Adarb1 Cas9-CKO Strategy

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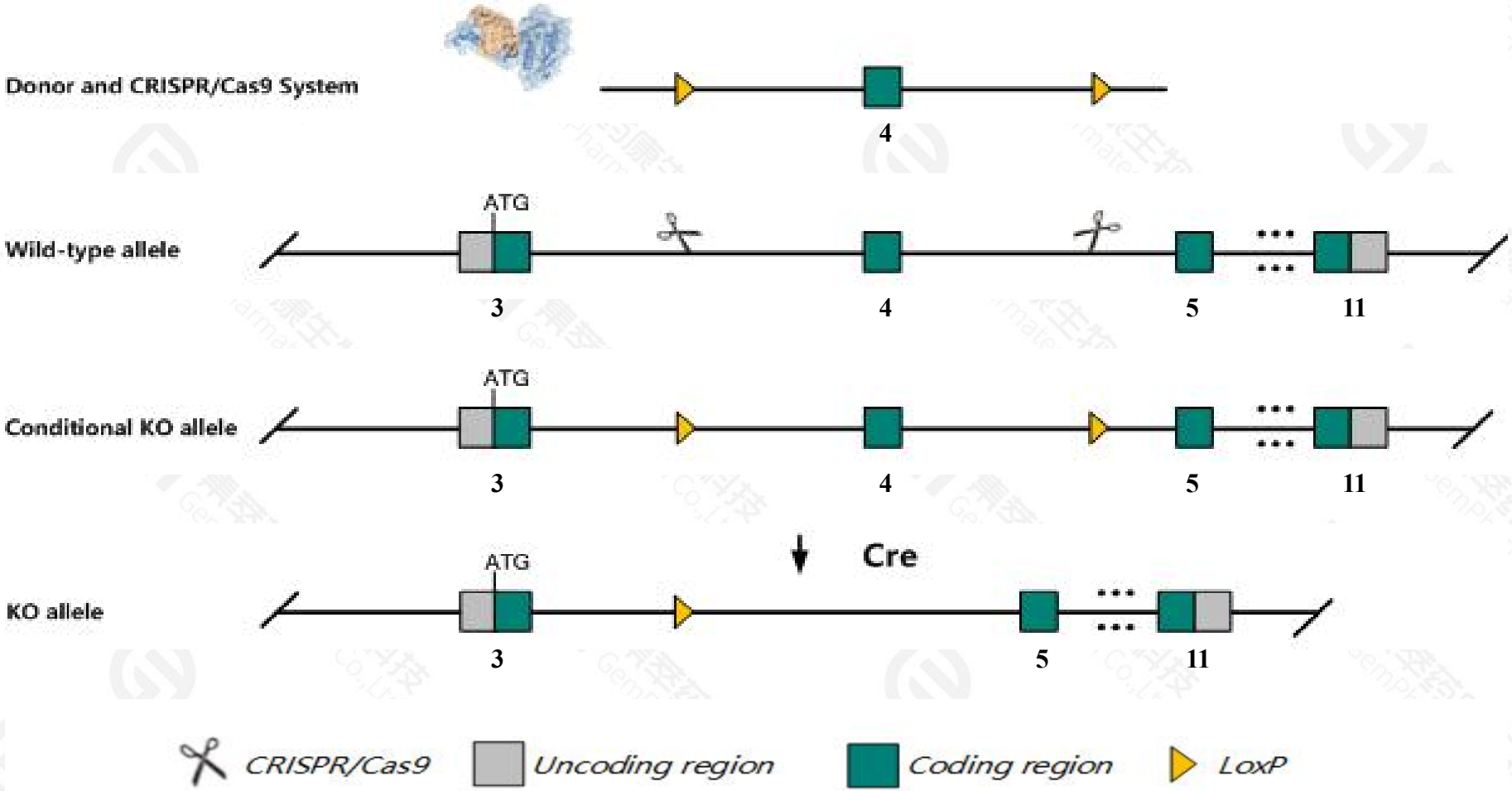
Project Overview

Project Name	***Adarb1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Adarb1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Adarb1* gene has 13 transcripts. According to the structure of *Adarb1* gene, exon4 of *Adarb1-201*(ENSMUST00000020496.14) transcript is recommended as the knockout region.The region contains 935bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Adarb1* 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,homozygous mutation of this gene results in progressive seizure susceptibility and death within 20 days of age. Mice homozygous for a conditional allele activated in neurons exhibit motor neuron degeneration, motor function abnormalities, and premature death.

➢ The *Adarb1* gene is located on the Chr10. 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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Adarb1 adenosine deaminase, RNA-specific, B1 [Mus musculus (house mouse)]

Gene ID: 110532, updated on 26-Jan-2021

Summary

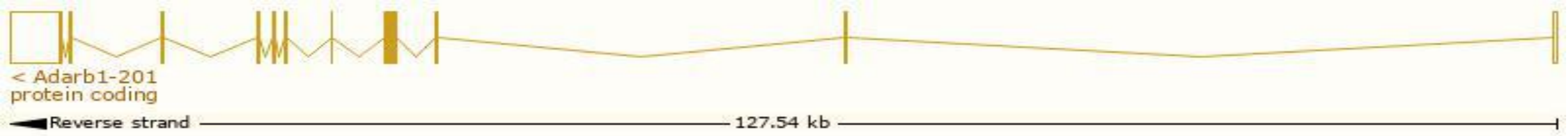
Official Symbol	Adarb1 provided by MGI
Official Full Name	adenosine deaminase, RNA-specific, B1 provided by MGI
Primary source	MGI:MGI:891999
See related	Ensembl:ENSMUSG00000020262
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1700057H01Rik, AD, AW124433, AW558573, Adar2, BB220382, D10Bwg0447e, RED, Red1
Summary	This gene encodes a double-stranded-RNA-specific adenosine deaminase that is involved in editing pre-mRNAs by site-specific conversion of adenosine (A) to inosine (I). Substrates for this enzyme include ionotropic glutamate receptors (GluR2-6) and serotonin receptor (5HT2C). Studies in rodents have shown that this protein can modify its own pre-mRNA by A->I editing to create a novel acceptor splice site, alternative splicing to which results in down regulation of its protein expression. Additional splicing events result in transcript variants encoding different isoforms. [provided by RefSeq, Jul 2008]
Expression	Broad expression in cerebellum adult (RPKM 24.4), frontal lobe adult (RPKM 19.8) and 18 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

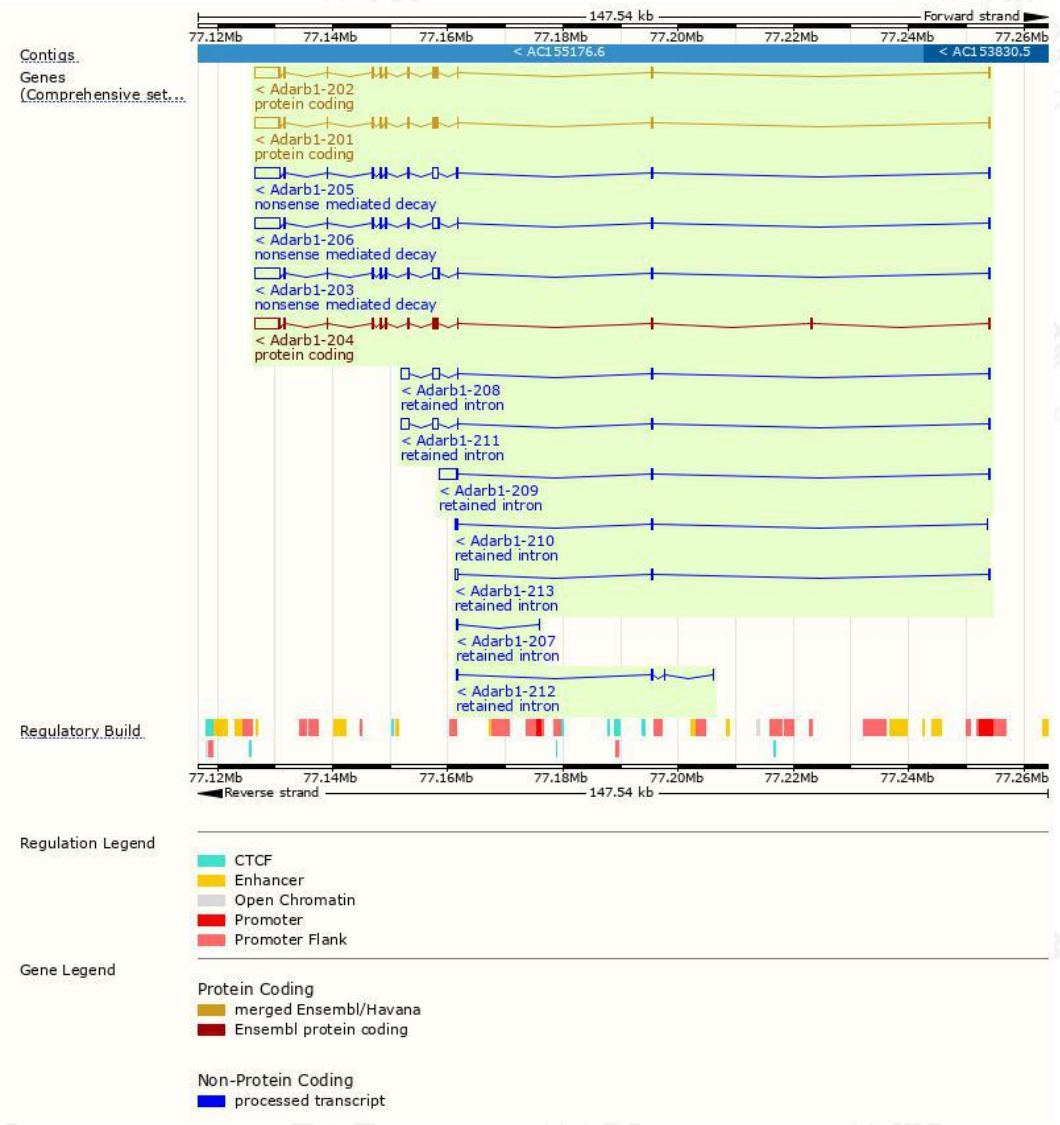
The gene has 13 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Adarb1-204	ENSMUST00000105406.8	6662	711aa	Protein coding	CCDS35948		TSL:1 , GENCODE basic ,
Adarb1-202	ENSMUST00000098374.9	6584	711aa	Protein coding	CCDS35948		TSL:1 , GENCODE basic ,
Adarb1-201	ENSMUST00000020496.14	6549	701aa	Protein coding	CCDS35949		TSL:1 , GENCODE basic , APPRIS P1 ,
Adarb1-206	ENSMUST00000144547.8	6630	82aa	Nonsense mediated decay	-		TSL:1 ,
Adarb1-205	ENSMUST00000126073.8	6583	38aa	Nonsense mediated decay	-		TSL:1 ,
Adarb1-203	ENSMUST00000105404.9	6557	82aa	Nonsense mediated decay	-		TSL:1 ,
Adarb1-209	ENSMUST00000150227.8	3289	No protein	Retained intron	-		TSL:1 ,
Adarb1-208	ENSMUST00000149738.8	2584	No protein	Retained intron	-		TSL:1 ,
Adarb1-211	ENSMUST00000154607.8	2577	No protein	Retained intron	-		TSL:1 ,
Adarb1-213	ENSMUST00000156583.8	704	No protein	Retained intron	-		TSL:1 ,
Adarb1-210	ENSMUST00000150512.8	670	No protein	Retained intron	-		TSL:3 ,
Adarb1-212	ENSMUST00000155117.2	451	No protein	Retained intron	-		TSL:1 ,
Adarb1-207	ENSMUST00000146319.2	383	No protein	Retained intron	-		TSL:3 ,

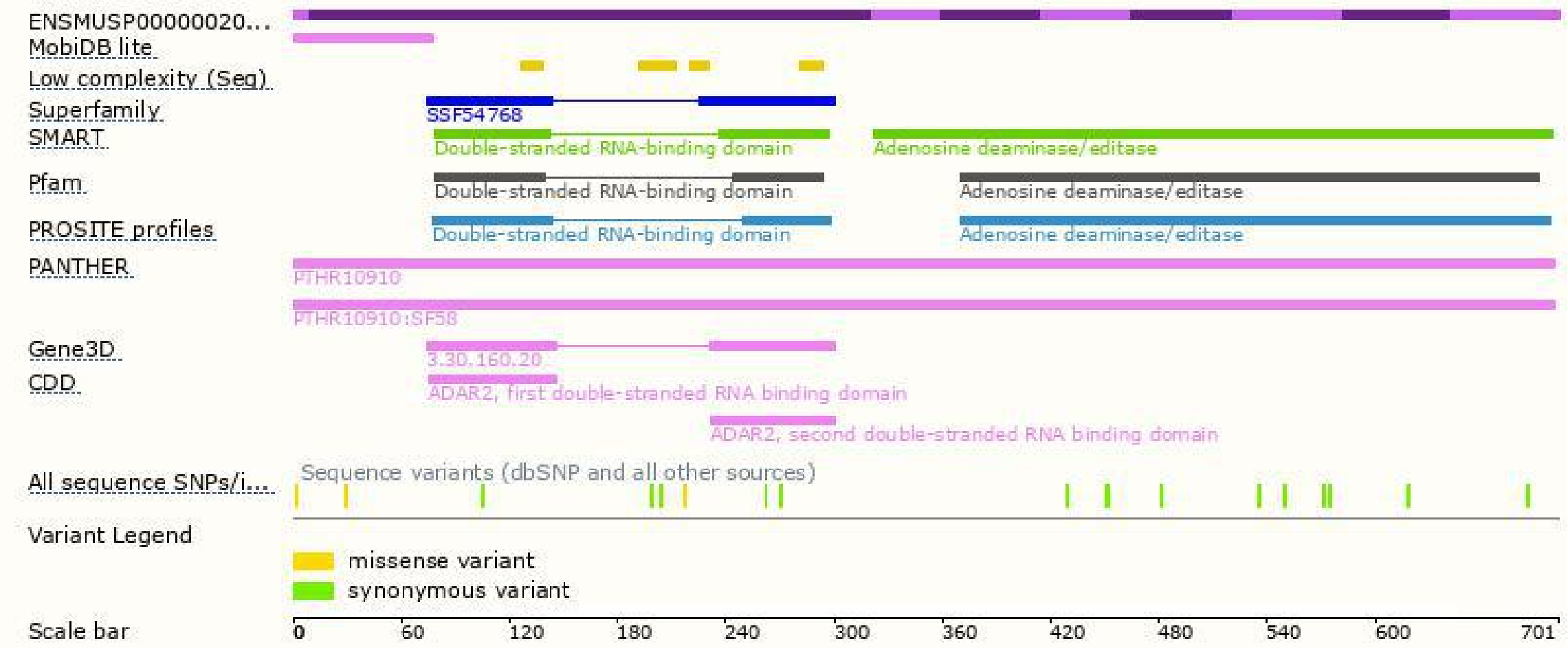
The strategy is based on the design of *Adarb1-201* transcript,the transcription is shown below:



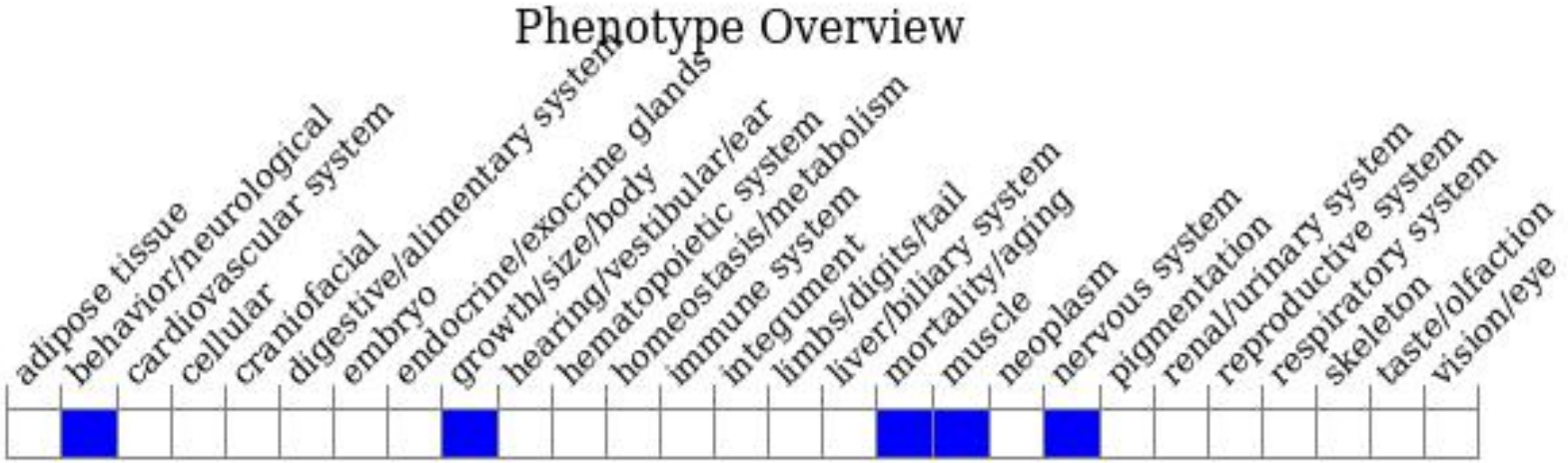
Genomic location distribution



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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