Brwd3 Cas9-CKO Strategy

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Project Overview

Project Name	***Brwd3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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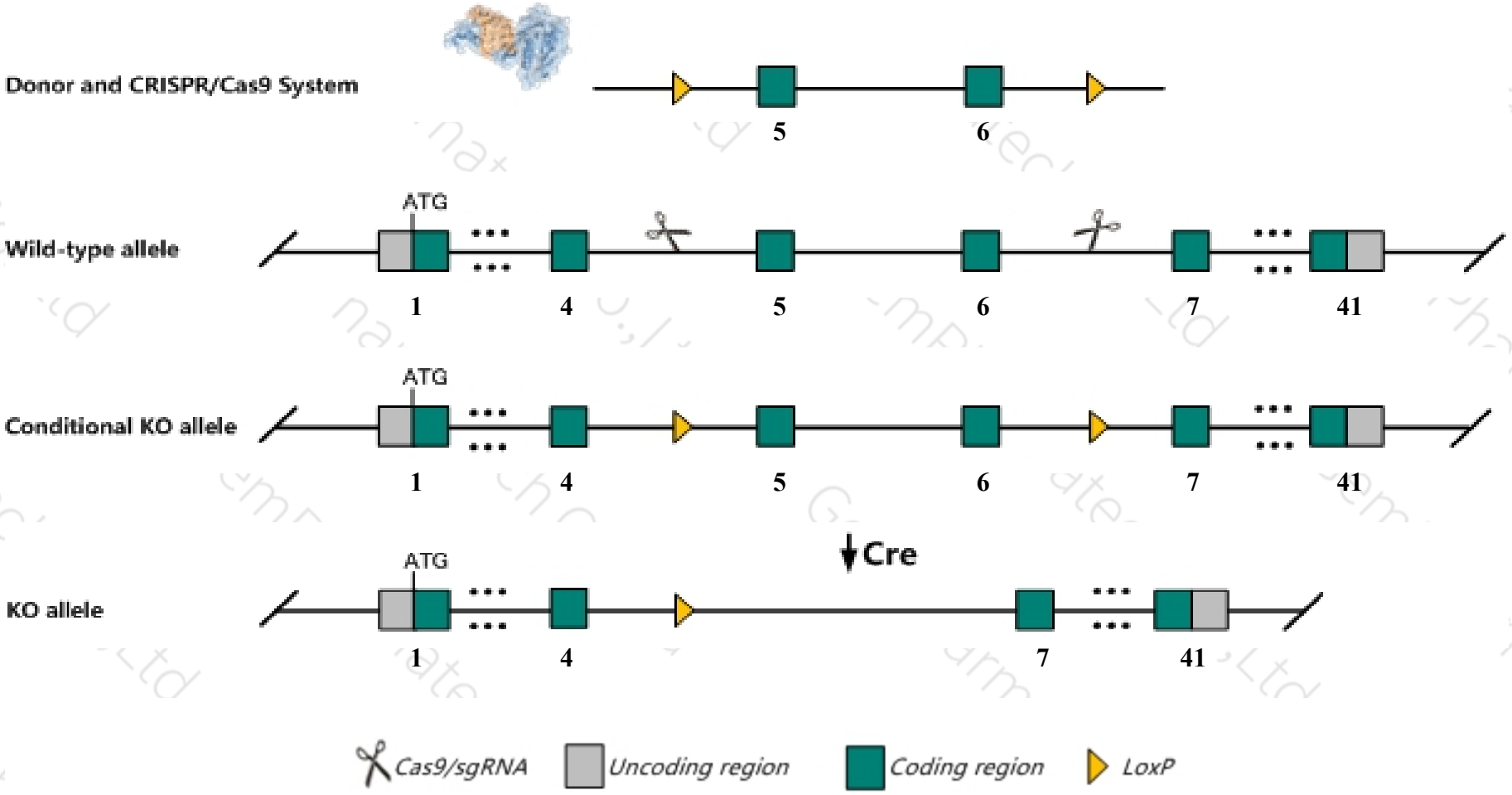
This model will use CRISPR/Cas9 technology to edit the *Brwd3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Brwd3* gene has 5 transcripts. According to the structure of *Brwd3* gene, exon5-exon6 of *Brwd3-205*(ENSMUST00000150434.7) transcript is recommended as the knockout region.The region contains 250bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Brwd3* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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,male chimeras hemizygous for a gene trapped allele exhibit short tail buds, microcephaly and, in some cases, embryonic growth retardation.

➢ The *Brwd3* gene is located on the ChrX. 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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Brwd3 bromodomain and WD repeat domain containing 3 [Mus musculus (house mouse)]

Gene ID: 382236, updated on 13-Mar-2020

Summary

Official Symbol	Brwd3 provided by MGI
Official Full Name	bromodomain and WD repeat domain containing 3 provided byMGI
Primary source	MGI:MGI:3029414
See related	Ensembl:ENSMUSG00000063663
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	Brodl, D030064D06Rik, Gm10452, Gm596
Expression	Broad expression in placenta adult (RPKM 3.9), CNS E11.5 (RPKM 1.8) and 20 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

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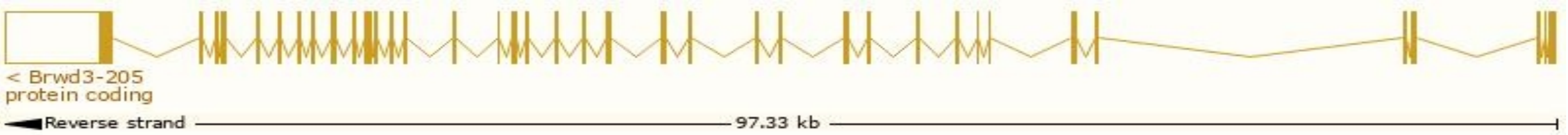
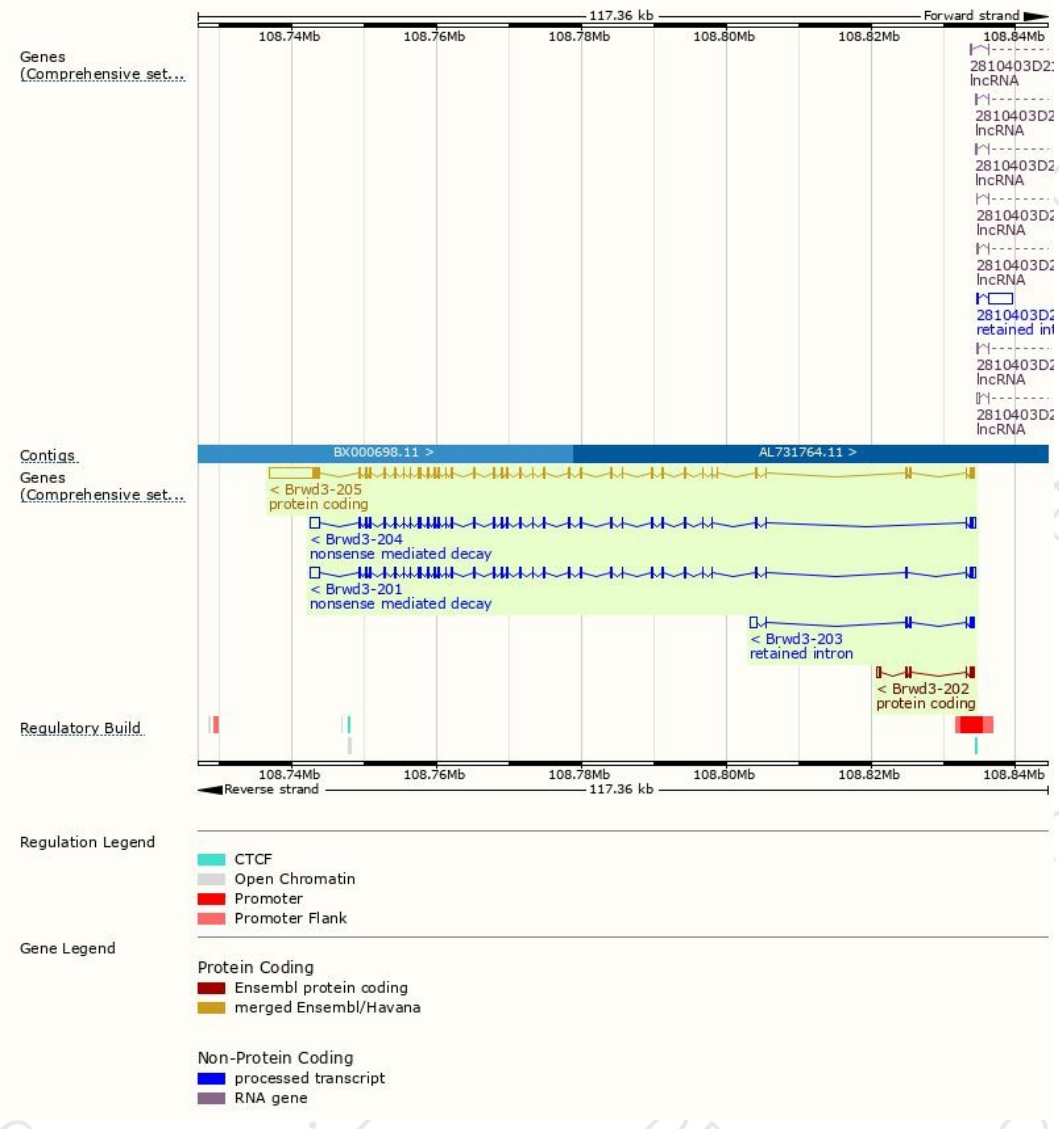
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Brwd3-205	ENSMUST00000150434.7	11619	1799aa	Protein coding	CCDS41100	A2AHJ4	TSL:5 GENCODE basic APPRIS P1
Brwd3-202	ENSMUST00000101283.3	1066	181aa	Protein coding	-	A2AHJ4	TSL:1 GENCODE basic
Brwd3-201	ENSMUST00000041866.11	5839	84aa	Nonsense mediated decay	-	G3UZT0	TSL:5
Brwd3-204	ENSMUST00000144521.7	5740	60aa	Nonsense mediated decay	-	G3UXF9	TSL:5
Brwd3-203	ENSMUST00000134434.1	1654	No protein	Retained intron	-	-	TSL:1

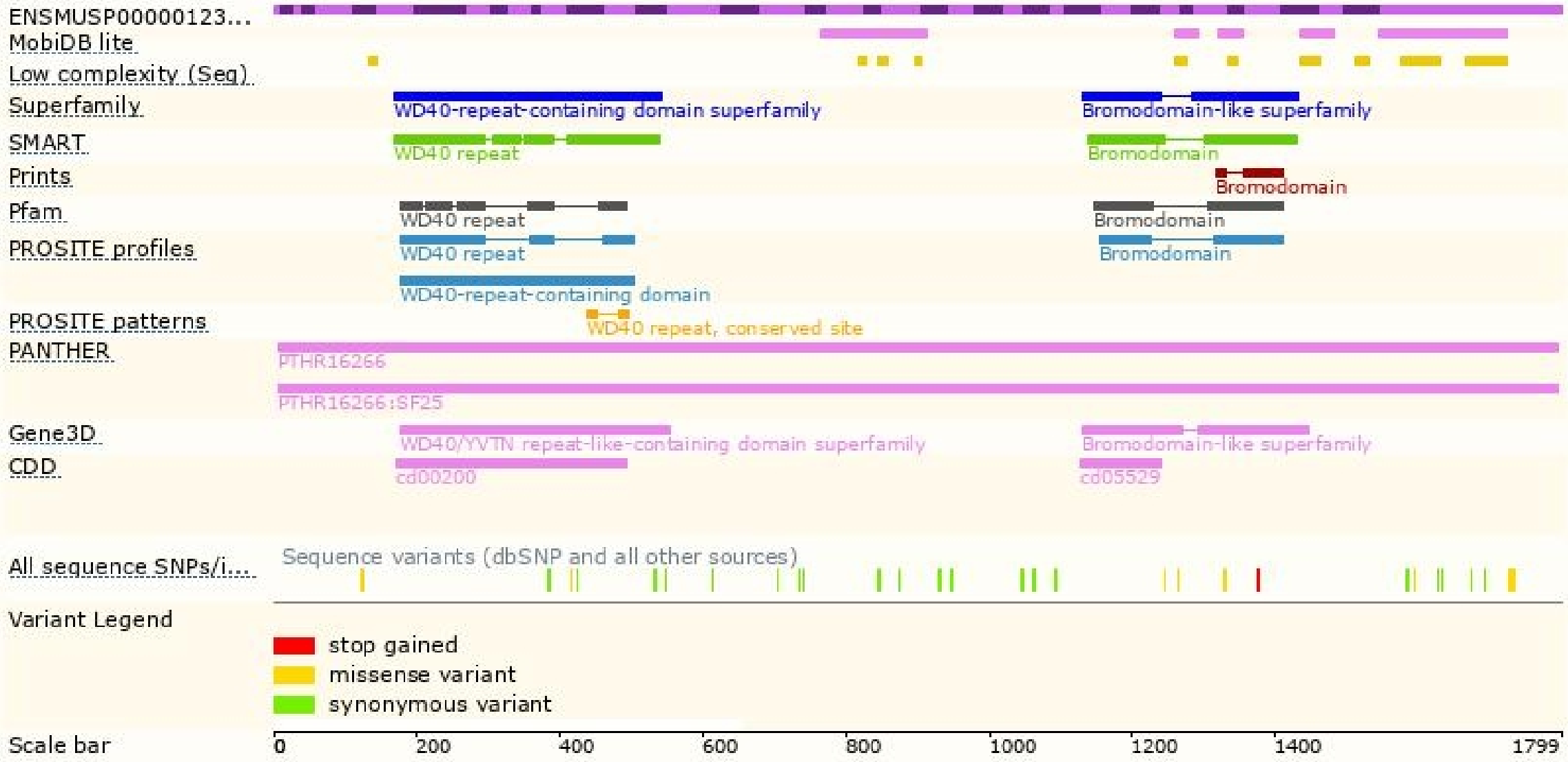
The strategy is based on the design of *Brwd3-205* transcript,the transcription is shown below:



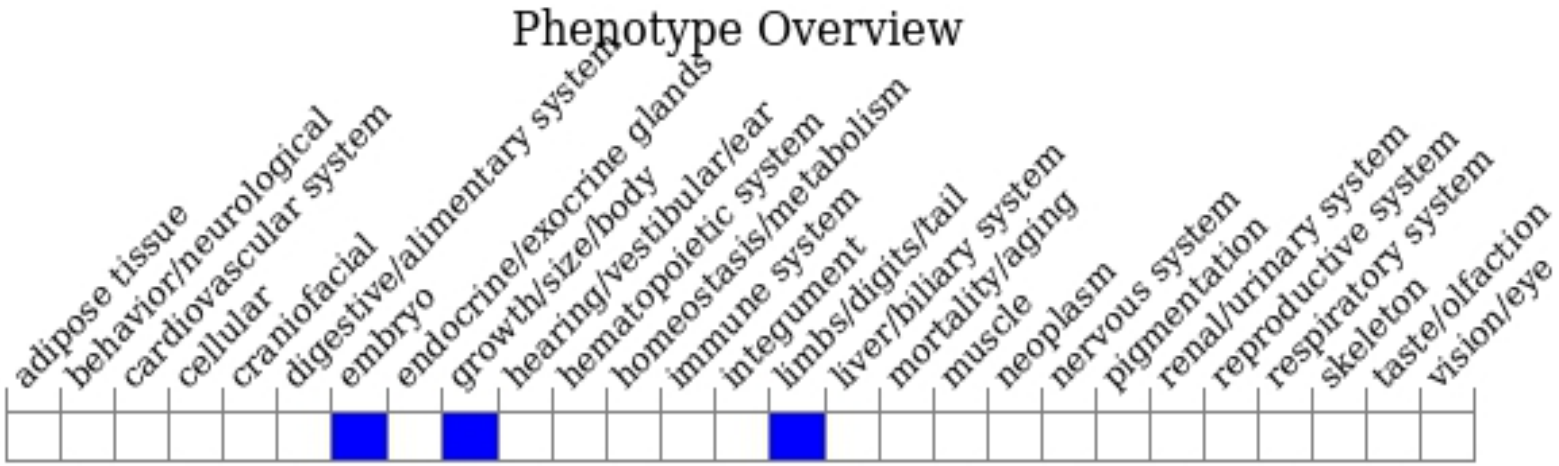
Genomic location distribution



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,male chimeras hemizygous for a gene trapped allele exhibit short tail buds, microcephaly and, in some cases, embryonic growth retardation.

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

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