

***Barx1* Cas9-CKO Strategy**

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Reviewer: Huimin Su

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Project Overview

Project Name

Barx1

Project type

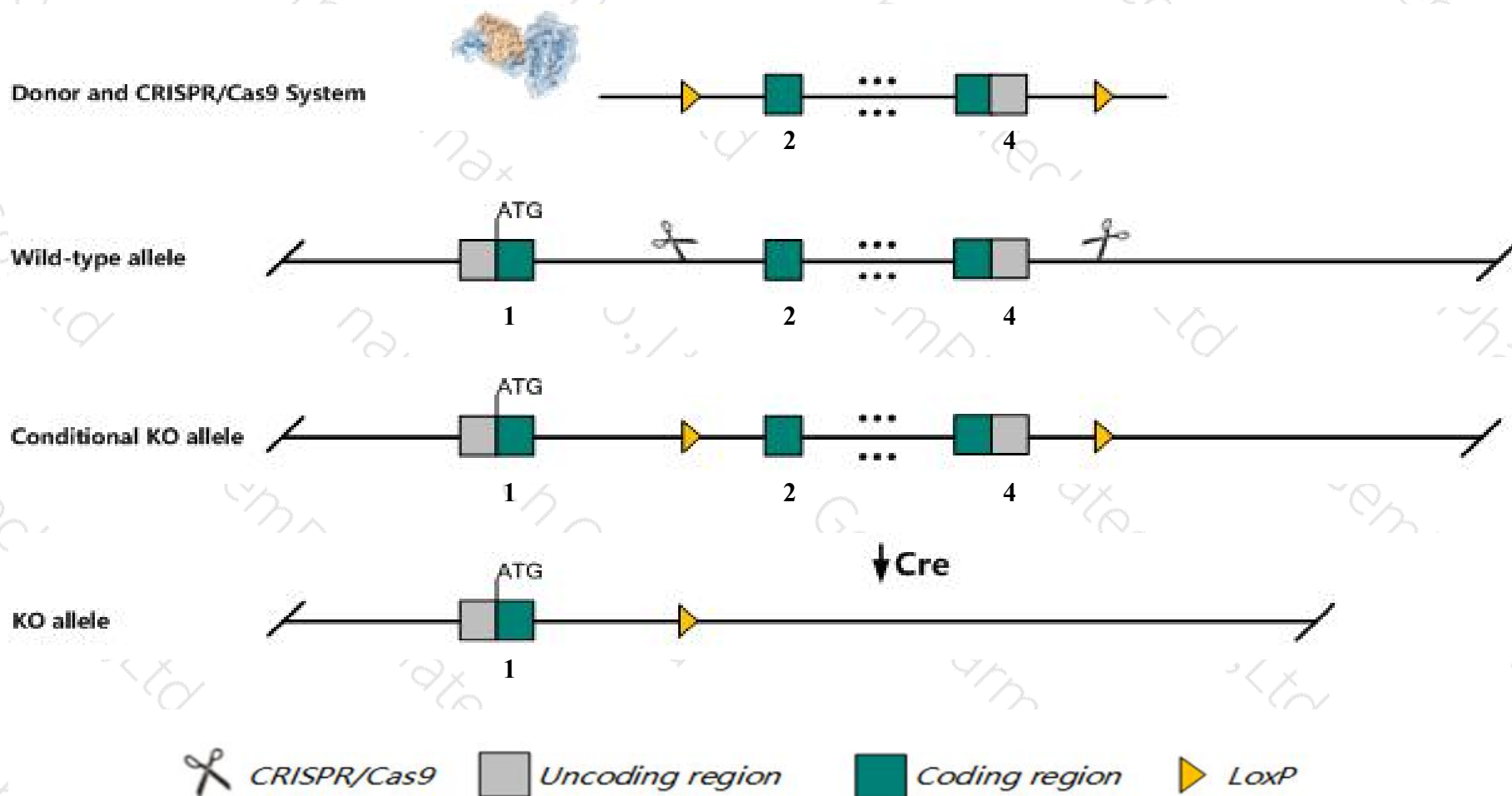
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Barx1* gene. The schematic diagram is as follows:



- The *Barx1* gene has 1 transcript. According to the structure of *Barx1* gene, exon2-exon4 of *Barx1-201* (ENSMUST00000021813.4) transcript is recommended as the knockout region. The region contains the stop codon. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Barx1* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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.

- According to the existing MGI data, mice homozygous for a null mutation die around e13 embryonic stage with shrunk and malformed stomach or shortly after birth with cleft palate and abnormal tooth development depending on strain background.
- The *Barx1* gene is located on the Chr13. 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)

Barx1 BarH-like homeobox 1 [Mus musculus (house mouse)]

Gene ID: 12022, updated on 13-Mar-2020

Summary



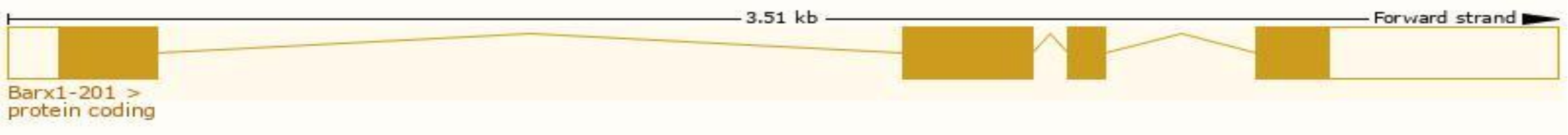
Official Symbol	Barx1 provided by MGI
Official Full Name	BarH-like homeobox 1 provided by MGI
Primary source	MGI:MGI:103124
See related	Ensembl:ENSMUSG000000021381
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Biased expression in stomach adult (RPKM 96.9) and CNS E11.5 (RPKM 5.6) See more
Orthologs	human all

Transcript information (Ensembl)

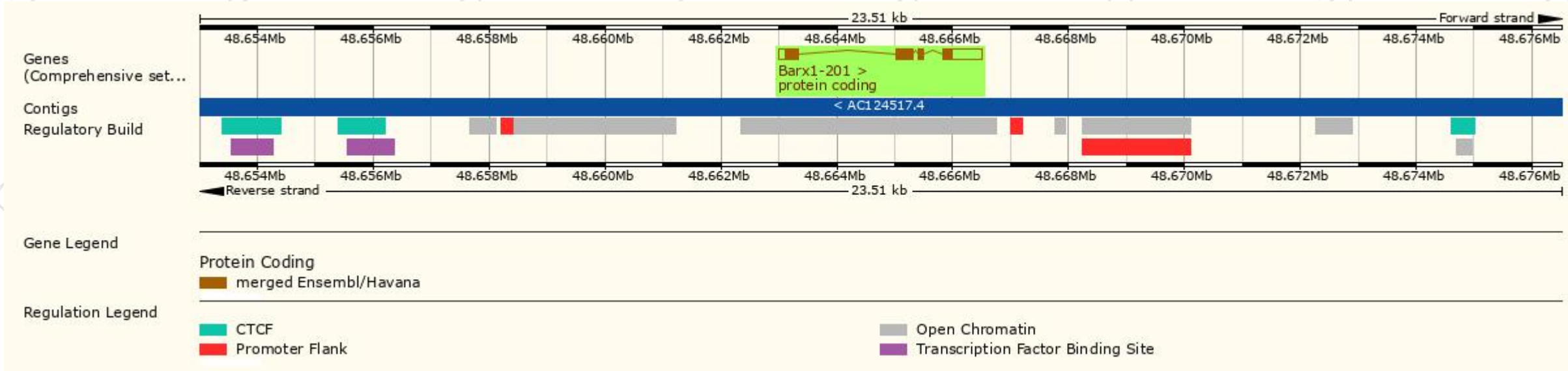
The gene has 1 transcript, and the transcript is shown below:

Show/hide columns (1 hidden)							Filter	
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Barx1-201	ENSMUST00000021813.4	1399	254aa	Protein coding	CCDS49258	Q9ER42	TSL:1	GENCODE basic APPRIS P1

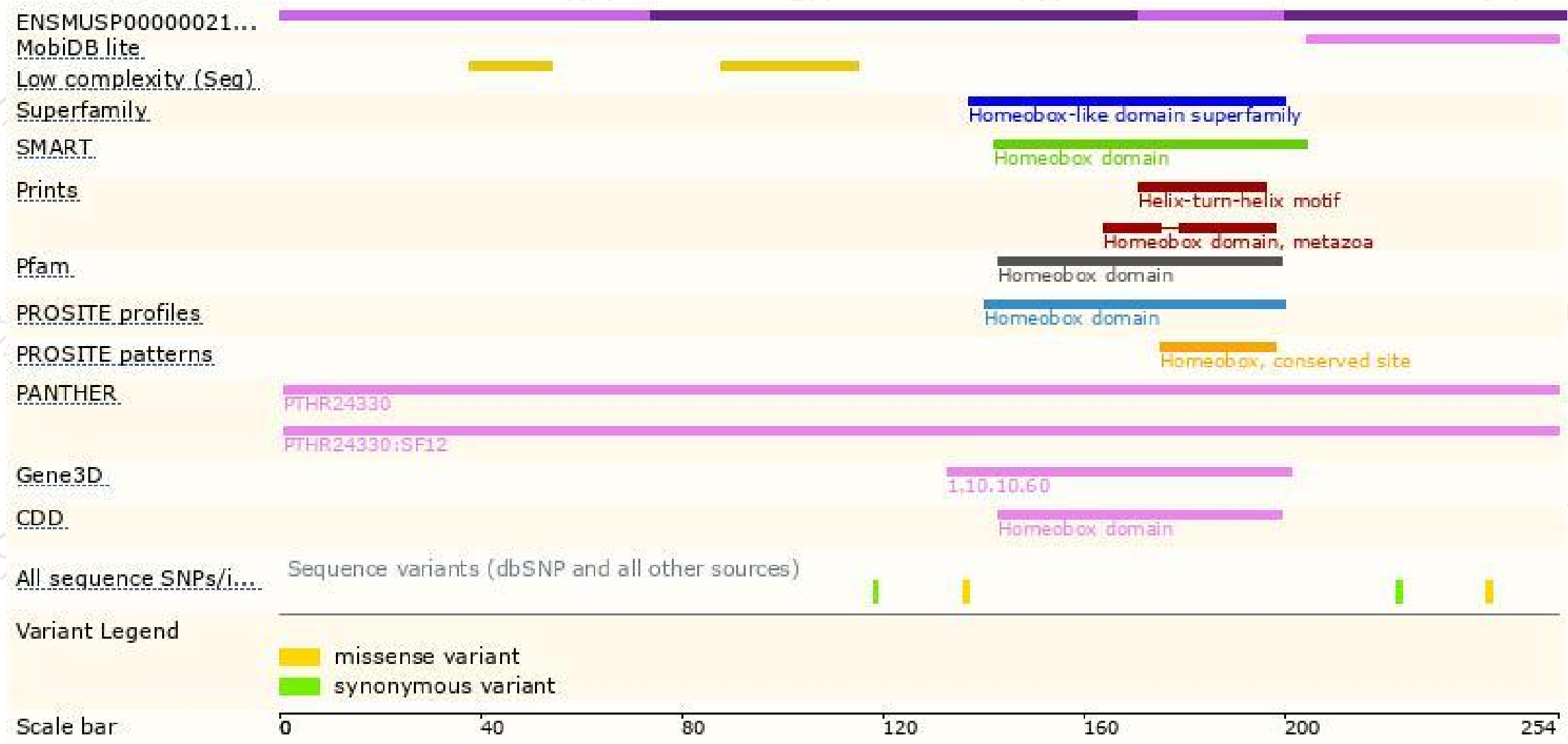
The strategy is based on the design of *Barx1-201* transcript, the transcription is shown below:



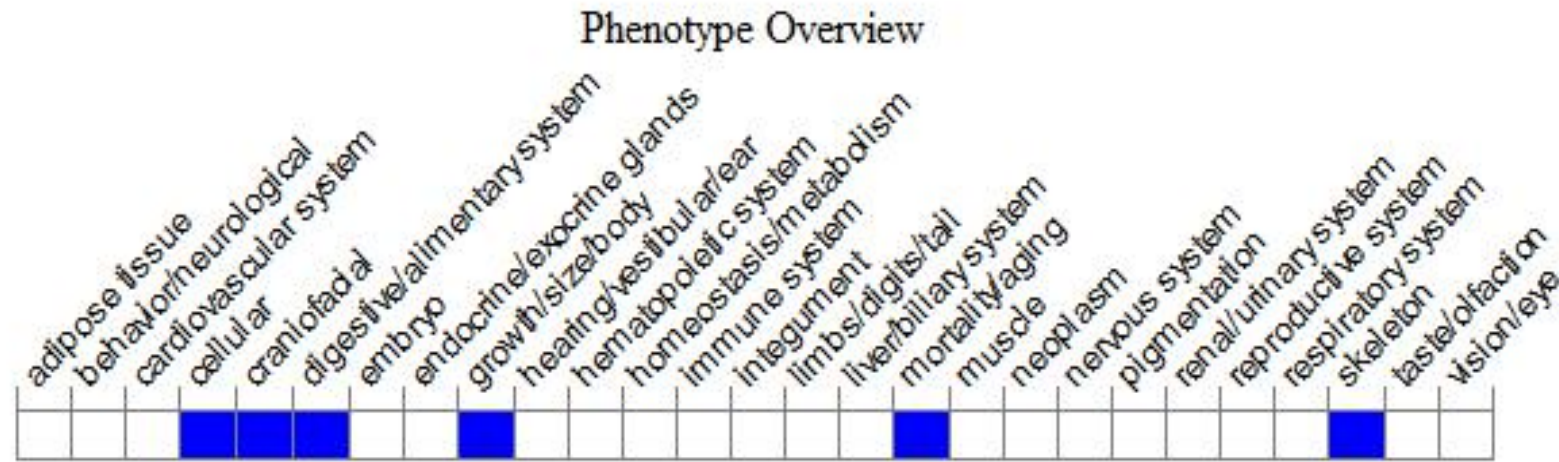
Genomic location distribution



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, mice homozygous for a null mutation die around E13 embryonic stage with shrunk and malformed stomach or shortly after birth with cleft palate and abnormal tooth development depending on strain background.

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

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