

Ehd1 Cas9-CKO Strategy

Designer: Miaomiao Cui

Reviewer: Lingyan Wu

Design Date: 2021-4-19

Project Overview

Project Name

Ehd1

Project type

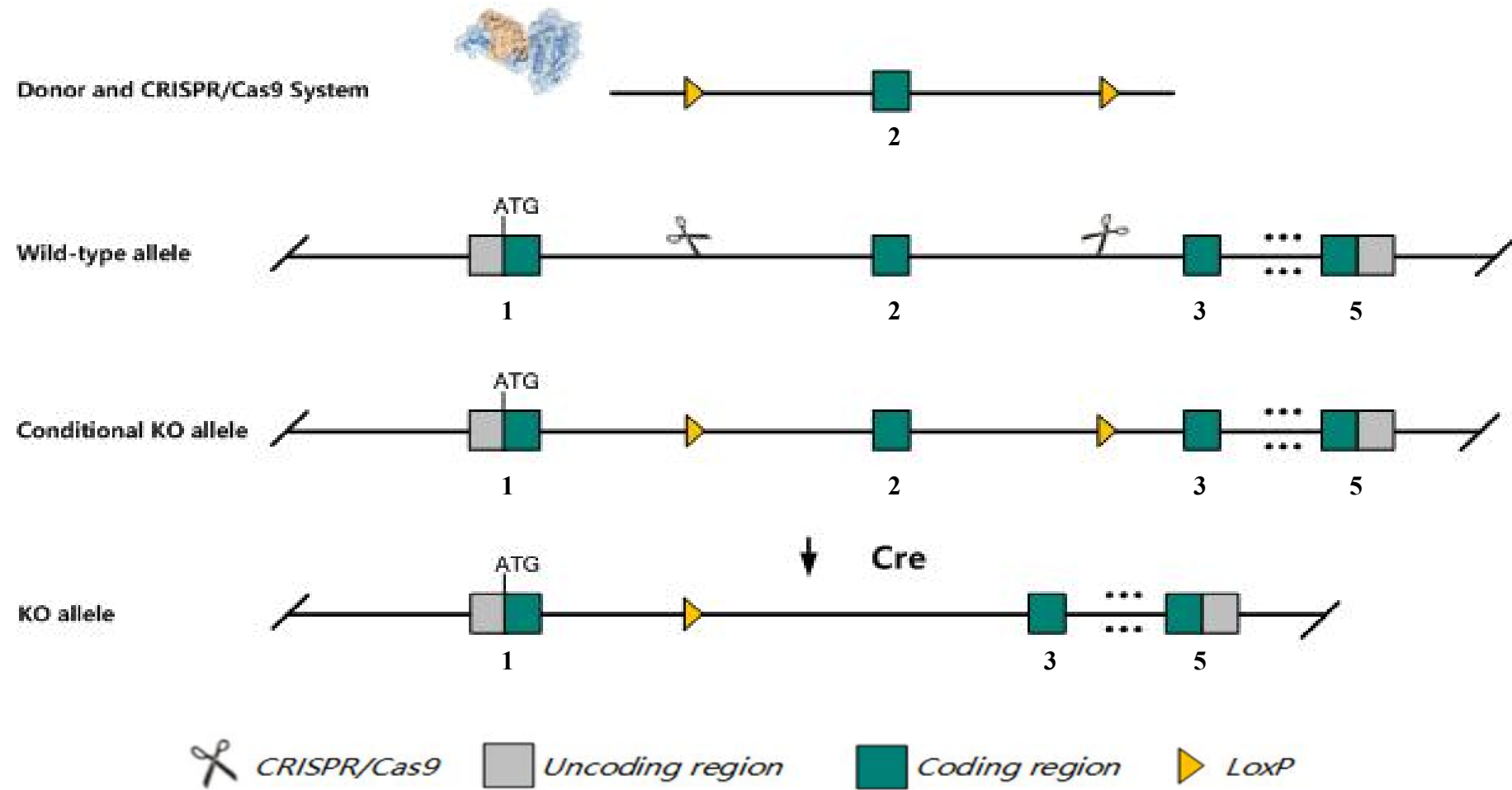
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



The *Ehd1* gene has 4 transcripts. According to the structure of *Ehd1* gene, exon2 of *Ehd1-201*(ENSMUST00000025684.4) transcript is recommended as the knockout region. The region contains 98bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Ehd1* gene. The brief process is as follows: CRISPR/Cas9 system 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 knock-out allele show perinatal and postnatal lethality, decreased body weight, and male infertility due to defective spermatogenesis; female homozygotes may display malocclusion and variable ocular defects, including congenital central cataracts.

The *Ehd1* gene is located on the Chr19. 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.

Ehd1 EH-domain containing 1 [Mus musculus (house mouse)]

Gene ID: 13660, updated on 12-Feb-2021

Summary



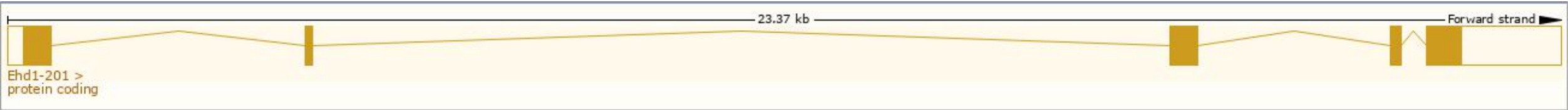
Official Symbol	Ehd1 provided by MGI
Official Full Name	EH-domain containing 1 provided by MGI
Primary source	MGI:MGI:1341878
See related	Ensembl:ENSMUSG00000024772
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AA409636, Pa, Past1, RME-, RME-1
Expression	Ubiquitous expression in testis adult (RPKM 79.5), duodenum adult (RPKM 51.9) and 26 other tissues See more
Orthologs	human all

Transcript information Ensembl

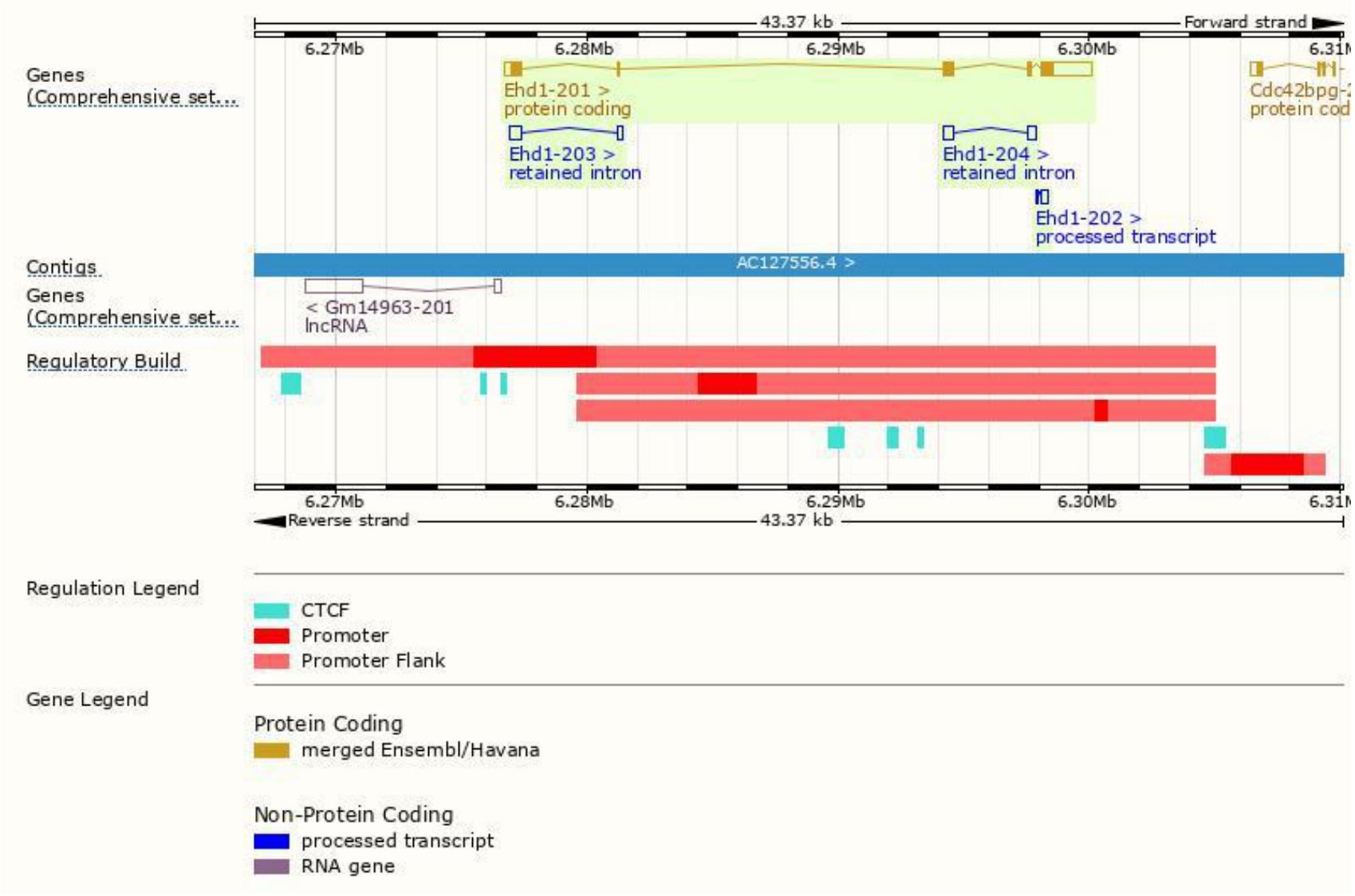
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ehd1-201	ENSMUST00000025684.4	3353	534aa	Protein coding	CCDS29501		TSL:1 , GENCODE basic , APPRIS P1 ,
Ehd1-202	ENSMUST00000148547.2	349	No protein	Processed transcript	-		TSL:2 ,
Ehd1-204	ENSMUST00000171203.2	791	No protein	Retained intron	-		TSL:3 ,
Ehd1-203	ENSMUST00000165797.2	629	No protein	Retained intron	-		TSL:1 ,

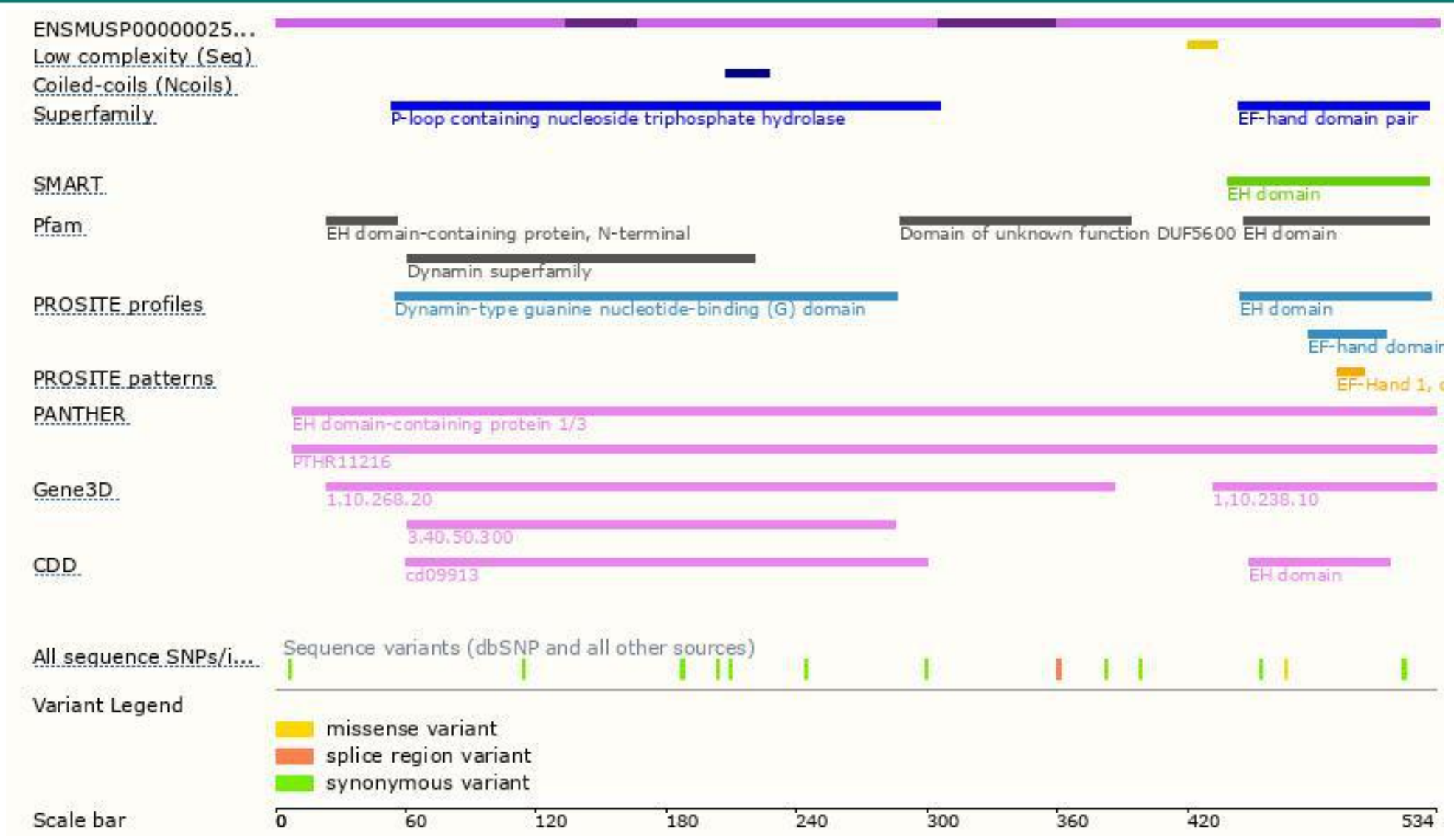
The strategy is based on the design of *Ehd1-201* transcript,the transcription is shown below:



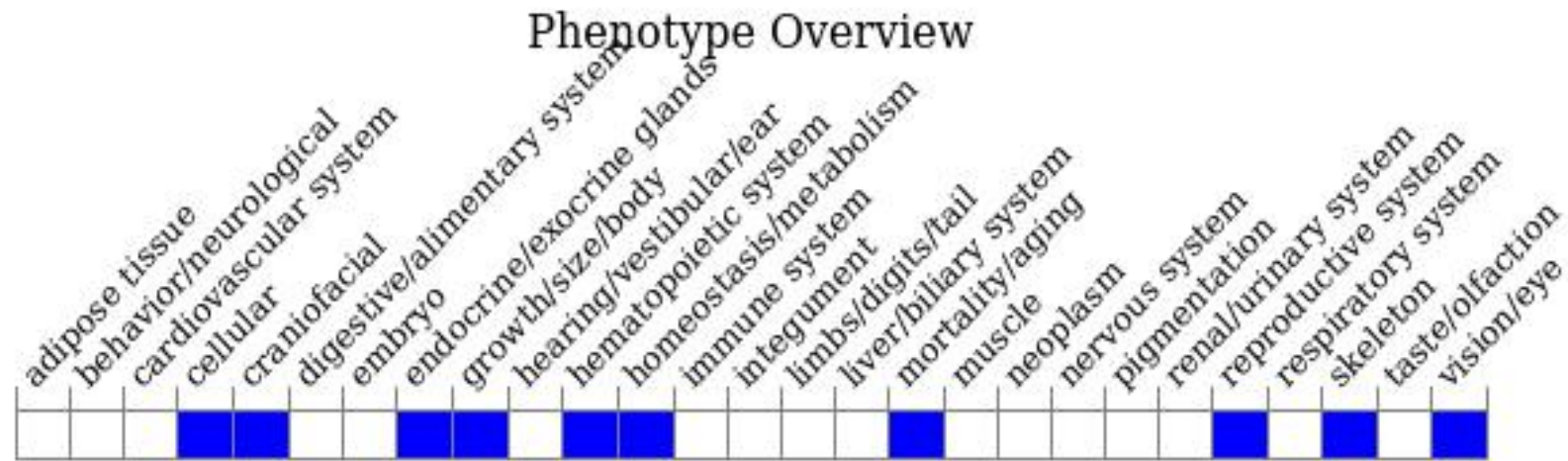
Genomic location distribution



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 show perinatal and postnatal lethality, decreased body weight, and male infertility due to defective spermatogenesis; female homozygotes may display malocclusion and variable ocular defects, including congenital central cataracts.

If you have any questions, you are welcome to inquire.
Tel: 400-9660890

