Brd4 Cas9-CKO Strategy

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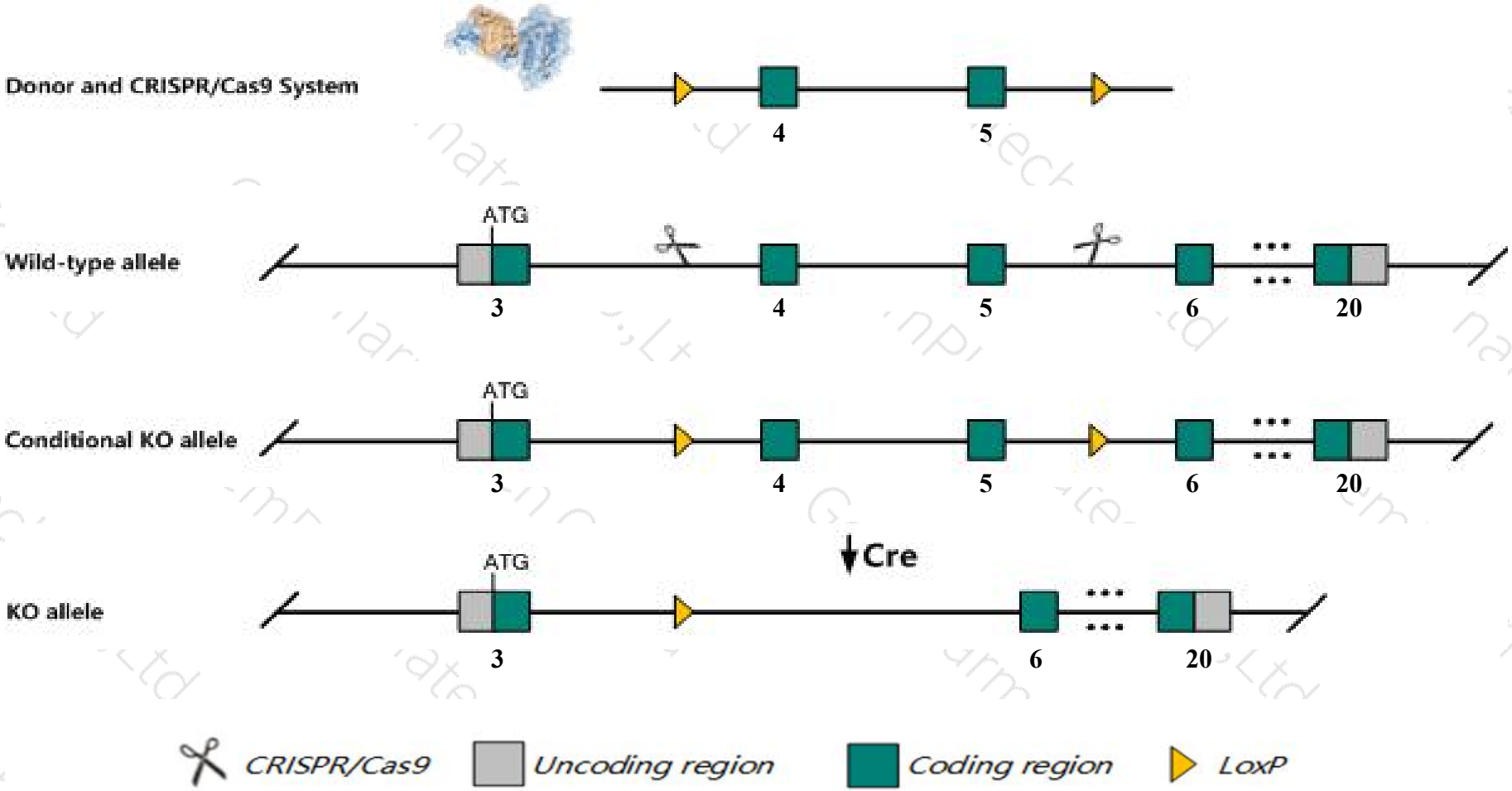
Project Overview

Project Name	***Brd4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Brd4* gene. The schematic diagram is as follows:



Technical routes

- The *Brd4* gene has 12 transcripts. According to the structure of *Brd4* gene, exon4-exon5 of *Brd4-201* (ENSMUST00000003726.15) transcript is recommended as the knockout region.The region contains 277bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Brd4* 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,Homozygotes for a gene-trap null mutation die soon after implantation. Heterozygotes exhibit impaired pre- and postnatal growth, head malformations, lack of subcutaneous fat, cataracts, and abnormal liver cells.

- The *Brd4* gene is located on the Chr17. 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

Brd4 bromodomain containing 4 [Mus musculus (house mouse)]

Gene ID: 57261, updated on 3-Feb-2019

Summary

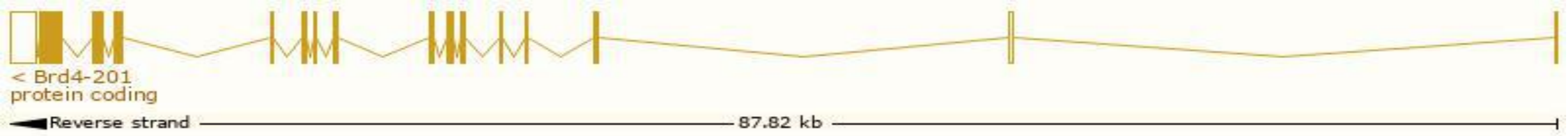
Official Symbol	Brd4 provided by MGI
Official Full Name	bromodomain containing 4 provided by MGI
Primary source	MGI:MGI:1888520
See related	Ensembl:ENSMUSG00000024002
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Brd5, HUNK1, MCAP, WI-11513
Summary	This gene was temporarily named bromodomain-containing 5 (Brd5) and was renamed bromodomain-containing 4 (Brd4). [provided by RefSeq, Jul 2008]
Expression	Ubiquitous expression in thymus adult (RPKM 17.8), spleen adult (RPKM 13.4) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

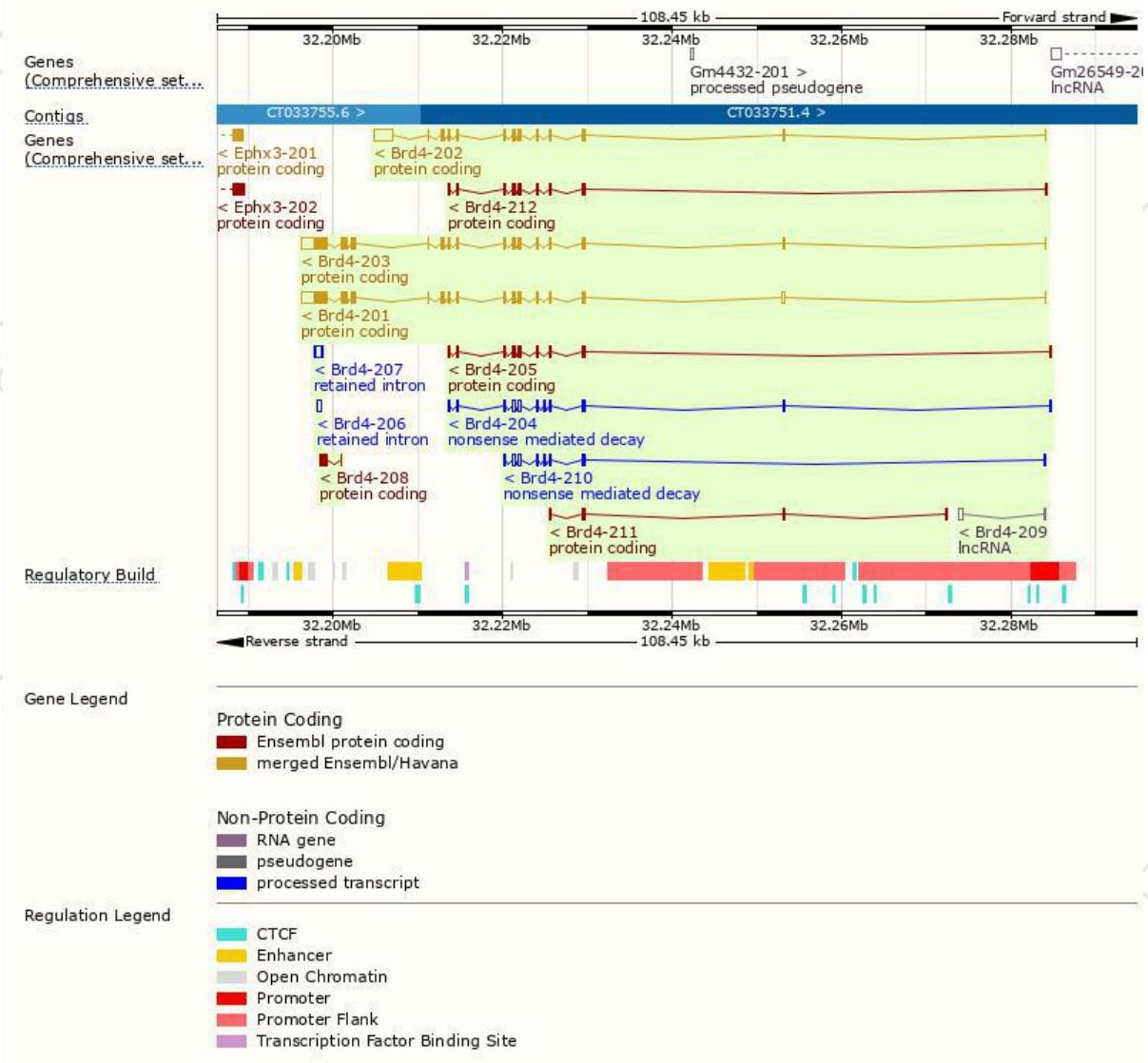
The gene has 12 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Brd4-201	ENSMUST00000003726.15	5957	1401aa	Protein coding	CCDS70784	Q3UH70	TSL:1 GENCODE basic APPRIS ALT2
Brd4-203	ENSMUST00000121285.7	5922	1400aa	Protein coding	CCDS50057	Q9ESU6	TSL:1 GENCODE basic APPRIS P3
Brd4-202	ENSMUST00000120276.8	4486	723aa	Protein coding	CCDS37554	B0V2V8 B2RSE4 Q9ESU6	TSL:5 GENCODE basic
Brd4-205	ENSMUST00000127893.7	1910	557aa	Protein coding	-	B0V2V6	CDS 3' incomplete TSL:1
Brd4-212	ENSMUST00000237692.1	1823	572aa	Protein coding	-	-	CDS 3' incomplete
Brd4-211	ENSMUST00000237008.1	731	133aa	Protein coding	-	-	CDS 3' incomplete
Brd4-208	ENSMUST00000230858.1	507	169aa	Protein coding	-	A0A2R8VHW4	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete
Brd4-204	ENSMUST00000125899.1	2118	146aa	Nonsense mediated decay	-	D6RGP2	TSL:1
Brd4-210	ENSMUST00000235598.1	1757	146aa	Nonsense mediated decay	-	-	
Brd4-207	ENSMUST00000230565.1	747	No protein	Retained intron	-	-	
Brd4-206	ENSMUST00000229020.1	438	No protein	Retained intron	-	-	
Brd4-209	ENSMUST00000230982.1	618	No protein	lncRNA	-	-	

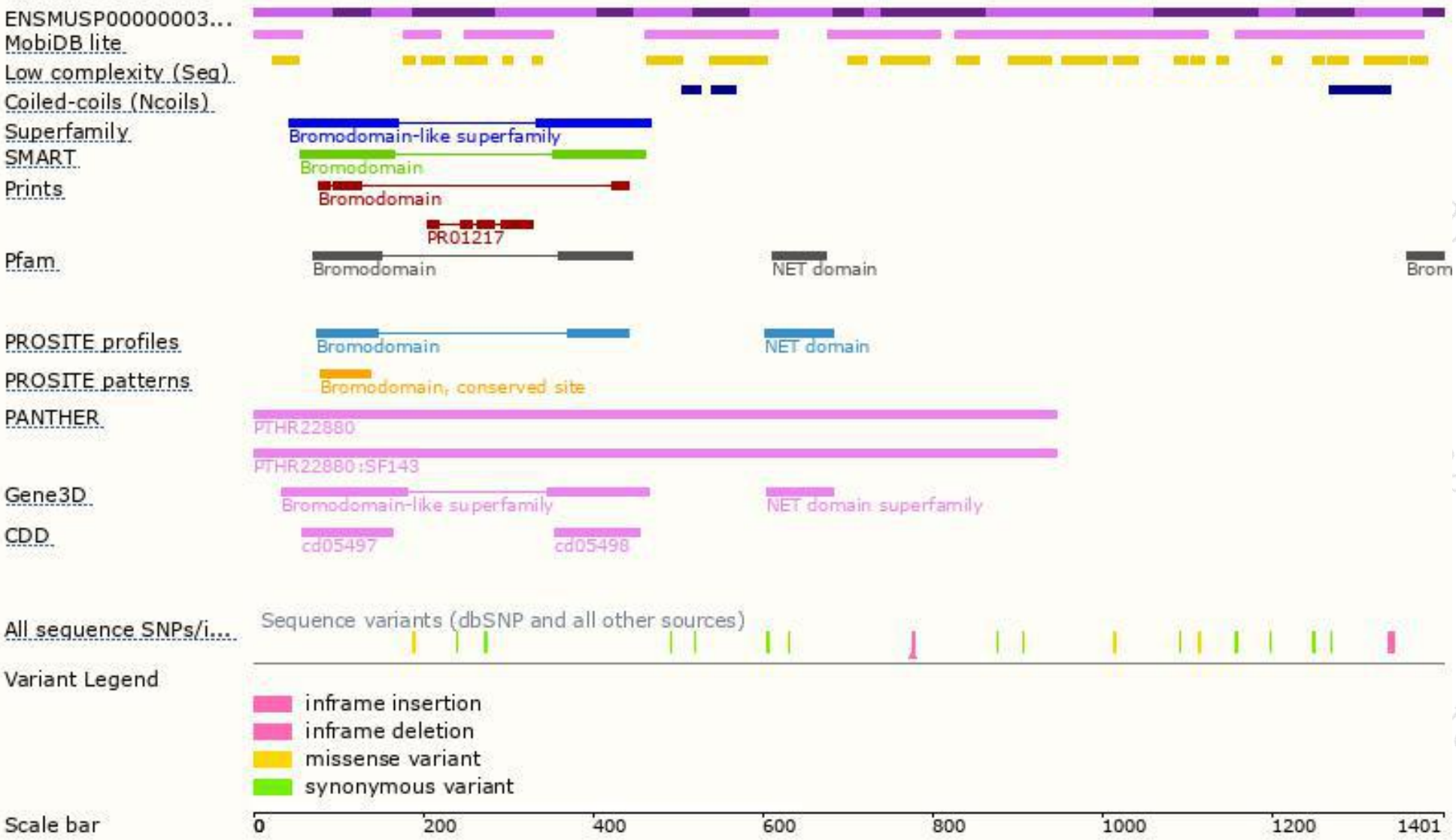
The strategy is based on the design of *Brd4-201* transcript,The transcription is shown below



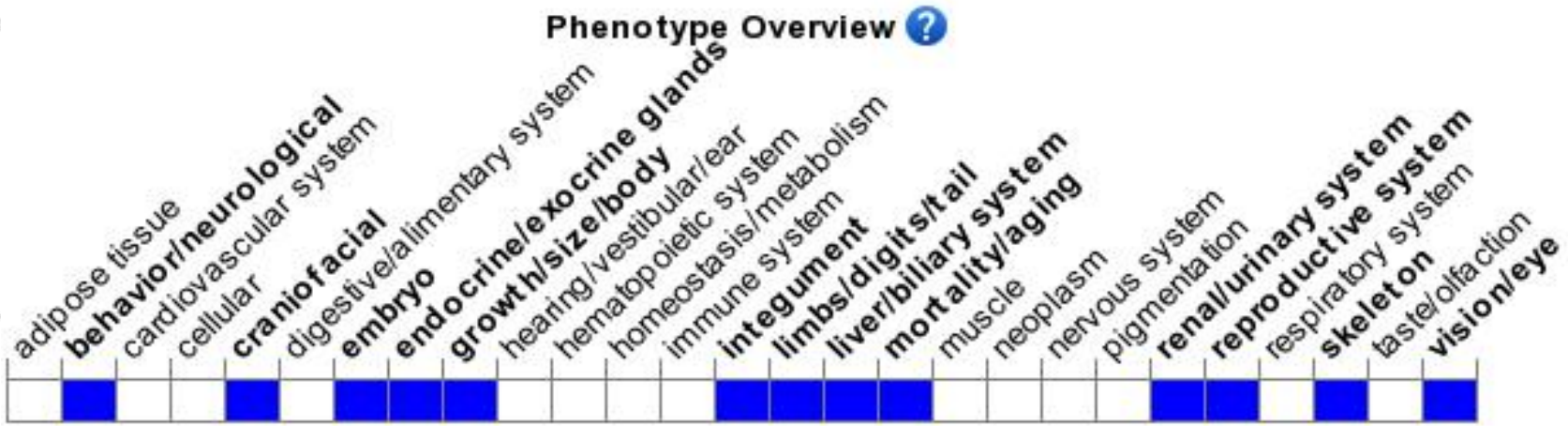
Genomic location distribution



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,Homozygotes for a gene-trap null mutation die soon after implantation. Heterozygotes exhibit impaired pre- and postnatal growth, head malformations, lack of subcutaneous fat, cataracts, and abnormal liver cells.

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

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