Aff1 Cas9-KO Strategy

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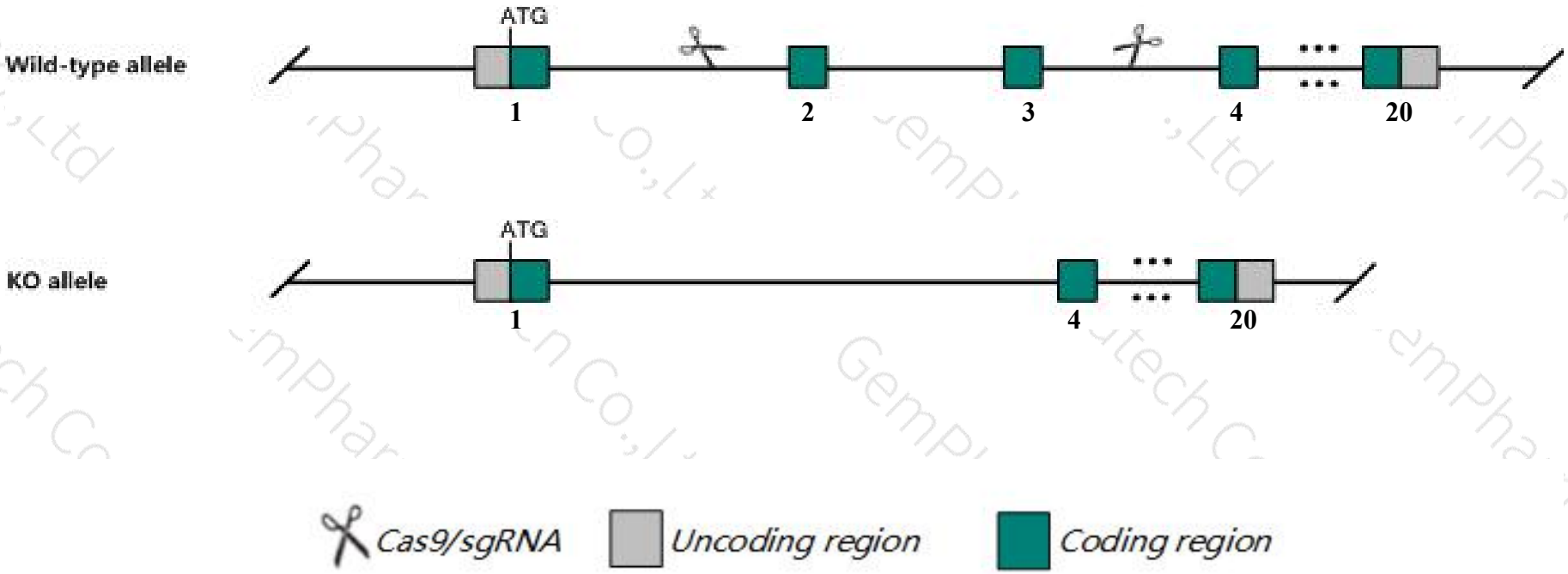
Project Overview

Project Name	***Aff1***
Project type	**Cas9-KO**
Strain background	**C57BL/6J**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Aff1* gene. The schematic diagram is as follows:



Technical routes

- The *Aff1* gene has 5 transcripts. According to the structure of *Aff1* gene, exon2-exon3 of *Aff1-202* (ENSMUST00000054979.9) transcript is recommended as the knockout region.The region contains 979bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Aff1* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.

Notice

- According to the existing MGI data,Homozygotes for a targeted null mutation exhibit impaired B and T cell development. Heterozygotes for an ENU-induced mutation exhibit small size, ataxia, adult-onset Purkinje cell loss, cataracts, reduced survival, and low fertility.

- The *Aff1* gene is located on the Chr5. 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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Aff1 AF4/FMR2 family, member 1 [Mus musculus (house mouse)]

Gene ID: 17355, updated on 31-Jan-2019

Summary

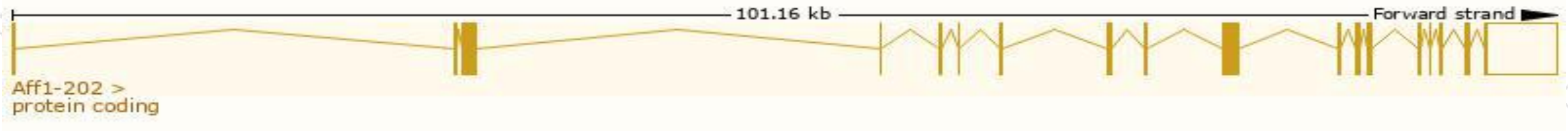
Official Symbol	Aff1 provided by MGI
Official Full Name	AF4/FMR2 family, member 1 provided by MGI
Primary source	MGI:MGI:1100819
See related	Ensembl:ENSMUSG00000029313
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	9630032B01Rik, AW319193, Af4, Mllt2h, Rob
Summary	This gene encodes a member of the AF4/ lymphoid nuclear protein related to the Fragile X E syndrome (FRAXE) family of proteins, which have been implicated in human childhood lymphoblastic leukemia, fragile chromosome X intellectual disability, and ataxia. It is the prevalent mixed-lineage leukemia fusion gene associated with spontaneous acute lymphoblastic leukemia. Members of this family have three conserved domains: an N-terminal homology domain, an AF4/ lymphoid nuclear protein domain, and a C-terminal homology domain. Knockout of the mouse gene by homologous recombination severely affects early events in lymphopoiesis, including precursor proliferation or recruitment, but is dispensable for terminal differentiation. In addition, an autosomal dominant missense mutation results in several phenotypes including ataxia and adult-onset Purkinje cell loss in the cerebellum, indicating a role in Purkinje cell maintenance and function. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Jul 2017]
Expression	Ubiquitous expression in thymus adult (RPKM 11.6), lung adult (RPKM 6.6) and 25 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

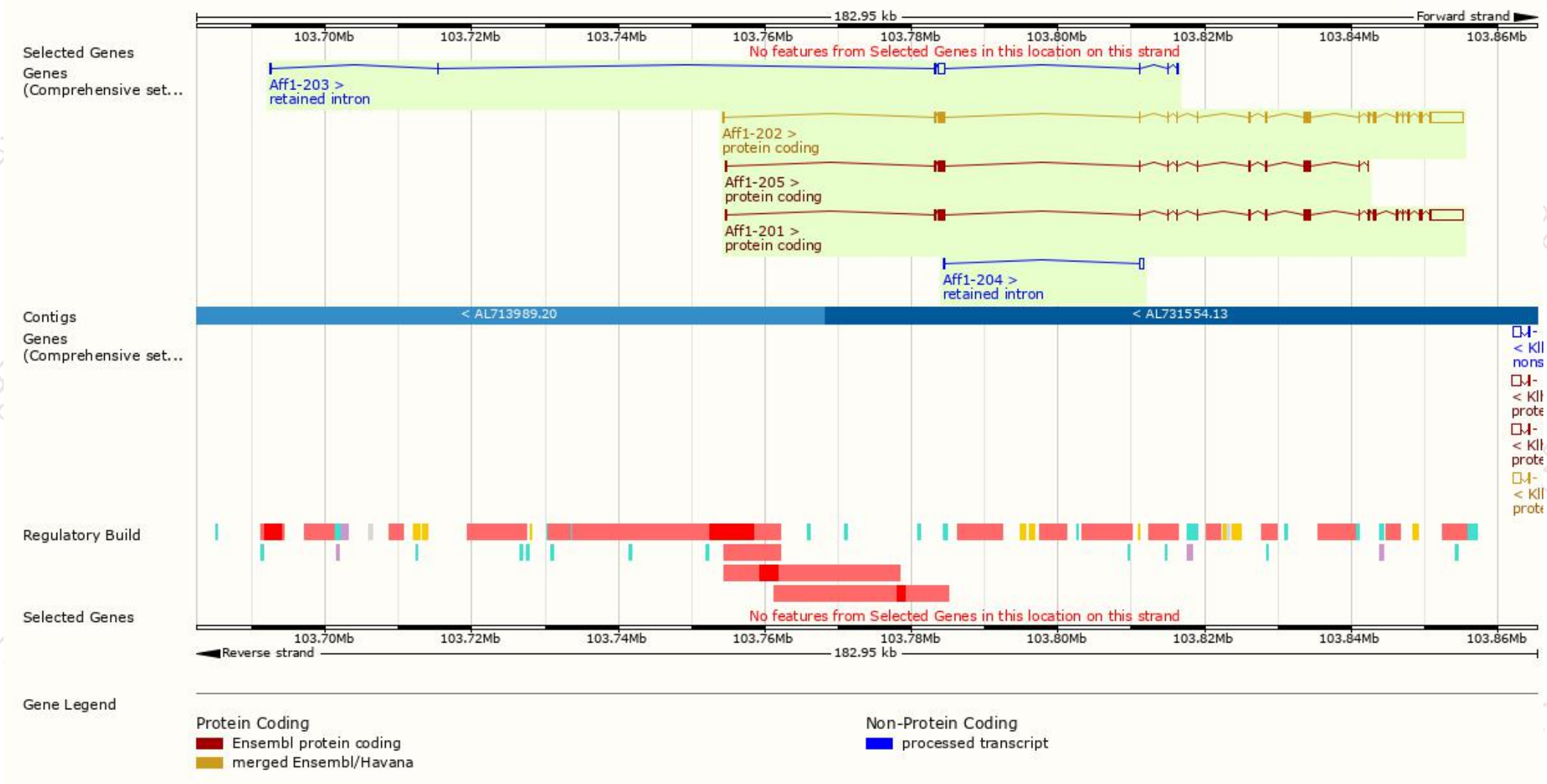
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Aff1-202	ENSMUST00000054979.9	8323	1218aa	Protein coding	CCDS39188	A3KMF4	TSL:1 GENCODE basic APPRIS ALT2
Aff1-201	ENSMUST00000031256.5	8312	1226aa	Protein coding	CCDS39189	E9Q921	TSL:5 GENCODE basic APPRIS P4
Aff1-205	ENSMUST00000153165.7	2753	870aa	Protein coding	-	B1AVP1	CDS 3' incomplete TSL:1
Aff1-203	ENSMUST00000126335.1	1613	No protein	Retained intron	-	-	TSL:1
Aff1-204	ENSMUST00000152145.1	688	No protein	Retained intron	-	-	TSL:3

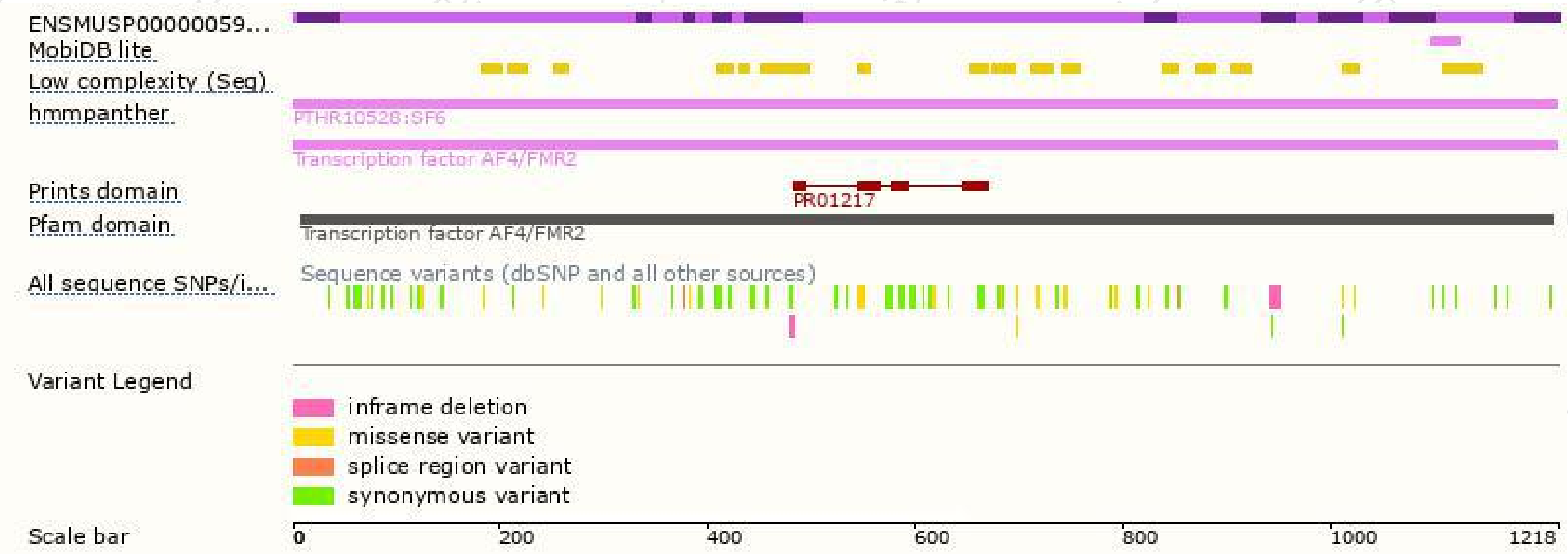
The strategy is based on the design of *Aff1-202* transcript, the transcription is shown below:



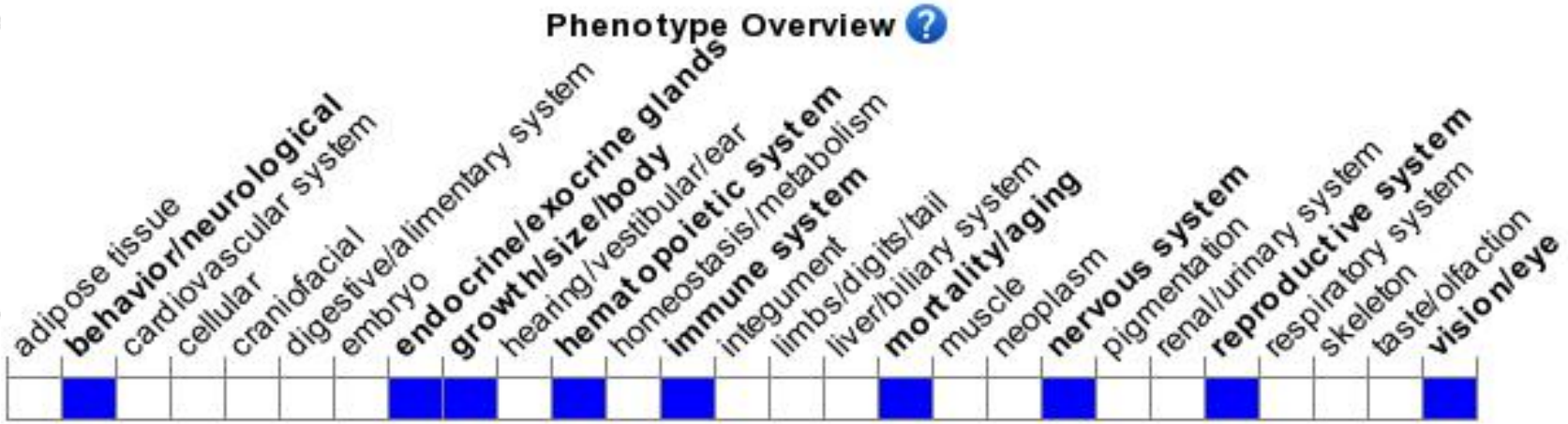
Genomic location distribution



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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