

***Brdt* Cas9-KO Strategy**

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Project Overview

Project Name

Brdt

Project type

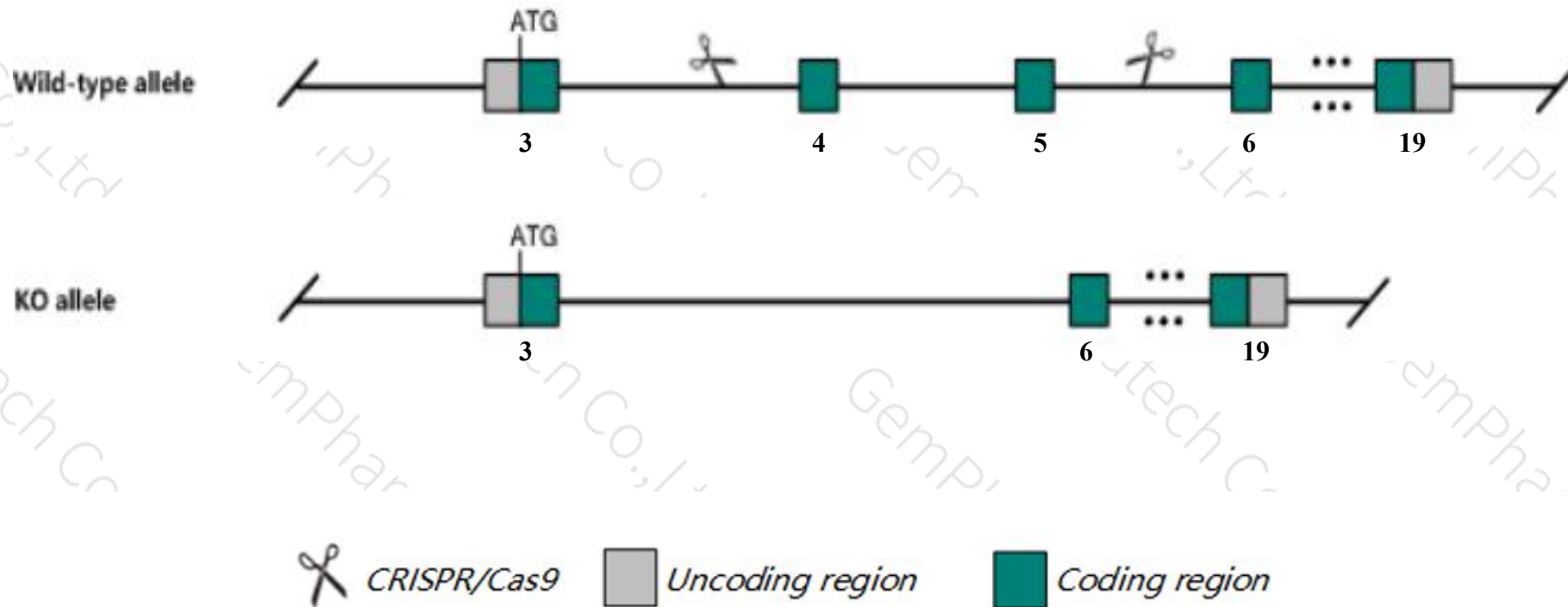
Cas9-KO

Strain background

C57BL/6JGpt

Knockout strategy

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



- The *Brdt* gene has 4 transcripts. According to the structure of *Brdt* gene, exon4-exon5 of *Brdt*-201(ENSMUST00000031215.14) transcript is recommended as the knockout region. The region contains 253bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Brdt* gene. The brief process is as follows: gRNA was transcribed in vitro. Cas9 and gRNA 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.

- According to the existing MGI data, homozygous inactivation of this genes leads to arrest of spermatogenesis and male infertility.
- The *Brdt* 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)

Brdtd bromodomain, testis-specific [*Mus musculus* (house mouse)]

Gene ID: 114642, updated on 26-Jun-2020

Summary

Official Symbol Brdtd provided by MGI

Official Full Name bromodomain, testis-specific provided by MGI

Primary source MGI:MGI:1891374

See related Ensembl:ENSMUSG00000029279

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 Brd6; Fsr3; 7420412D09Rik

Summary The protein encoded by this gene belongs to the BET protein family. BET proteins have two N-terminal bromodomains and one C-terminal extraterminal domain (ET domain). BET proteins regulate chromatin reorganization via binding to acetylated histones. This gene is thought to play a role in the transcriptional regulation of spermatogenesis. Although referred to as testis-specific bromodomain (Brdtd) protein, RT-PCR indicates that this gene is expressed in both mouse oocytes and testes. Alternative splicing results in multiple transcript variants encoding different proteins. [provided by RefSeq, Jul 2008]

Expression Biased expression in testis adult (RPKM 12.3), cortex adult (RPKM 1.6) and 6 other tissues [See more](#)

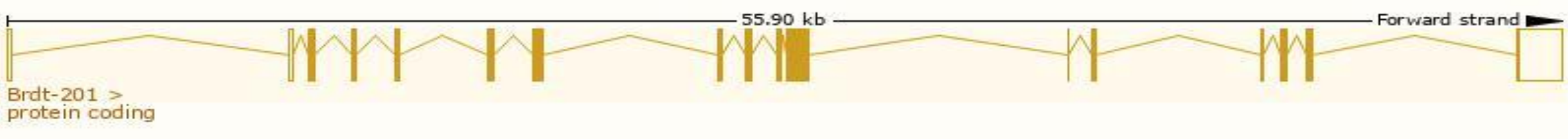
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

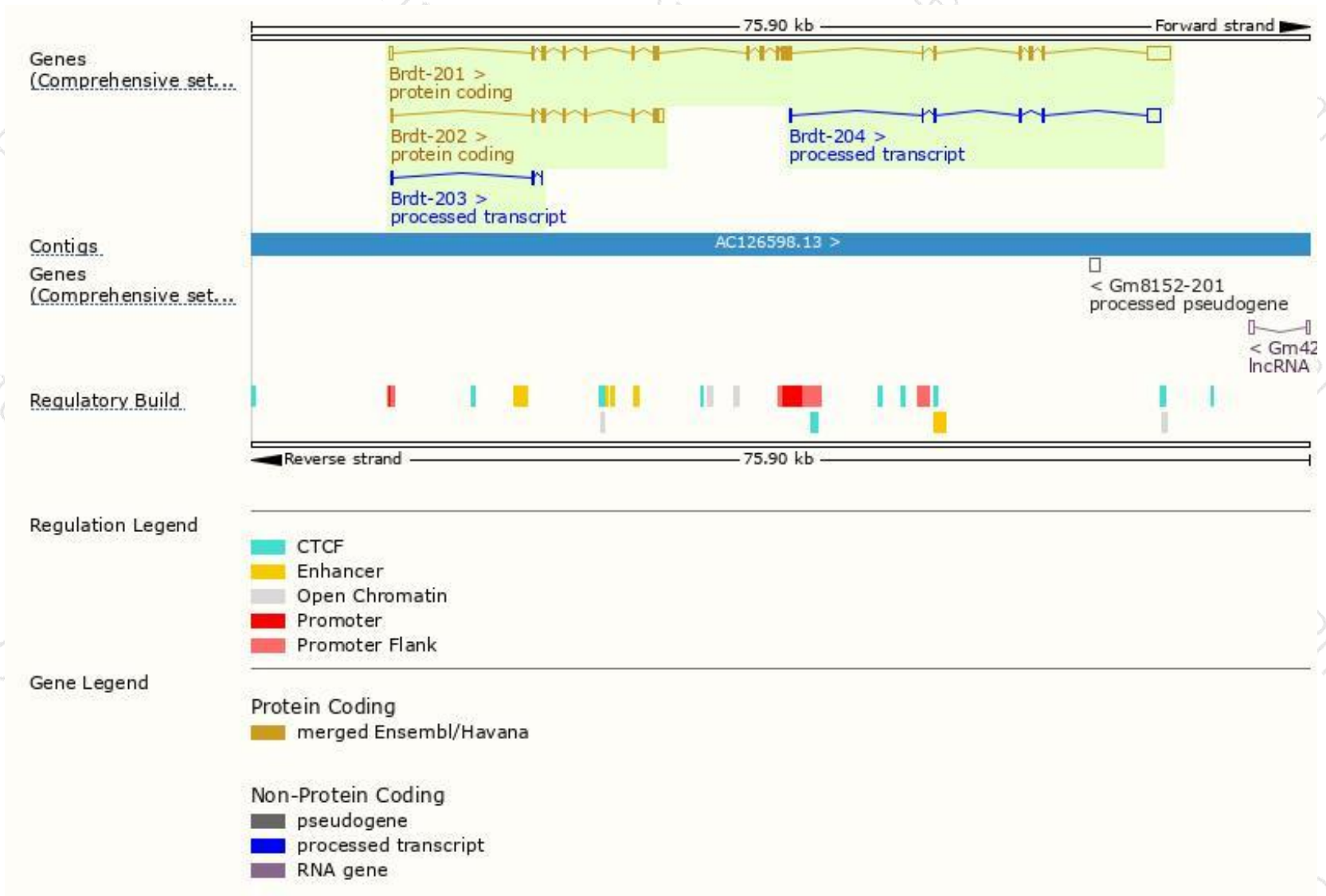
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Brdt-201	ENSMUST00000031215.14	4745	956aa	Protein coding	CCDS19500	Q91Y44	TSL:1 GENCODE basic APPRIS P1
Brdt-202	ENSMUST00000112677.9	1607	326aa	Protein coding	CCDS39197	Q91Y44	TSL:1 GENCODE basic
Brdt-204	ENSMUST00000162804.1	1568	No protein	Processed transcript	-	-	TSL:1
Brdt-203	ENSMUST00000161377.1	369	No protein	Processed transcript	-	-	TSL:3

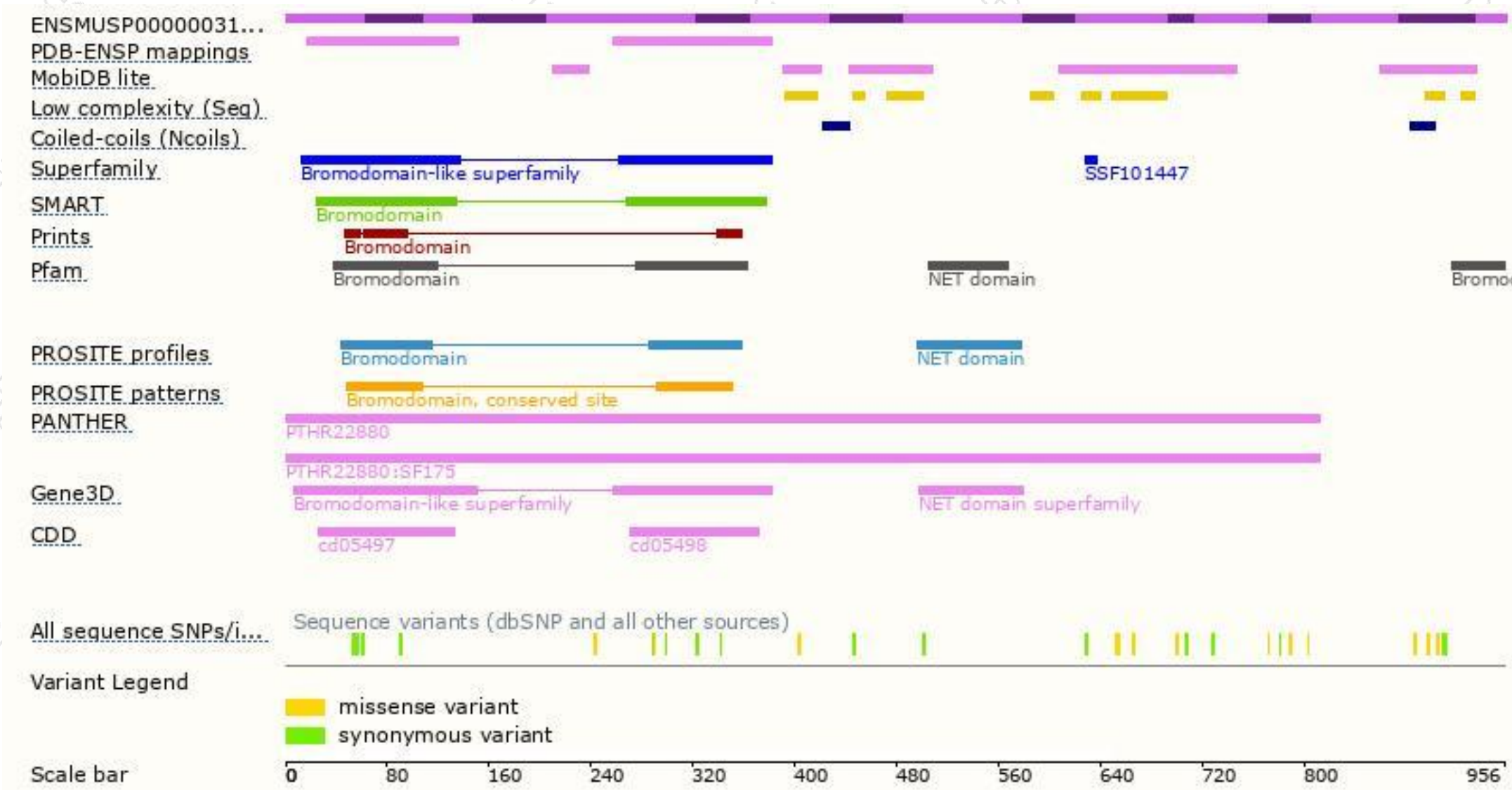
The strategy is based on the design of *Brdt-201* transcript,the transcription is shown below:



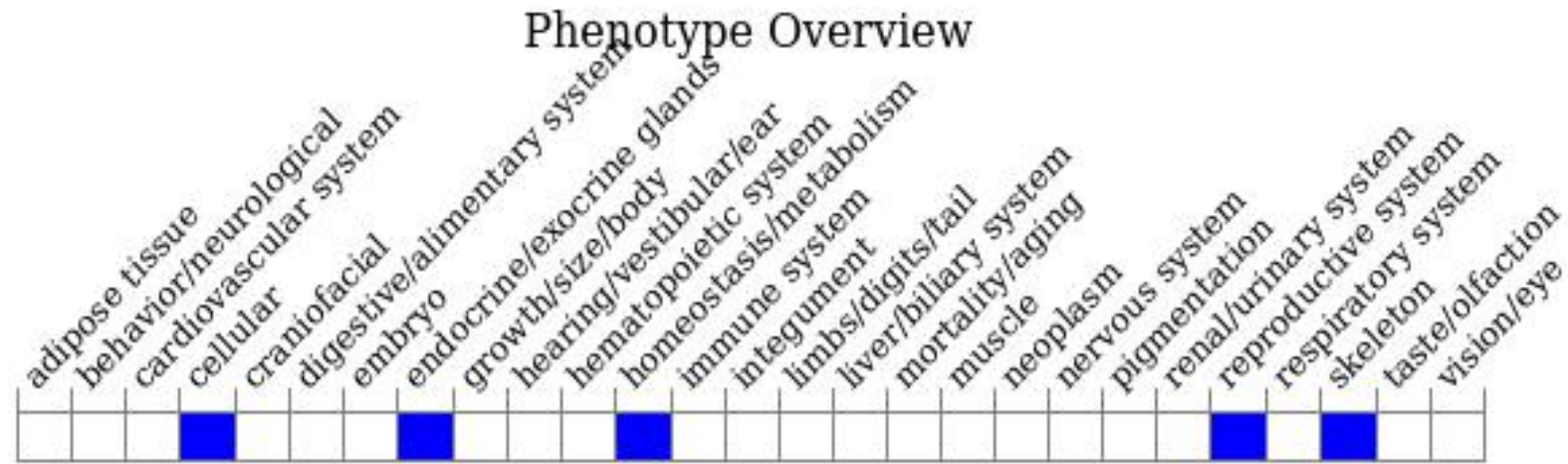
Genomic location distribution



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, homozygous inactivation of this genes leads to arrest of spermatogenesis and male infertility.

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

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