Triobp Cas9-CKO Strategy

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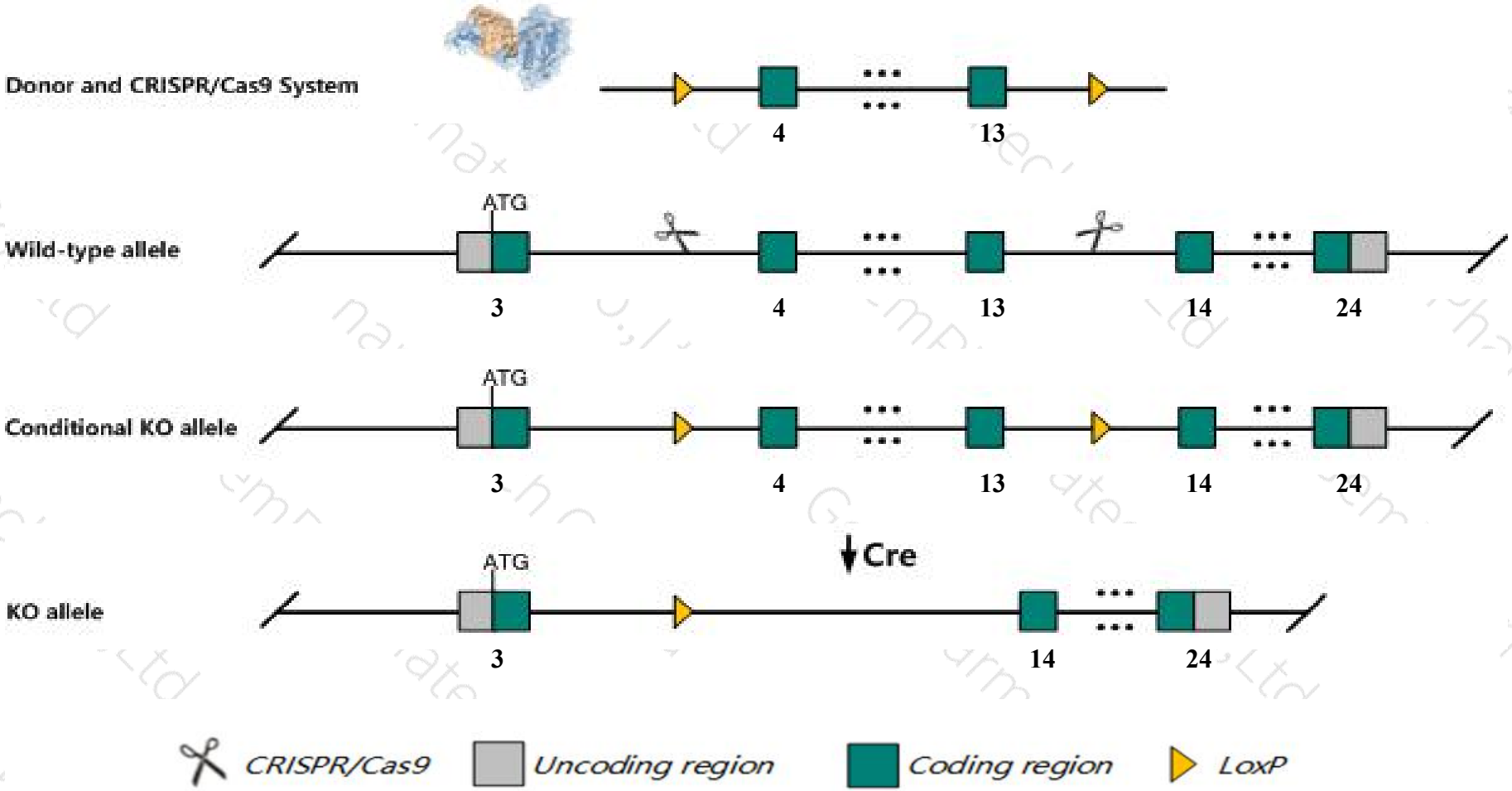
Project Overview

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

Conditional Knockout strategy

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



Technical routes

➢ The *Triobp* gene has 12 transcripts. According to the structure of *Triobp* gene, exon4-exon13 of *Triobp-204*(ENSMUST00000109690.10) transcript is recommended as the knockout region.The region contains 4342bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Triobp* 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 gene trapped alleles exhibit embryonic lethality. Mice homozygous for a targeted allele eliminating isoforms 4 and 5 exhibit profound deafness associated with stereocilia fragility and degeneration.

➢ The *Triobp* gene is located on the Chr15. 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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Triobp TRIO and F-actin binding protein [Mus musculus (house mouse)]

Gene ID: 110253, updated on 13-Mar-2020

Summary

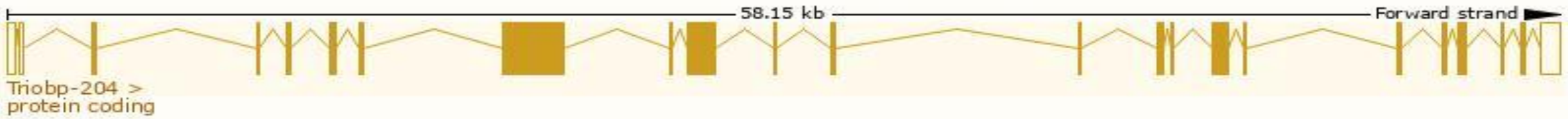
Official Symbol	Triobp provided by MGI
Official Full Name	TRIO and F-actin binding protein provided byMGI
Primary source	MGI:MGI:1349410
See related	Ensembl:ENSMUSG00000033088
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI428493, EST478828, Tara, mKIAA1662
Summary	This gene encodes a protein that interacts with trio, which is involved with neural tissue development and controlling actin cytoskeleton organization, cell motility, and cell growth. The encoded protein also associates with F-actin and stabilizes F-actin structures. Domains contained in this encoded protein are an N-terminal pleckstrin homology domain and a C-terminal coiled-coil region. Mutations in the human gene have been associated with a form of autosomal recessive nonsyndromic deafness. Multiple alternatively spliced transcript variants have been described [provided by RefSeq, Sep 2012]
Expression	Ubiquitous expression in adrenal adult (RPKM 14.9), ovary adult (RPKM 12.4) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

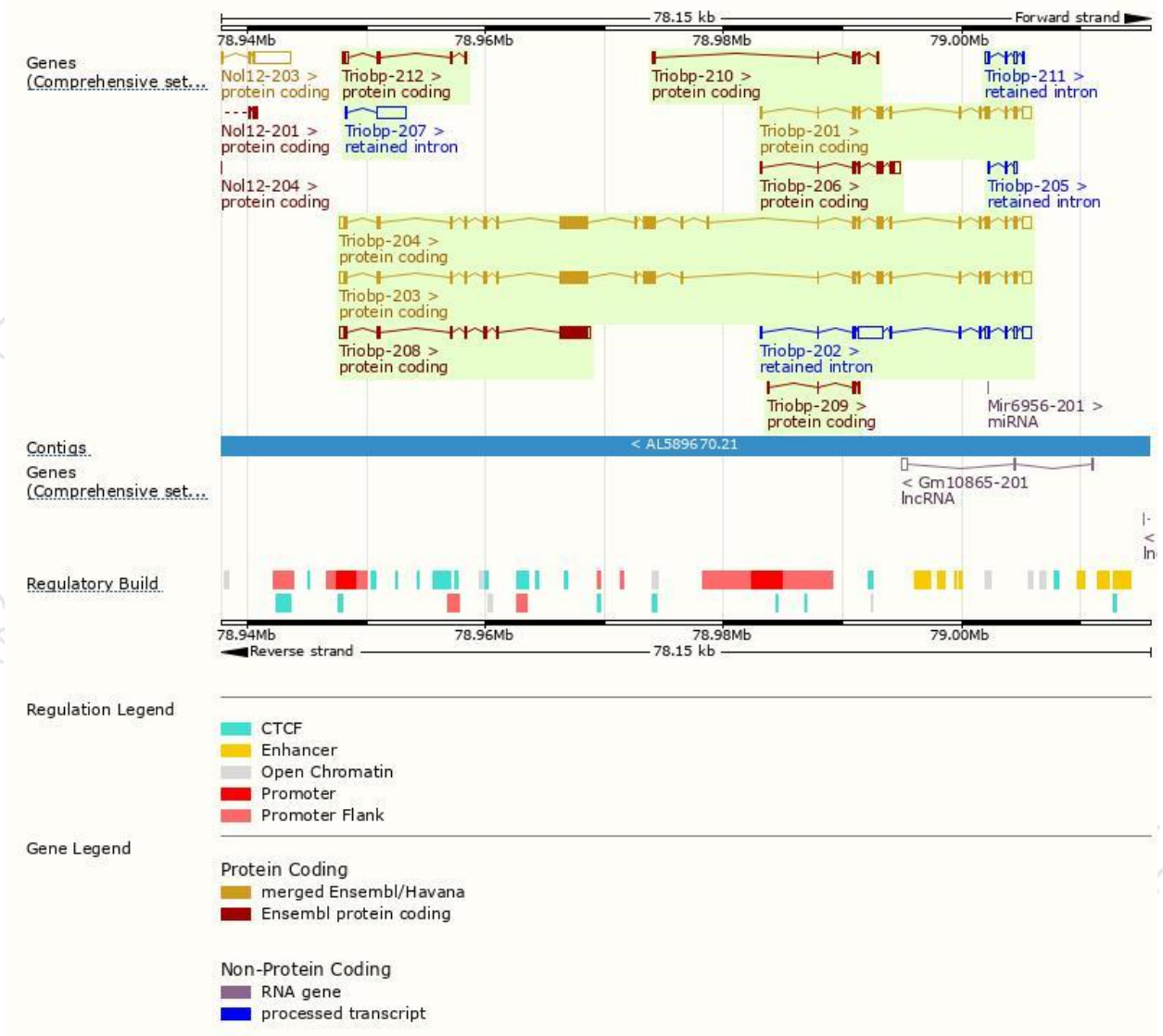
The gene has 12 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Triobp-204	ENSMUST00000109690.10	7327	2014aa	Protein coding	CCDS37136	Q99KW3	TSL:1 GENCODE basic APPRIS P4
Triobp-203	ENSMUST00000109689.8	7187	1968aa	Protein coding	CCDS37137	Q99KW3	TSL:1 GENCODE basic APPRIS ALT2
Triobp-201	ENSMUST00000109687.8	2570	627aa	Protein coding	CCDS37138	A0A2U3TZ82	TSL:1 GENCODE basic APPRIS ALT2
Triobp-208	ENSMUST00000140228.7	3807	980aa	Protein coding	-	Q99KW3	TSL:1 GENCODE basic
Triobp-206	ENSMUST00000130663.9	1660	402aa	Protein coding	-	G5E8W1	CDS 5' incomplete TSL:5
Triobp-212	ENSMUST00000229943.1	762	90aa	Protein coding	-	A0A2R8VI64	CDS 3' incomplete
Triobp-210	ENSMUST00000229270.1	745	248aa	Protein coding	-	A0A2R8VI29	CDS 5' and 3' incomplete APPRIS ALT2
Triobp-209	ENSMUST00000144151.2	404	98aa	Protein coding	-	J9JI90	CDS 3' incomplete TSL:3
Triobp-202	ENSMUST00000109688.8	3980	No protein	Retained intron	-	-	TSL:1
Triobp-207	ENSMUST00000137934.1	2446	No protein	Retained intron	-	-	TSL:1
Triobp-211	ENSMUST00000229535.1	676	No protein	Retained intron	-	-	
Triobp-205	ENSMUST00000129922.1	480	No protein	Retained intron	-	-	TSL:2

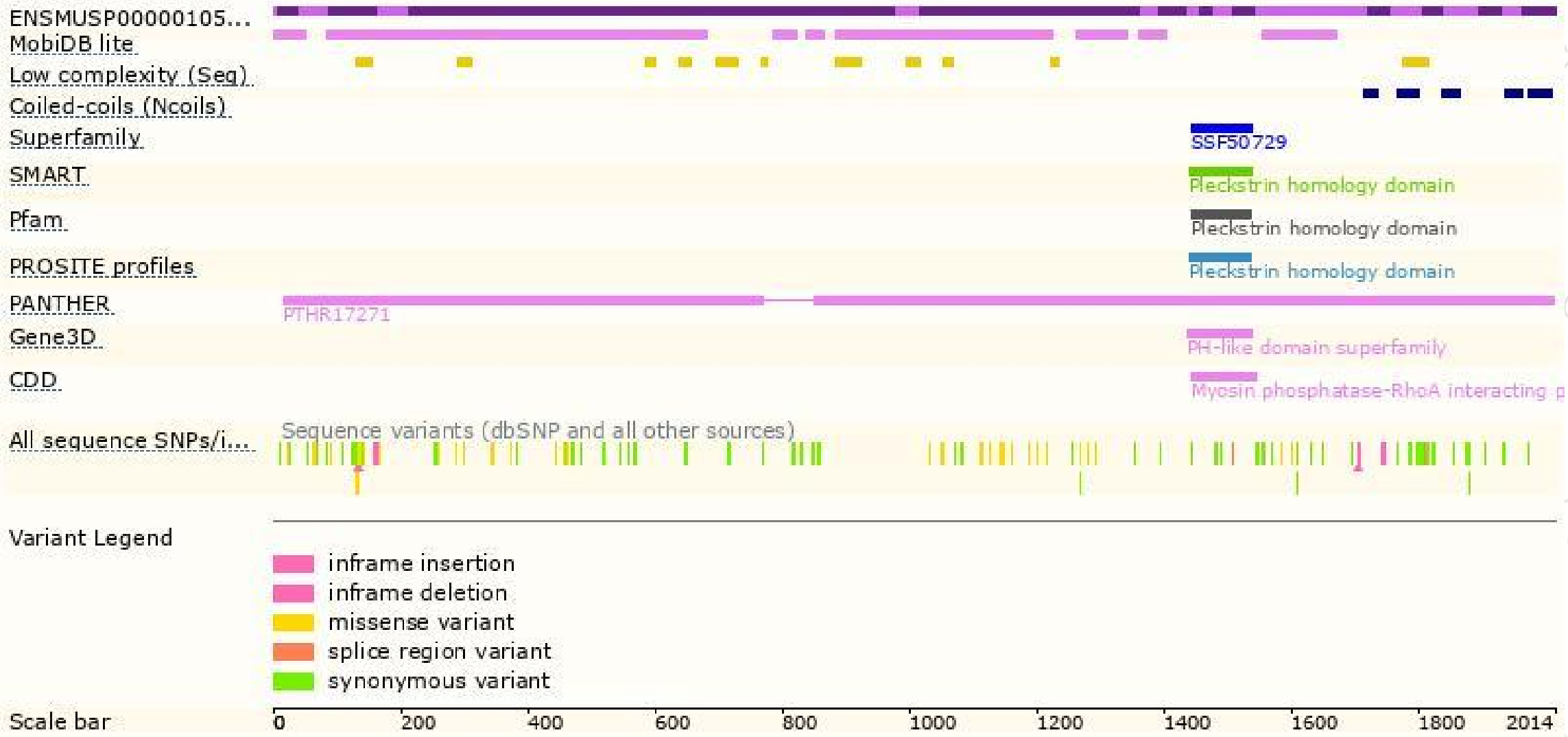
The strategy is based on the design of *Triobp-204* transcript,the transcription is shown below:



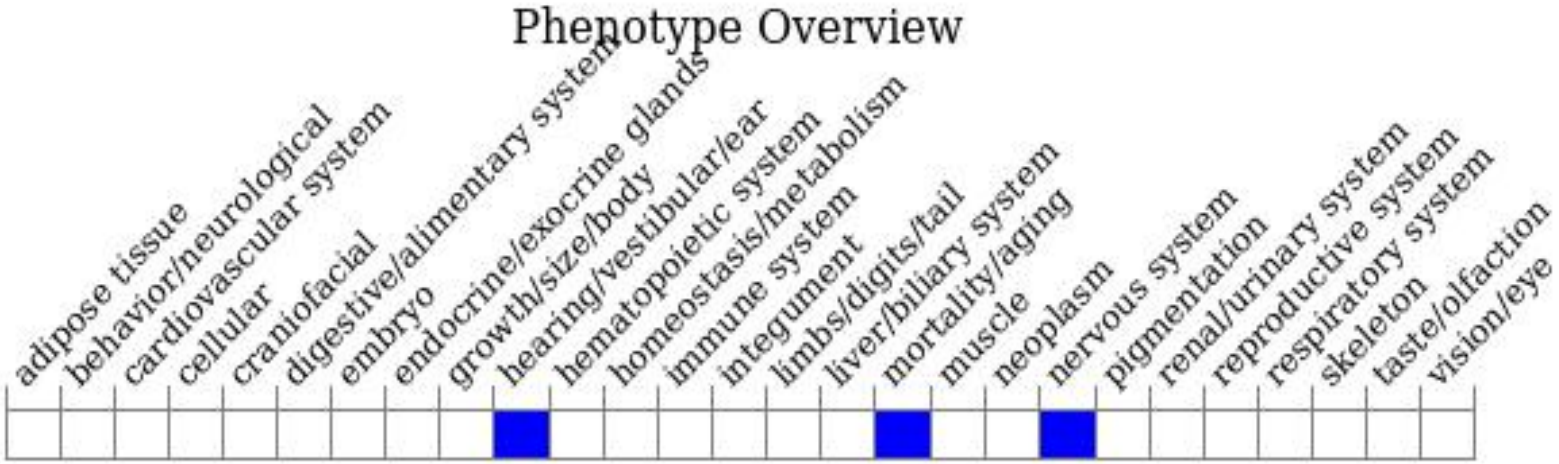
Genomic location distribution



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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