Edn2 Cas9-CKO Strategy

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Project Overview

Project Name	***Edn2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6J**

Conditional Knockout strategy

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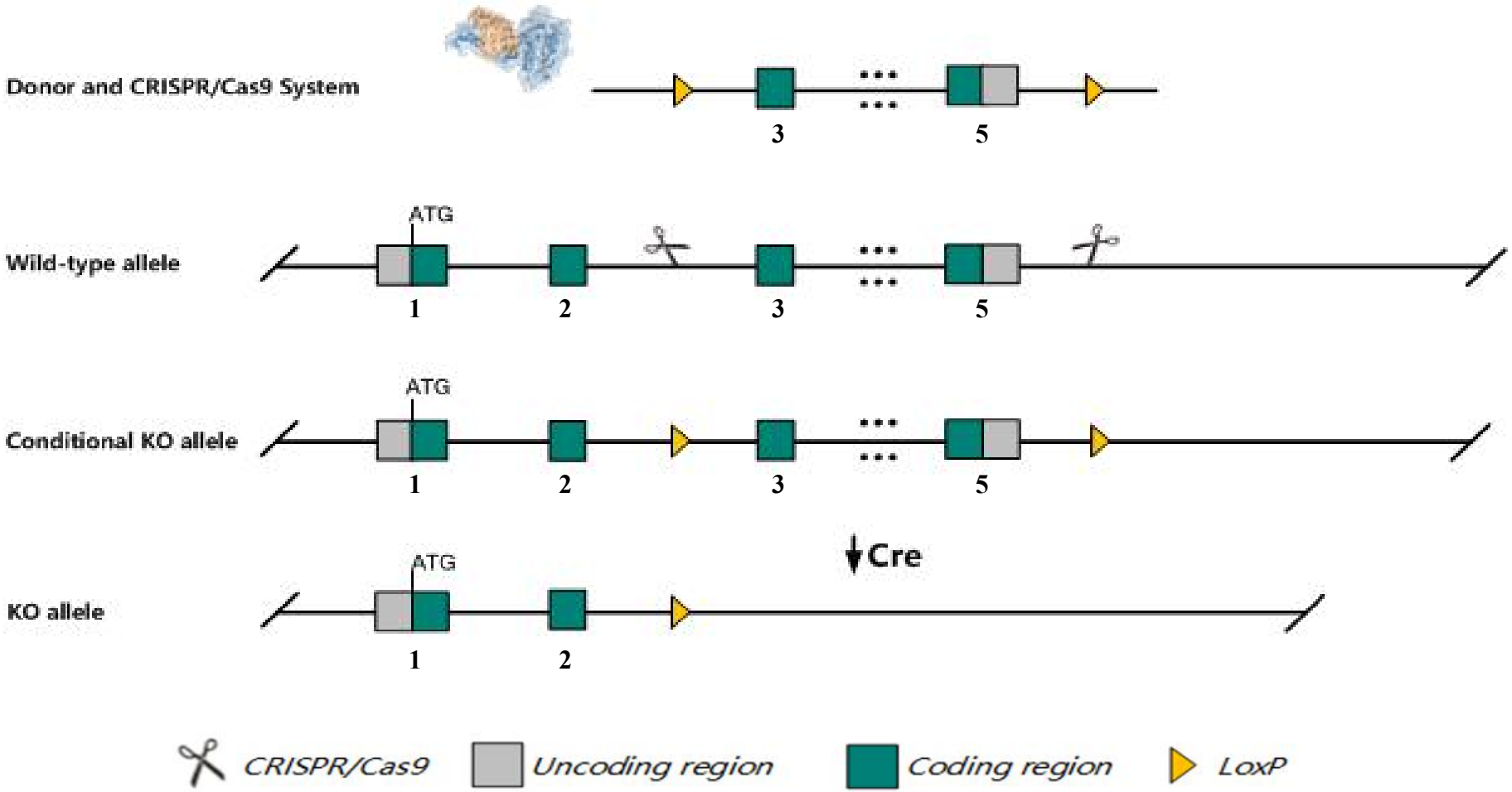
This model will use CRISPR/Cas9 technology to edit the *Edn2* gene. The schematic diagram is as follows:



Technical routes

The *Edn2* gene has 1 transcript. According to the structure of *Edn2* gene, exon3-exon5 of *Edn2-201* (ENSMUST00000030384.4) transcript is recommended as the knockout region.The region contains 316bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Edn2* gene. The brief process is as follows:CRISPR/Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6J 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/6J 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,Mice homozygous for a knock-out allele exhibit growth retardation, hypothermia, hypoxemic hypoxia, hypercapnia, emphysema and premature death.

The *Edn2* gene is located on the Chr4. 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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Edn2 endothelin 2 [Mus musculus (house mouse)]

Gene ID: 13615, updated on 31-Jan-2019

Summary

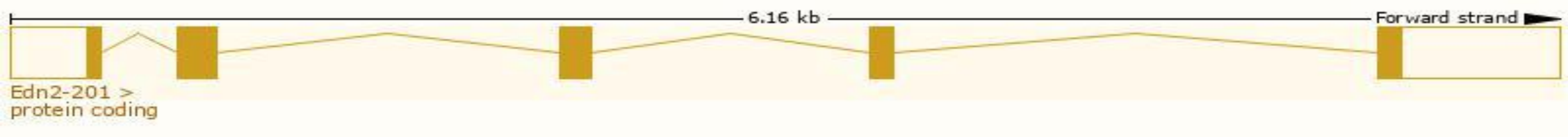
Official Symbol	Edn2 provided by MGI
Official Full Name	endothelin 2 provided by MGI
Primary source	MGI:MGI:95284
See related	Ensembl:ENSMUSG00000028635
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	ET-2, PPET2, VIC
Summary	This gene encodes a member of the endothelin family of peptides. The encoded preproprotein undergoes proteolytic processing to generate a potent vasoconstrictive peptide. This gene is abundantly expressed in the gastrointestinal tract, strongly induced in photorecepteror cells in retinal diseases and injury, and produced by microglia and macrophages in the early stages of glaucoma. Mice lacking the encoded protein exhibit severe growth retardation, hypothermia and juvenile lethality. [provided by RefSeq, Feb 2016]
Expression	Biased expression in large intestine adult (RPKM 14.8), small intestine adult (RPKM 13.6) and 5 other tissuesSee more
Orthologs	human all

Transcript information Ensembl

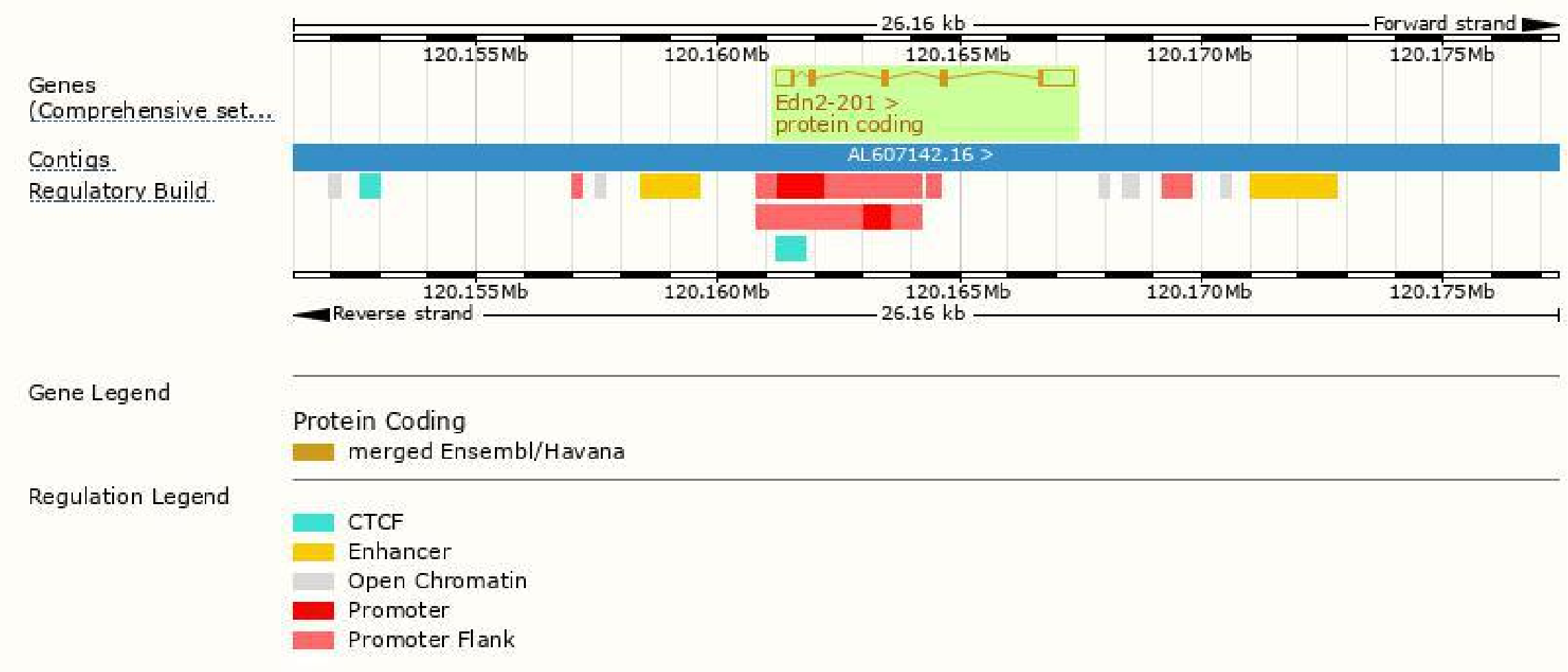
The gene has 1 transcript,and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Edn2-201	ENSMUST00000030384.4	1462	175aa	Protein coding	CCDS38864	P22389	TSL:1 GENCODE basic APPRIS P1

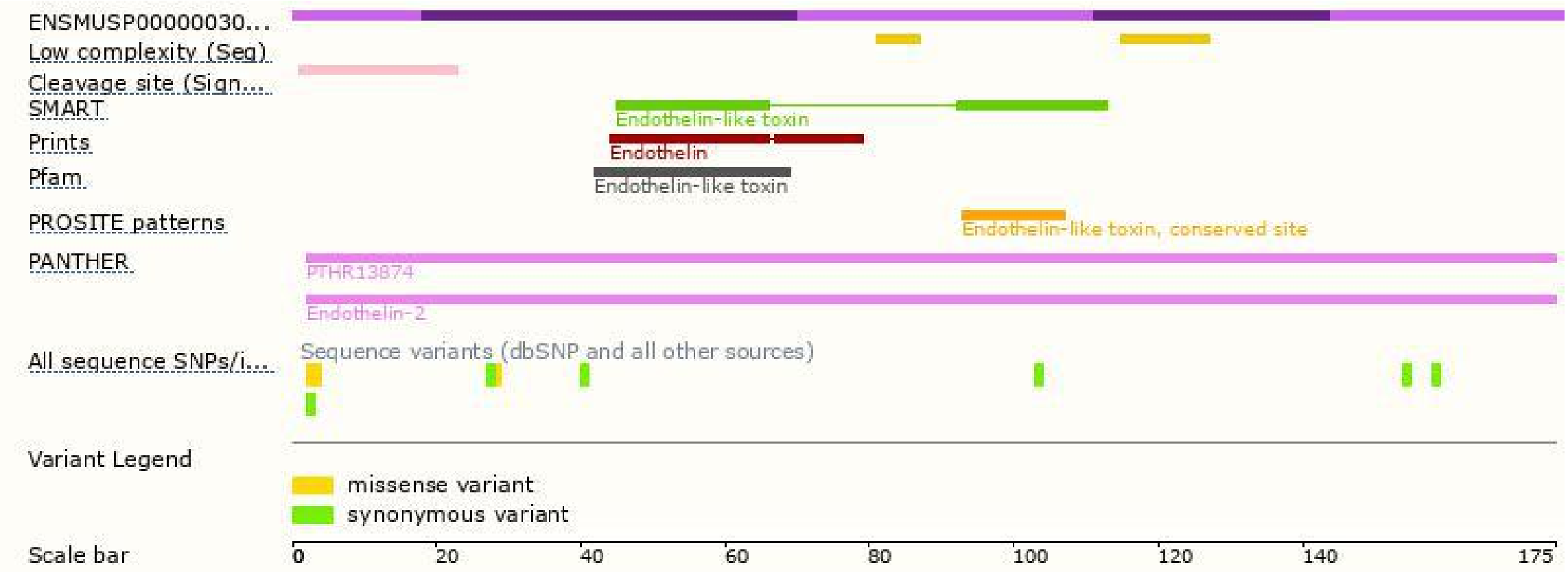
The strategy is based on the design of *Edn2-201* transcript,The transcription is shown below



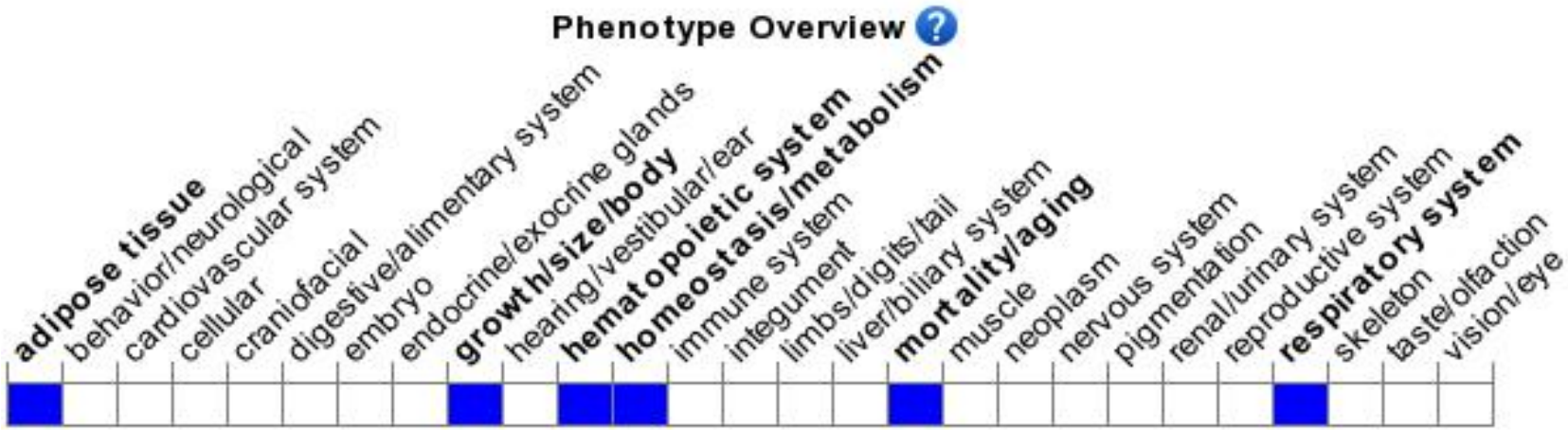
Genomic location distribution



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 knock-out allele exhibit growth retardation, hypothermia, hypoxemic hypoxia, hypercapnia, emphysema and premature death.

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