Afap1 Cas9-CKO Strategy

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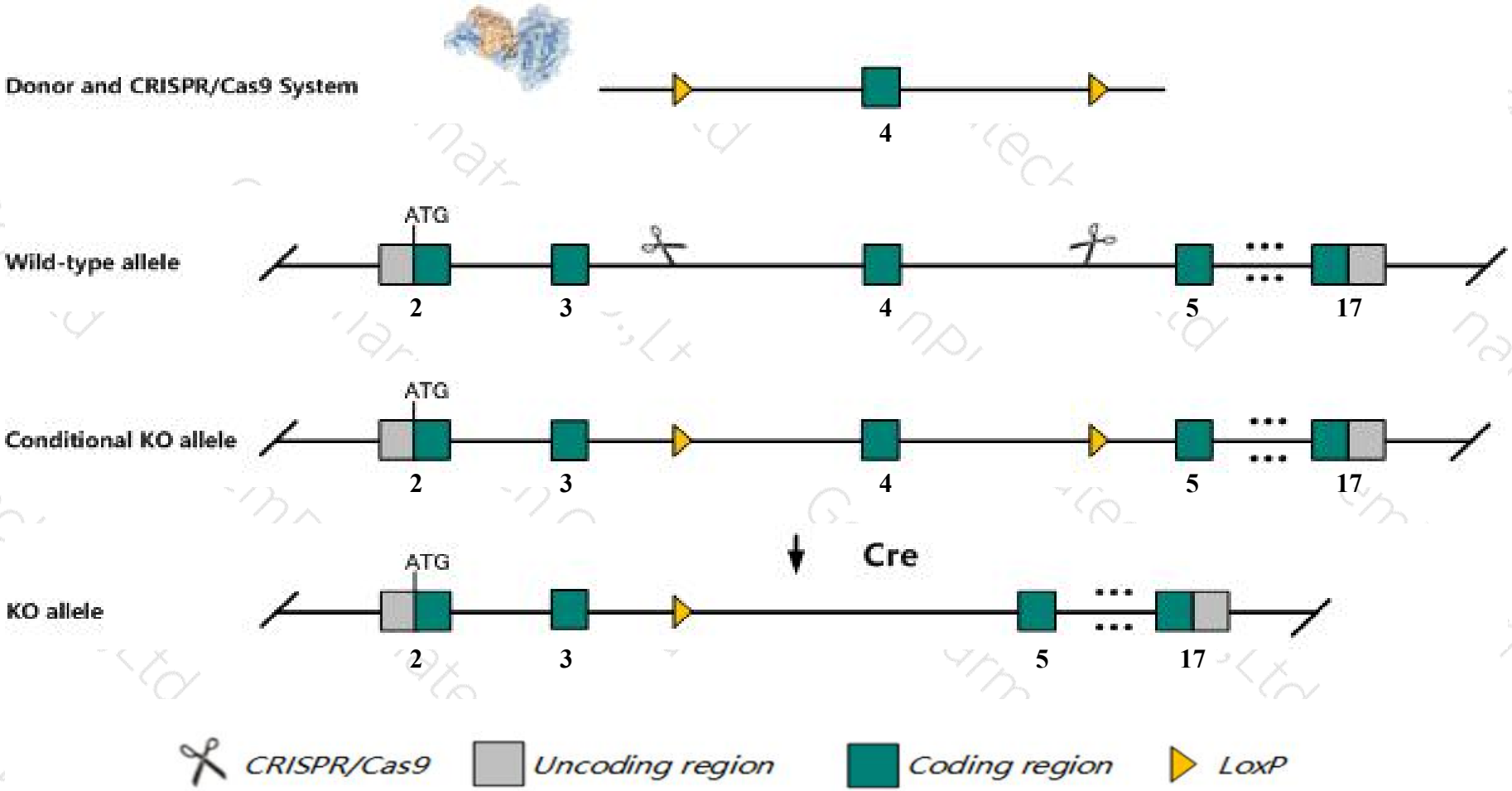
Design Date: 2020-2-17

Project Overview

Project Name	***Afap1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Afap1* gene. The schematic diagram is as follows:



Technical routes

- The *Afap1* gene has 5 transcripts. According to the structure of *Afap1* gene, exon4 of *Afap1-201* (ENSMUST00000064571.10) transcript is recommended as the knockout region.The region contains 109bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Afap1* 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 knock-out allele exhibit inability to nurse pups due to failed secretory activation, reduced milk lipid synthesis and precocious mammary gland involution.

- Transcript 205 CDS 5' and 3' incomplete the influences is unknown.

- The *Afap1* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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Afap1 actin filament associated protein 1 [Mus musculus (house mouse)]

Gene ID: 70292, updated on 31-Jan-2019

Summary

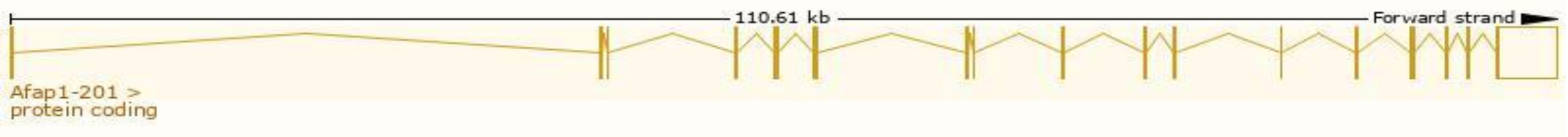
Official Symbol	Afap1 provided by MGI
Official Full Name	actin filament associated protein 1 provided byMGI
Primary source	MGI:MGI:1917542
See related	Ensembl:ENSMUSG00000029094
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2600003E23Rik, 9630044L16Rik, AI848729, Afap, mKIAA3018
Expression	Ubiquitous expression in subcutaneous fat pad adult (RPKM 12.9), whole brain E14.5 (RPKM 12.4) and 24 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

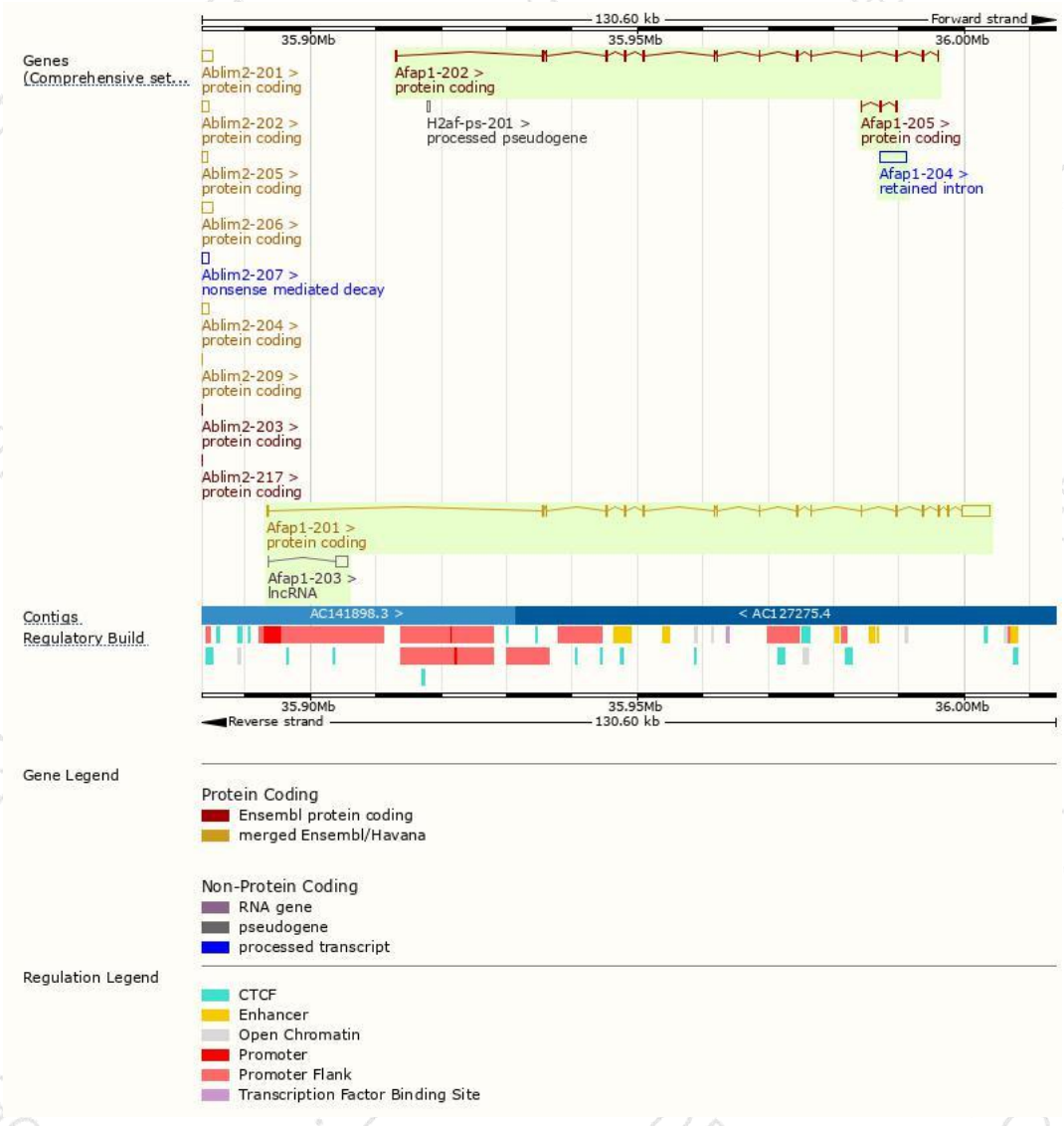
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Afap1-201	ENSMUST00000064571.10	6618	731aa	Protein coding	CCDS19236	Q80YS6	TSL:1 GENCODE basic APPRIS P1
Afap1-202	ENSMUST00000141824.1	1994	627aa	Protein coding	-	E9Q8X9	CDS 3' incomplete TSL:2
Afap1-205	ENSMUST00000212374.1	501	167aa	Protein coding	-	A0A1D5RLL0	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Afap1-204	ENSMUST00000201482.1	4222	No protein	Retained intron	-	-	TSL:NA
Afap1-203	ENSMUST00000146300.1	1890	No protein	lncRNA	-	-	TSL:1

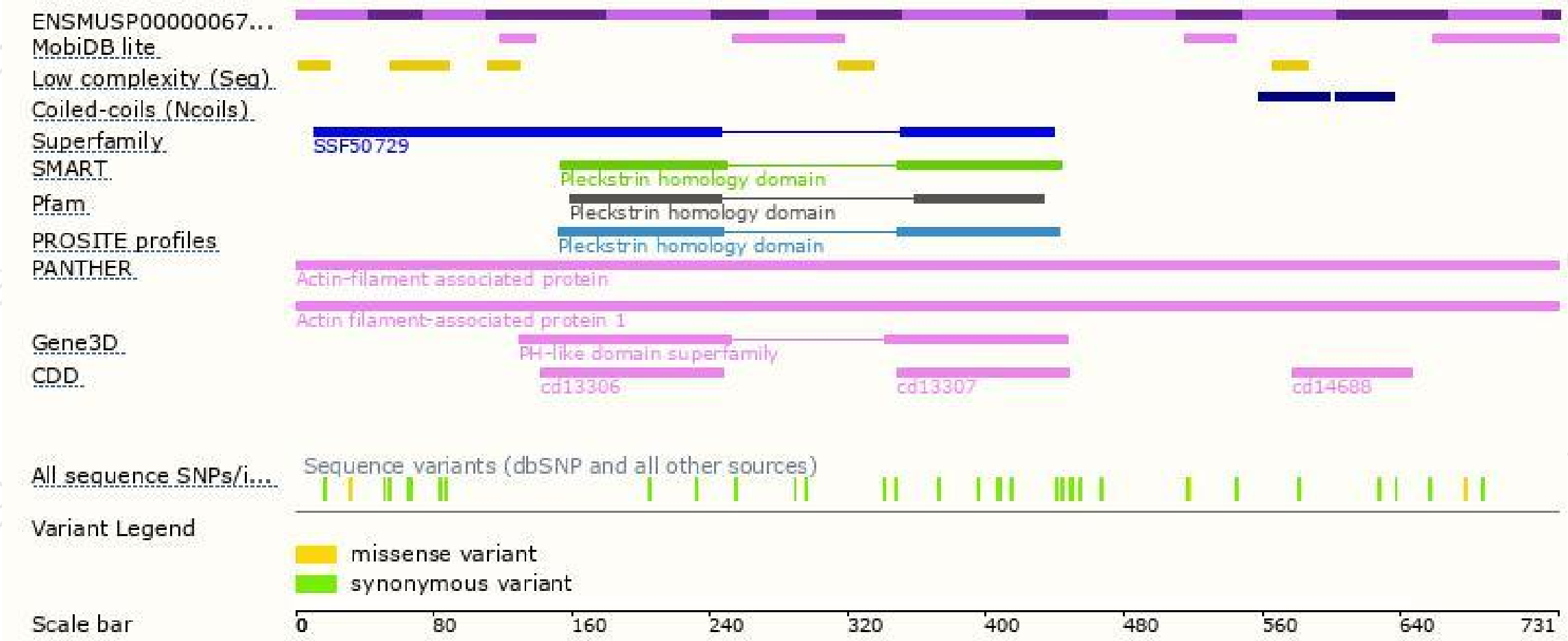
The strategy is based on the design of *Afap1-201* transcript,The transcription is shown below



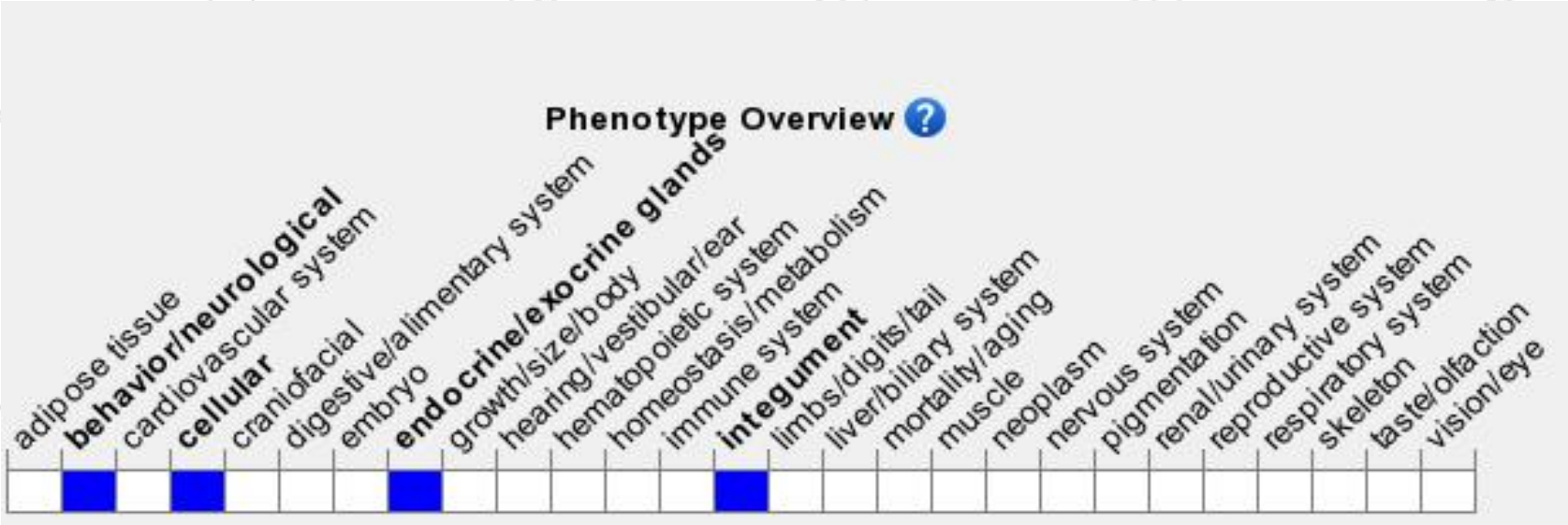
Genomic location distribution



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 exhibit inability to nurse pups due to failed secretory activation, reduced milk lipid synthesis and precocious mammary gland involution.

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

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