Pbx1 Cas9-KO *Strategy*

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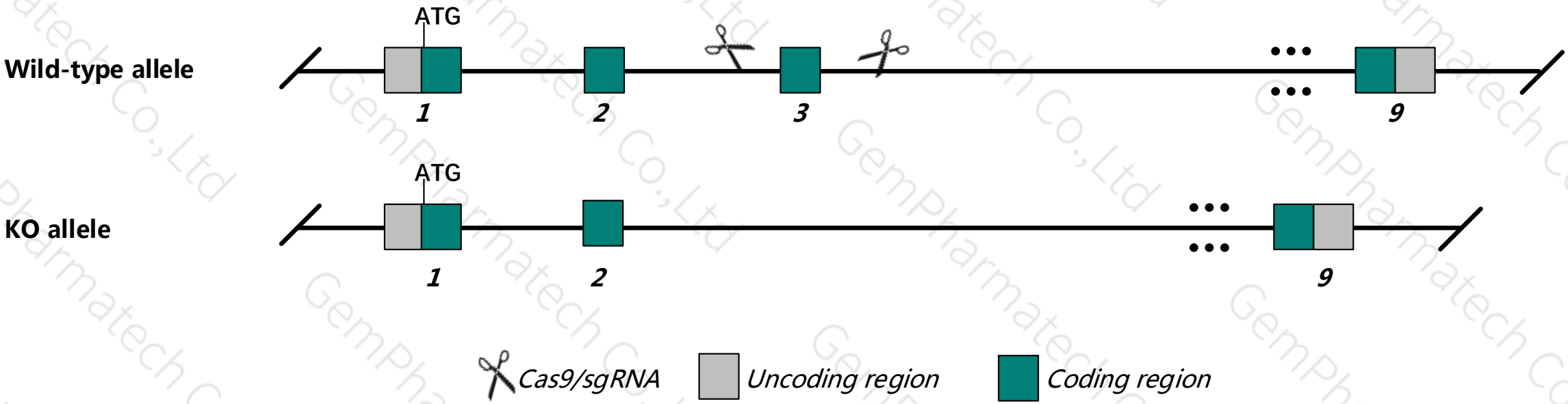
Project Overview

Project Name	***Pbx1***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pbx1* gene. The schematic diagram is as follows:



Technical routes

- The *Pbx1* gene has 8 transcripts. According to the structure of *Pbx1* gene, exon3 of *Pbx1-205*(ENSMUST00000176540.7) transcript is recommended as the knockout region.The region contains 245bp coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Pbx1* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

Notice

- According to the existing MGI data，homozygous disruption of this gene causes late gestational death, hypoplasia/aplasia of many organs, impaired hematopoiesis, anemia, skin edema, axial and appendicular skeleton defects, absent adrenal glands, abnormal chondrocyte differentiation, and abnormal bone, kidney and pancreas development.
- The *Pbx1* gene is located on the Chr1. 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）

Pbx1 pre B cell leukemia homeobox 1 [*Mus musculus* (house mouse)]

Gene ID: 18514, updated on 20-Jul-2019

Summary

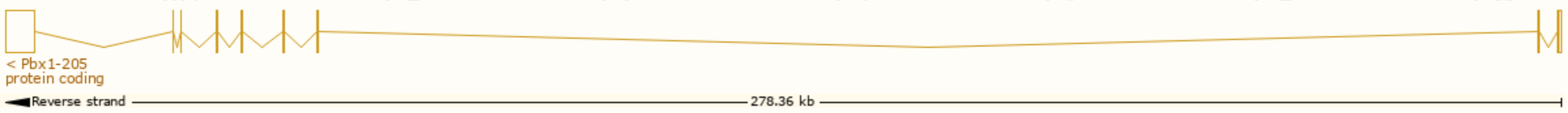
Official Symbol	Pbx1 provided by MGI
Official Full Name	pre B cell leukemia homeobox 1 provided by MGI
Primary source	MGI:MGI:97495
See related	Ensembl:ENSMUSG00000052534
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Pbx-1; 2310056B04Rik; D230003C07Rik
Summary	This gene encodes a homeobox protein that belongs to the three-amino-acid loop extension/Pre-B cell leukemia transcription factor (TALE/PBX) family of proteins. The encoded protein is involved in several biological processes during embryogenesis including steroidogenesis, sexual development and the maintenance of hematopoietic stem cells. This protein functions in the development of several organ systems and plays a role in skeletal patterning and programming. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Apr 2014]
Expression	Broad expression in whole brain E14.5 (RPKM 20.5), frontal lobe adult (RPKM 20.5) and 24 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

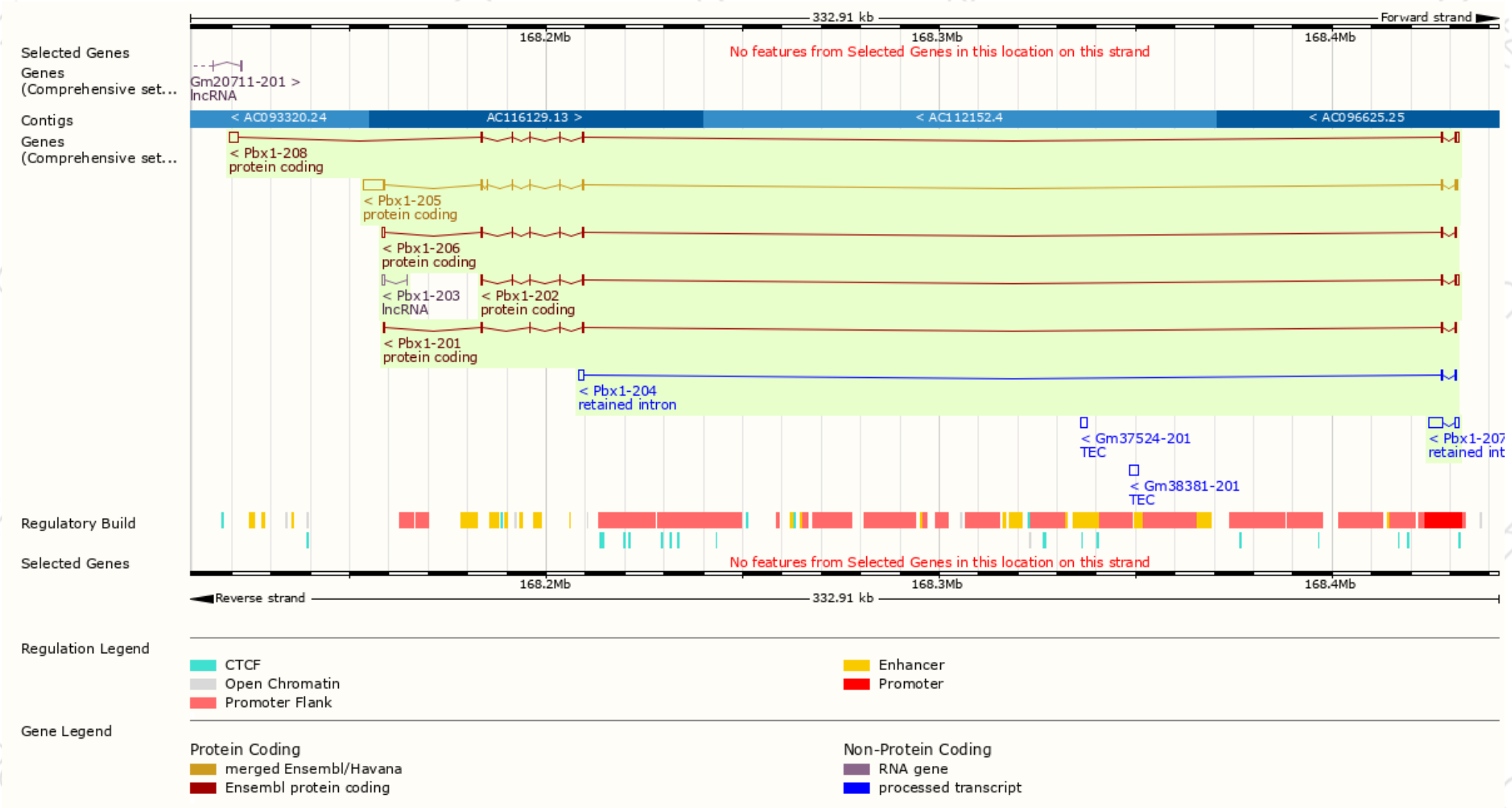
The gene has 8 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pbx1-208	ENSMUST00000188912.6	3970	347aa	Protein coding	CCDS15462	P41778 Q71VB4	TSL:1 GENCODE basic APPRIS ALT1
Pbx1-206	ENSMUST00000176790.7	1828	347aa	Protein coding	CCDS15462	P41778 Q71VB4	TSL:1 GENCODE basic APPRIS ALT1
Pbx1-202	ENSMUST00000072863.5	1709	347aa	Protein coding	CCDS15462	P41778 Q71VB4	TSL:1 GENCODE basic APPRIS ALT1
Pbx1-205	ENSMUST00000176540.7	6876	430aa	Protein coding	CCDS15461	P41778	TSL:1 GENCODE basic APPRIS P4
Pbx1-201	ENSMUST00000064438.10	1020	339aa	Protein coding	CCDS78739	D9J2V6	TSL:1 GENCODE basic
Pbx1-207	ENSMUST00000176791.1	4357	No protein	Retained intron	-	-	TSL:2
Pbx1-204	ENSMUST00000176482.1	1674	No protein	Retained intron	-	-	TSL:2
Pbx1-203	ENSMUST00000097471.3	498	No protein	lncRNA	-	-	TSL:1

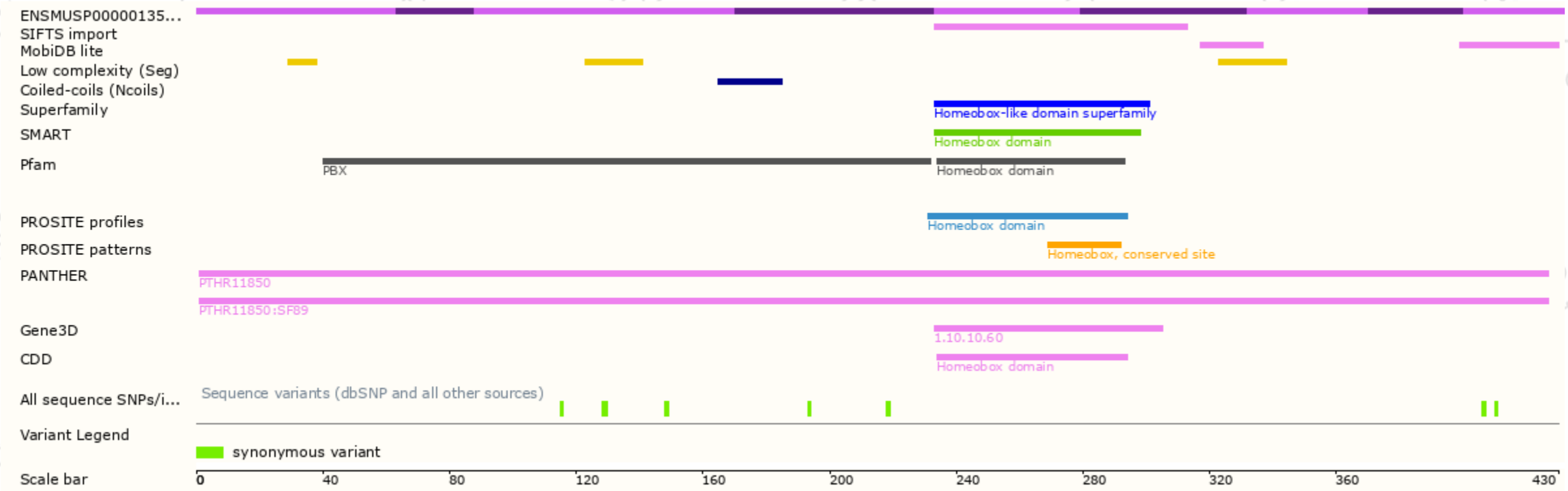
The strategy is based on the design of *Pbx1*-205 transcript,The transcription is shown below:



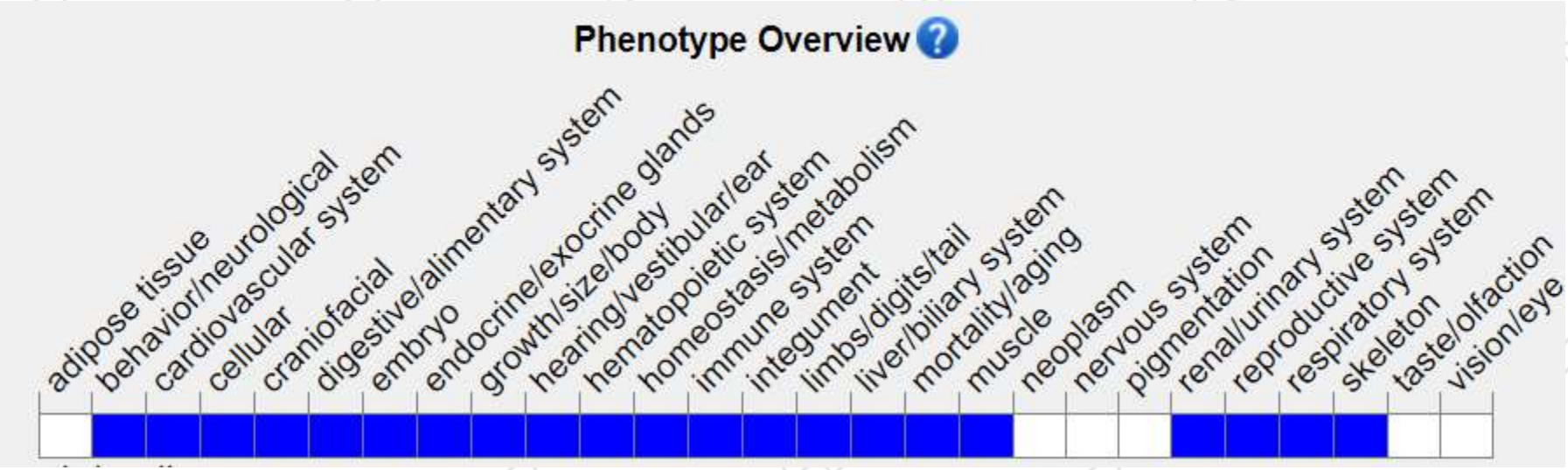
Genomic location (Ensembl)



Protein domain（Ensembl）



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/) .

Homozygous disruption of this gene causes late gestational death, hypoplasia/aplasia of many organs, impaired hematopoiesis, anemia, skin edema, axial and appendicular skeleton defects, absent adrenal glands, abnormal chondrocyte differentiation, and abnormal bone, kidney and pancreas development.

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

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