Abcd4 Cas9-CKO Strategy

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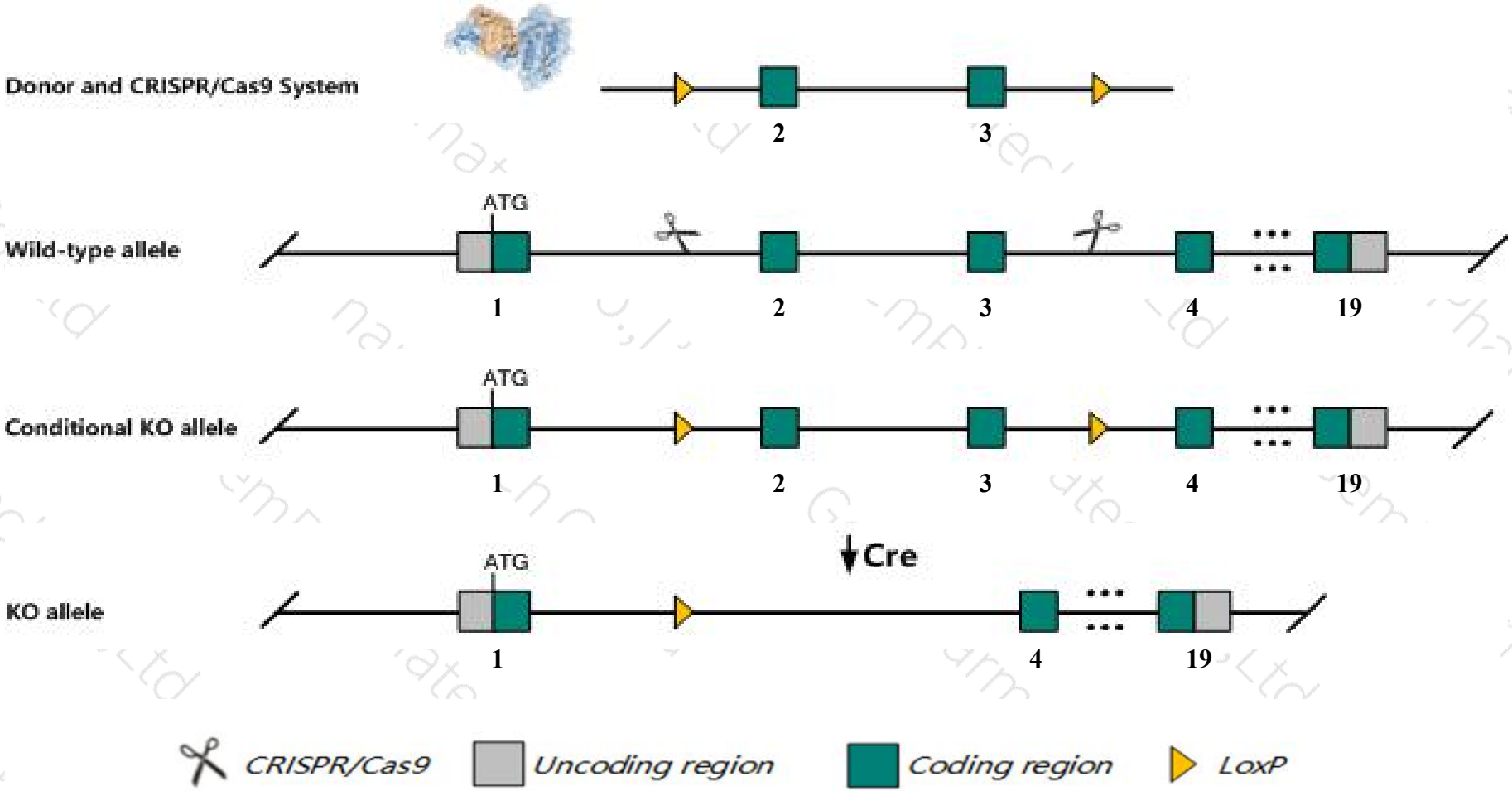
Design Date: 2020-3-5

Project Overview

Project Name	***Abcd4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Abcd4* gene. The schematic diagram is as follows:



Technical routes

- The *Abcd4* gene has 10 transcripts. According to the structure of *Abcd4* gene, exon2-exon3 of *Abcd4-201* (ENSMUST00000021666.5) transcript is recommended as the knockout region.The region contains 247bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Abcd4* 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

➢ The *Abcd4* gene is located on the Chr12. 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）

集萃药康 GemPharmatech

Abcd4 ATP-binding cassette, sub-family D (ALD), member 4 [Mus musculus (house mouse)]

Gene ID: 19300, updated on 31-Jan-2019

Summary

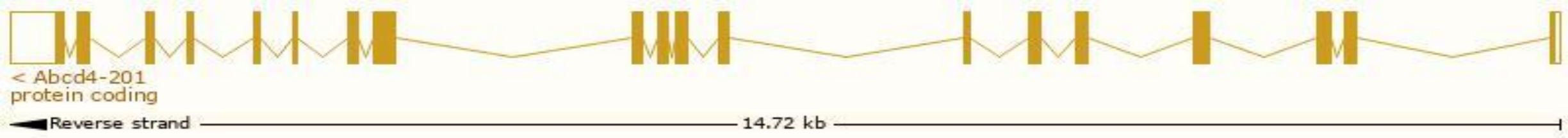
Official Symbol	Abcd4 provided by MGI
Official Full Name	ATP-binding cassette, sub-family D (ALD), member 4 provided by MGI
Primary source	MGI:MGI:1349217
See related	Ensembl:ENSMUSG00000021240
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	P69r, P70R, Pxmp1l
Summary	The membrane-associated protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intra-cellular membranes. ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White). This protein is a member of the ALD subfamily, which is involved in peroxisomal import of fatty acids and/or fatty acyl-CoAs in the organelle. All known peroxisomal ABC transporters are half transporters which require a partner half transporter molecule to form a functional homodimeric or heterodimeric transporter. The function of this peroxisomal membrane protein is unknown. However, it is speculated that the human protein may function as a heterodimer for another peroxisomal ABC transporter and, therefore, may modify the adrenoleukodystrophy phenotype. It may also play a role in the process of peroxisome biogenesis. [provided by RefSeq, Jul 2008]
Expression	Ubiquitous expression in kidney adult (RPKM 14.9), placenta adult (RPKM 11.8) and 27 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

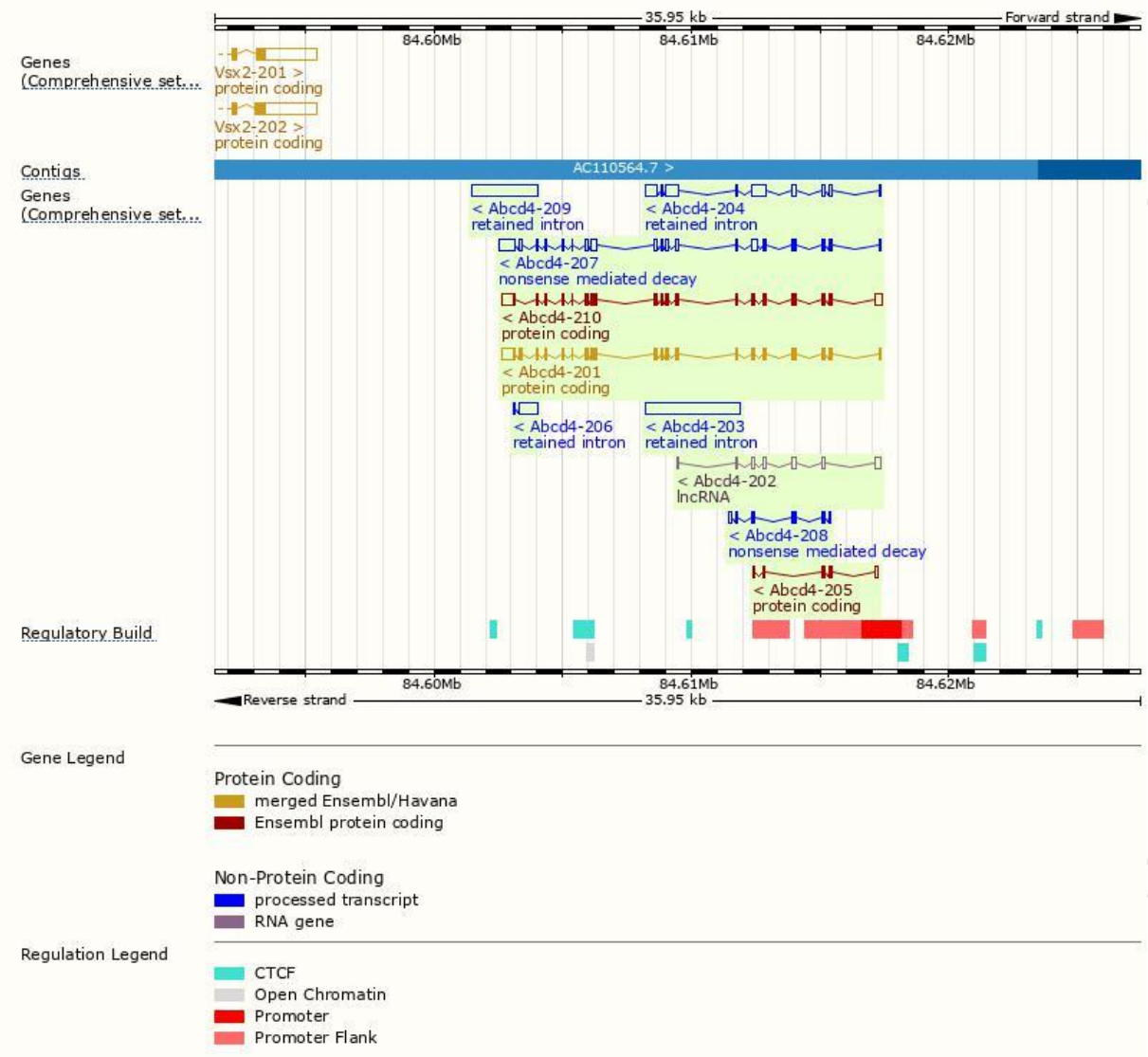
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Abcd4-201	ENSMUST00000021666.5	2309	606aa	Protein coding	CCDS26047	O89016	TSL:1 GENCODE basic APPRIS P1
Abcd4-210	ENSMUST00000223107.1	2410	558aa	Protein coding	-	A0A1Y7VMF7	TSL:1 GENCODE basic
Abcd4-205	ENSMUST00000221070.1	470	130aa	Protein coding	-	A0A1Y7VMS0	CDS 3' incomplete TSL:5
Abcd4-207	ENSMUST00000222581.1	2581	194aa	Nonsense mediated decay	-	A0A1Y7VM74	TSL:1
Abcd4-208	ENSMUST00000222889.1	640	150aa	Nonsense mediated decay	-	A0A1Y7VLL1	CDS 5' incomplete TSL:5
Abcd4-203	ENSMUST00000220678.1	3698	No protein	Retained intron	-	-	TSL:NA
Abcd4-209	ENSMUST00000222942.1	2589	No protein	Retained intron	-	-	TSL:NA
Abcd4-204	ENSMUST00000220952.1	2136	No protein	Retained intron	-	-	TSL:2
Abcd4-206	ENSMUST00000221948.1	825	No protein	Retained intron	-	-	TSL:2
Abcd4-202	ENSMUST00000220553.1	809	No protein	lncRNA	-	-	TSL:3

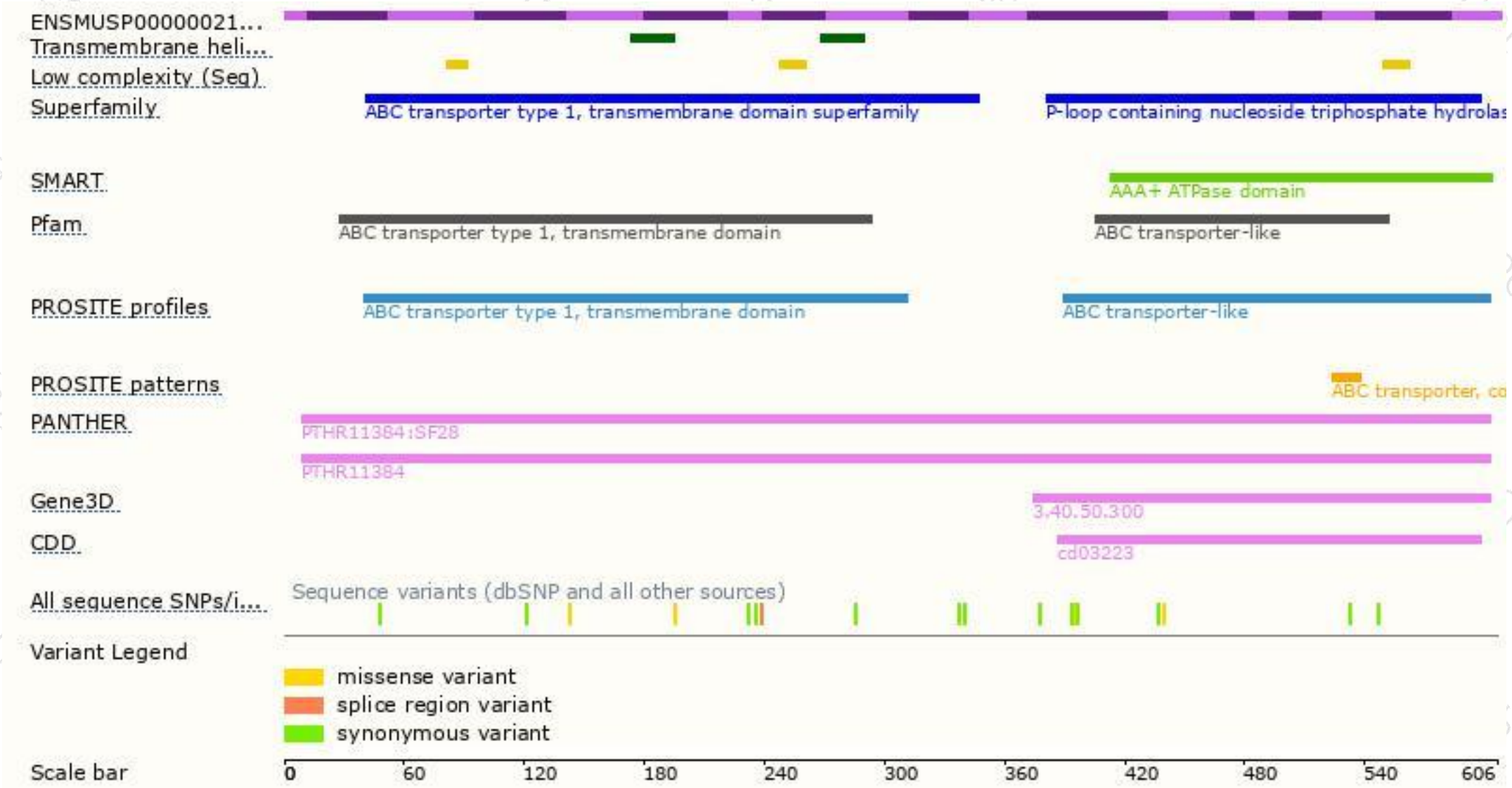
The strategy is based on the design of *Abcd4-201* transcript,The transcription is shown below



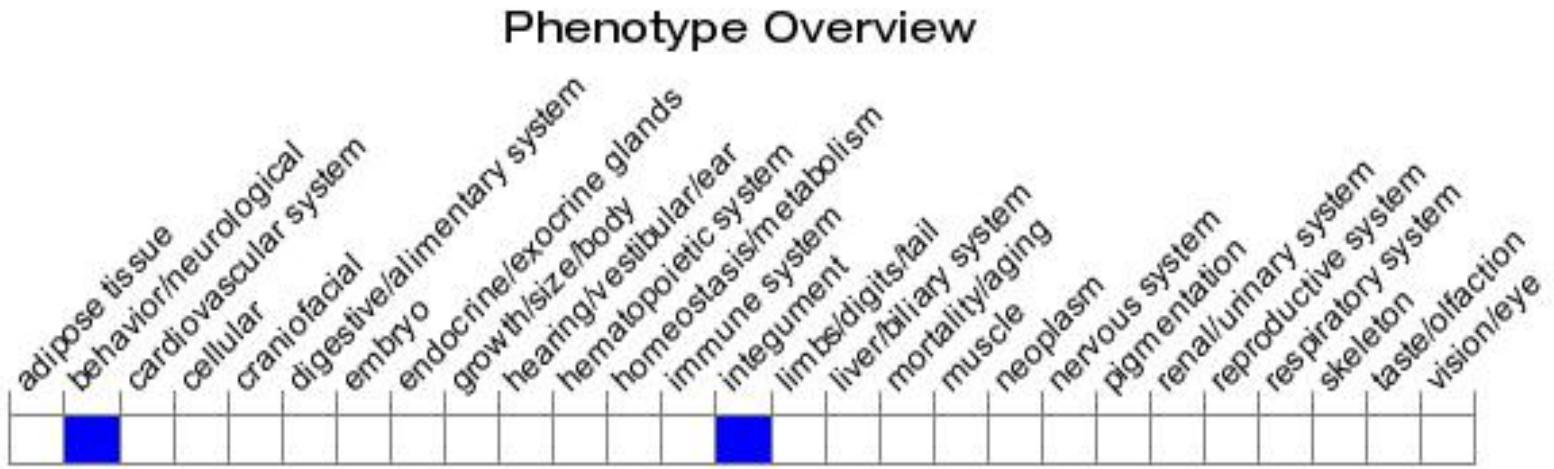
Genomic location distribution



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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