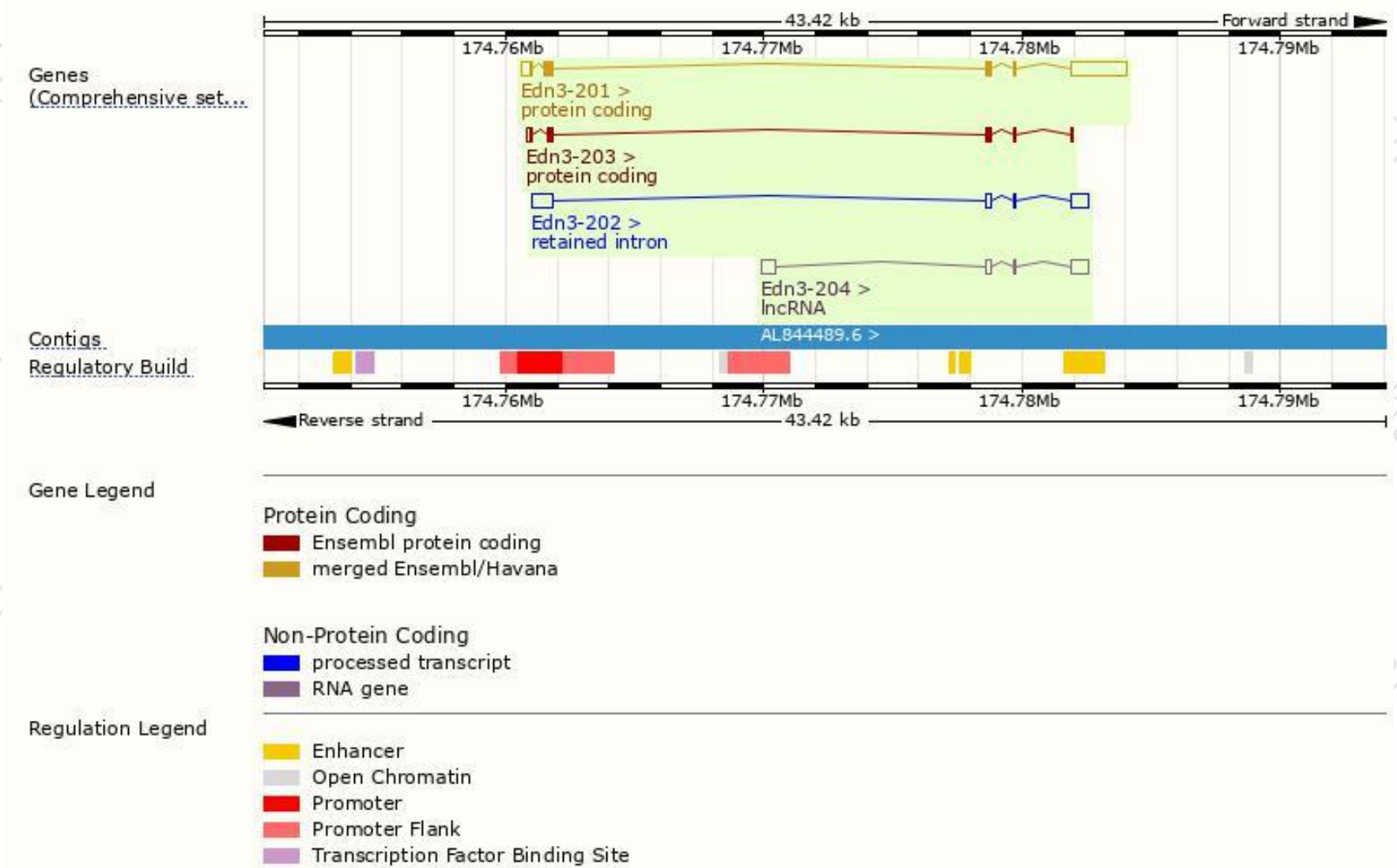
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Edn3-201	ENSMUST00000029030.8	3117	214aa	Protein coding	CCDS17156	A2APU5 P48299	TSL:1 GENCODE basic APPRIS P2
Edn3-203	ENSMUST00000140908.1	731	166aa	Protein coding	-	E0CZ86	TSL:5 GENCODE basic APPRIS ALT2
Edn3-202	ENSMUST00000137369.7	1721	No protein	Retained intron	-	-	TSL:1
Edn3-204	ENSMUST00000162473.1	1437	No protein	lncRNA	-	-	TSL:1

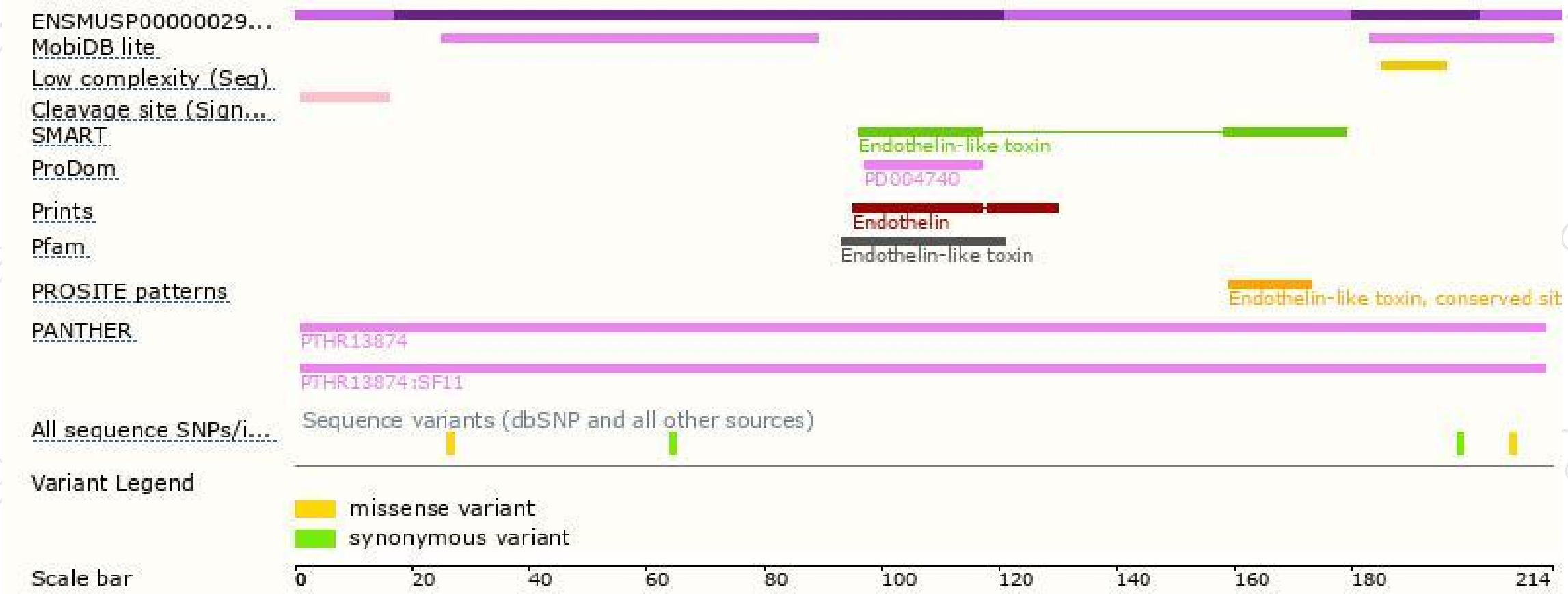
The strategy is based on the design of *Edn3-201* transcript,The transcription is shown below



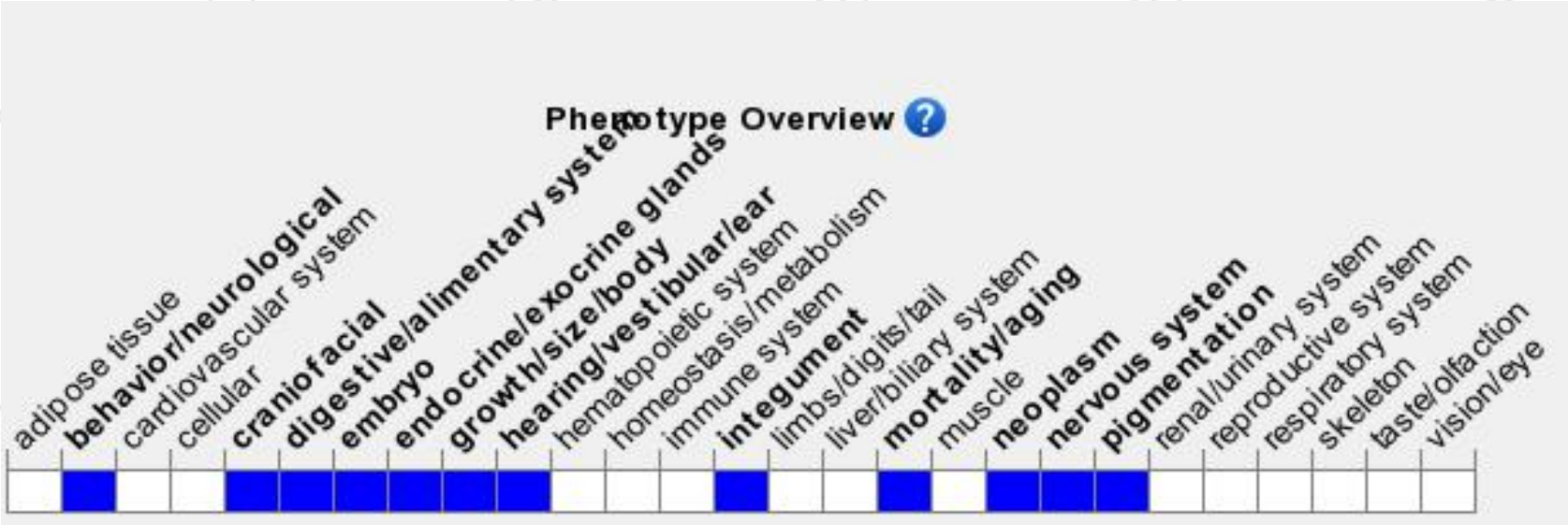
Genomic location distribution



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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