

Nceh1 Cas9-CKO Strategy

Designer: Xueting Zhang

Reviewer: Yanhua Shen

Date: 2020-02-12

Project Overview

Project Name

Nceh1

Project type

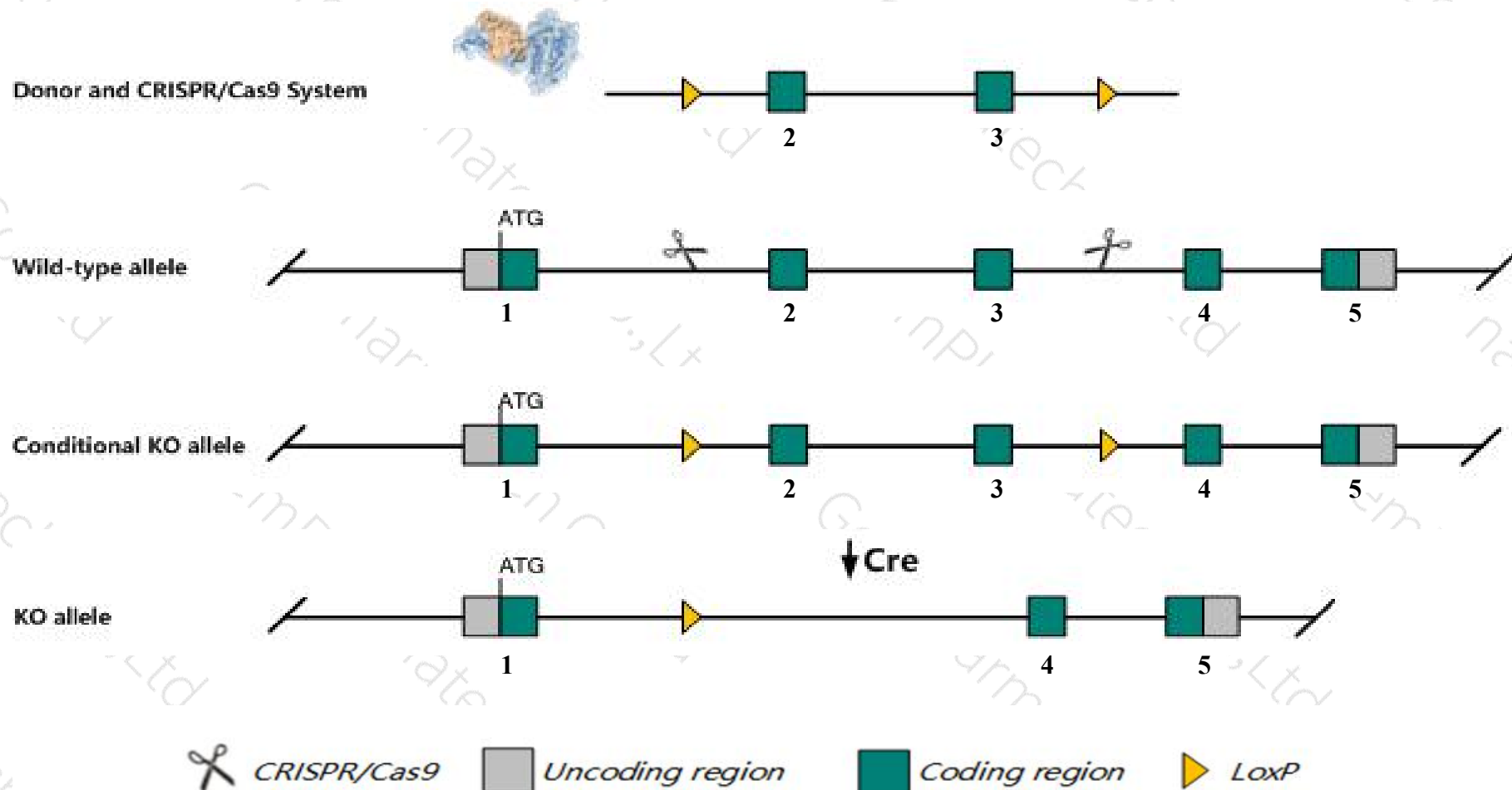
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ncehl1* gene. The schematic diagram is as follows:



Technical routes

- The *Nceh1* gene has 5 transcripts. According to the structure of *Nceh1* gene, exon2-exon3 of *Nceh1*-201 (ENSMUST00000046515.14) transcript is recommended as the knockout region. The region contains 299bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Nceh1* 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 exhibit abnormal organophosphorus metabolism and cholesterol homeostasis.
- The *Nceh1* gene is located on the Chr3. 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)

Nceh1 neutral cholesterol ester hydrolase 1 [*Mus musculus* (house mouse)]

Gene ID: 320024, updated on 5-Feb-2020

Summary

Official Symbol	Nceh1 provided by MGI
Official Full Name	neutral cholesterol ester hydrolase 1 provided by MGI
Primary source	MGI:MGI:2443191
See related	Ensembl:ENSMUSG000000027698
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Nceh; CPO-BP; Aadacl1; B230106I24Rik
Expression	Broad expression in kidney adult (RPKM 41.2), adrenal adult (RPKM 26.8) and 17 other tissues See more
Orthologs	human all

Genomic context

Location: 3; 3 A3

See Nceh1 in [Genome Data Viewer](#)

Exon count: 6

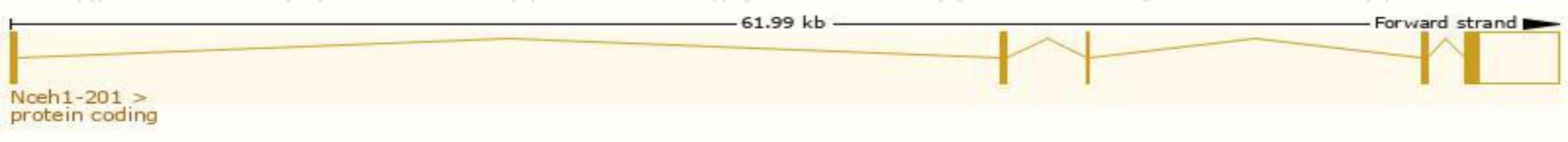
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	3	NC_000069.6 (27183004..27245266)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	3	NC_000069.5 (27081926..27143833)

Transcript information (Ensembl)

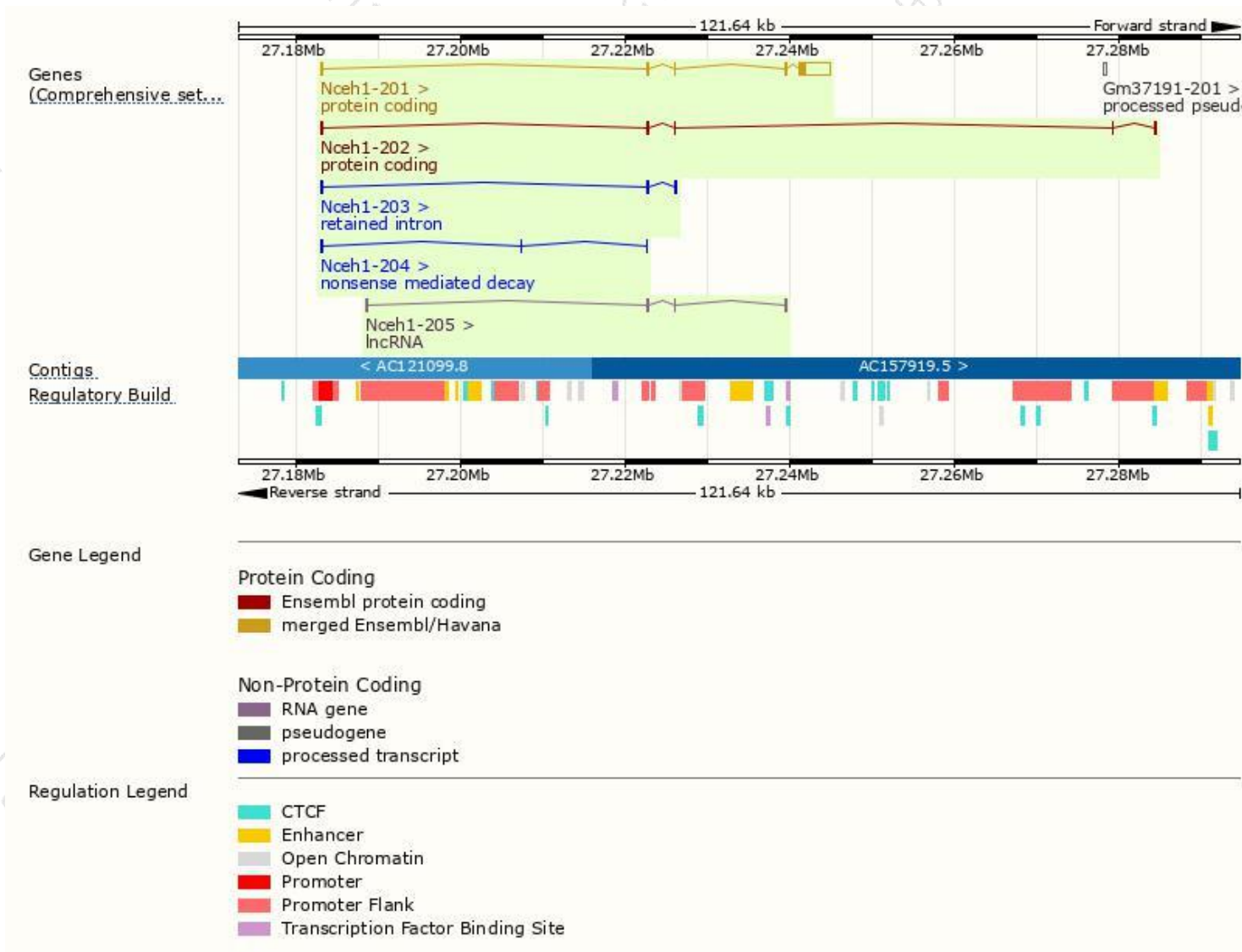
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nceh1-201	ENSMUST00000046515.14	4480	408aa	Protein coding	CCDS17271	Q8BLF1	TSL:1 GENCODE basic APPRIS P1
Nceh1-202	ENSMUST00000091284.4	830	199aa	Protein coding	-	Q8BYQ0	TSL:1 GENCODE basic
Nceh1-204	ENSMUST00000138947.7	339	51aa	Nonsense mediated decay	-	D6RGP7	TSL:3
Nceh1-203	ENSMUST00000129412.7	669	No protein	Retained intron	-	-	TSL:2
Nceh1-205	ENSMUST00000140872.1	577	No protein	lncRNA	-	-	TSL:3

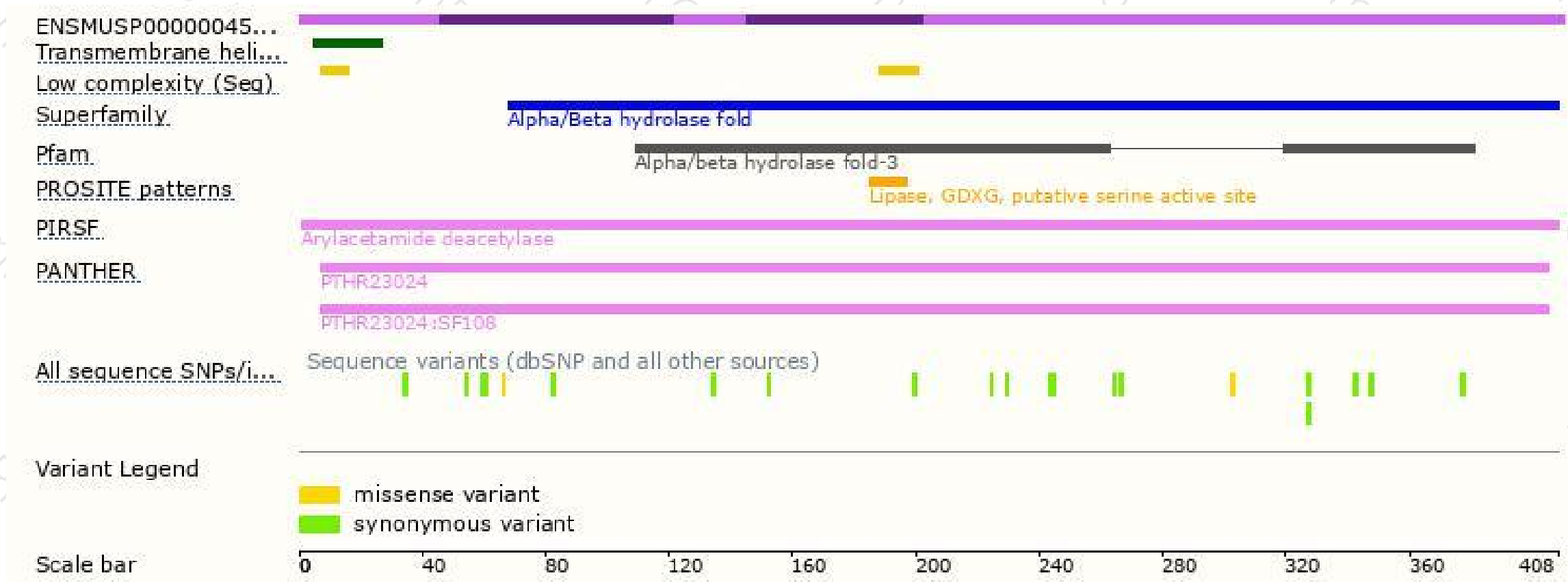
The strategy is based on the design of *Nceh1-201* transcript,The transcription is shown below



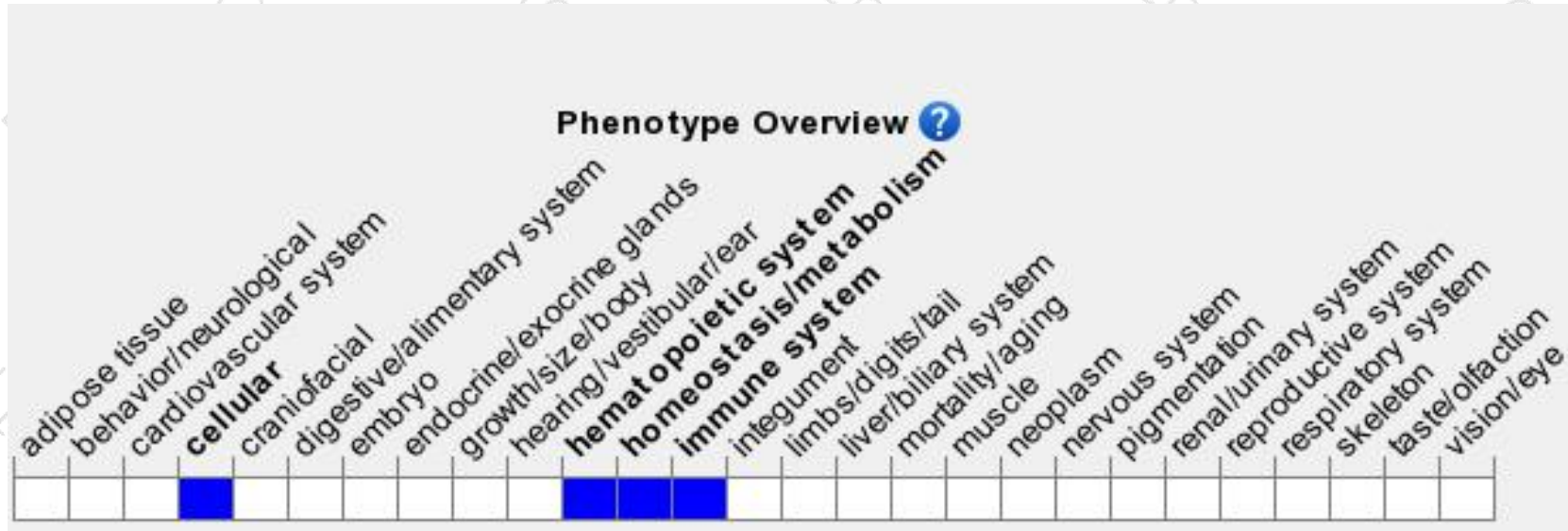
Genomic location distribution



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 abnormal organophosphorus metabolism and cholesterol homeostasis.

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

Tel: 400-9660890

