

Net1 Cas9-CKO Strategy

Designer:

Huimin Su

Reviewer:

Ruirui Zhang

Design Date:

2020/2/14

Project Overview

Project Name

Net1

Project type

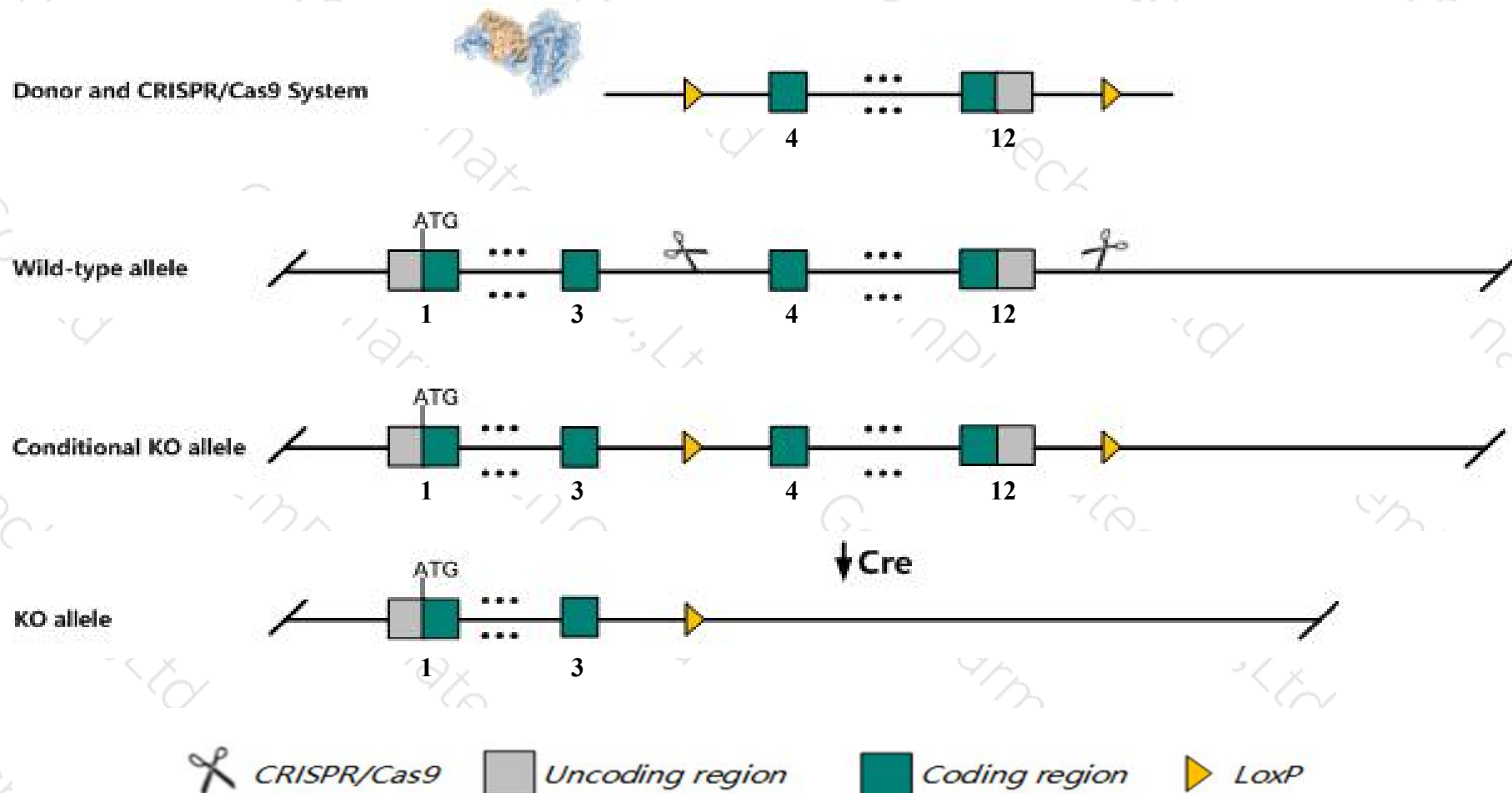
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Net1* gene. The schematic diagram is as follows:



Technical routes

- The *Net1* gene has 7 transcripts. According to the structure of *Net1* gene, exon4-exon12 of *Net1-201* (ENSMUST00000091853.11) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Net1* 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 delayed mammary gland development during puberty associated with slower ductal extension, reduced ductal branching and epithelial cell proliferation, disorganized myoepithelial and ductal epithelial cells, and increased collagen deposition.
- Transcript *Net1-207* may not be affected.
- The *Net1* 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.

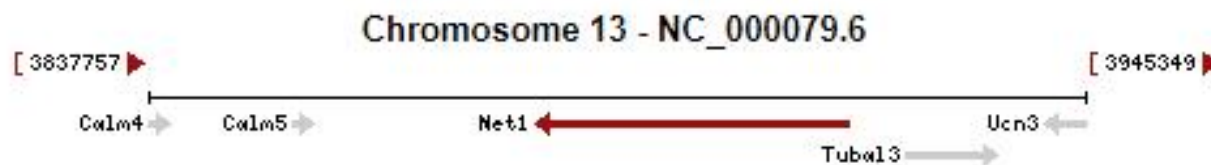
Gene information (NCBI)

Net1 neuroepithelial cell transforming gene 1 [*Mus musculus* (house mouse)]

Gene ID: 56349, updated on 12-Nov-2019

Summary

Official Symbol	Net1 provided by MGI
Official Full Name	neuroepithelial cell transforming gene 1 provided by MGI
Primary source	MGI:MGI:1927138
See related	Ensembl:ENSMUSG00000021215
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	Net1a; mNET1
Expression	Ubiquitous expression in large intestine adult (RPKM 25.9), limb E14.5 (RPKM 18.9) and 28 other tissues See more
Orthologs	human all

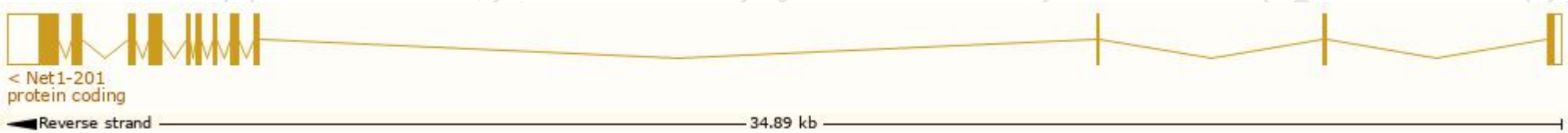


Transcript information (Ensembl)

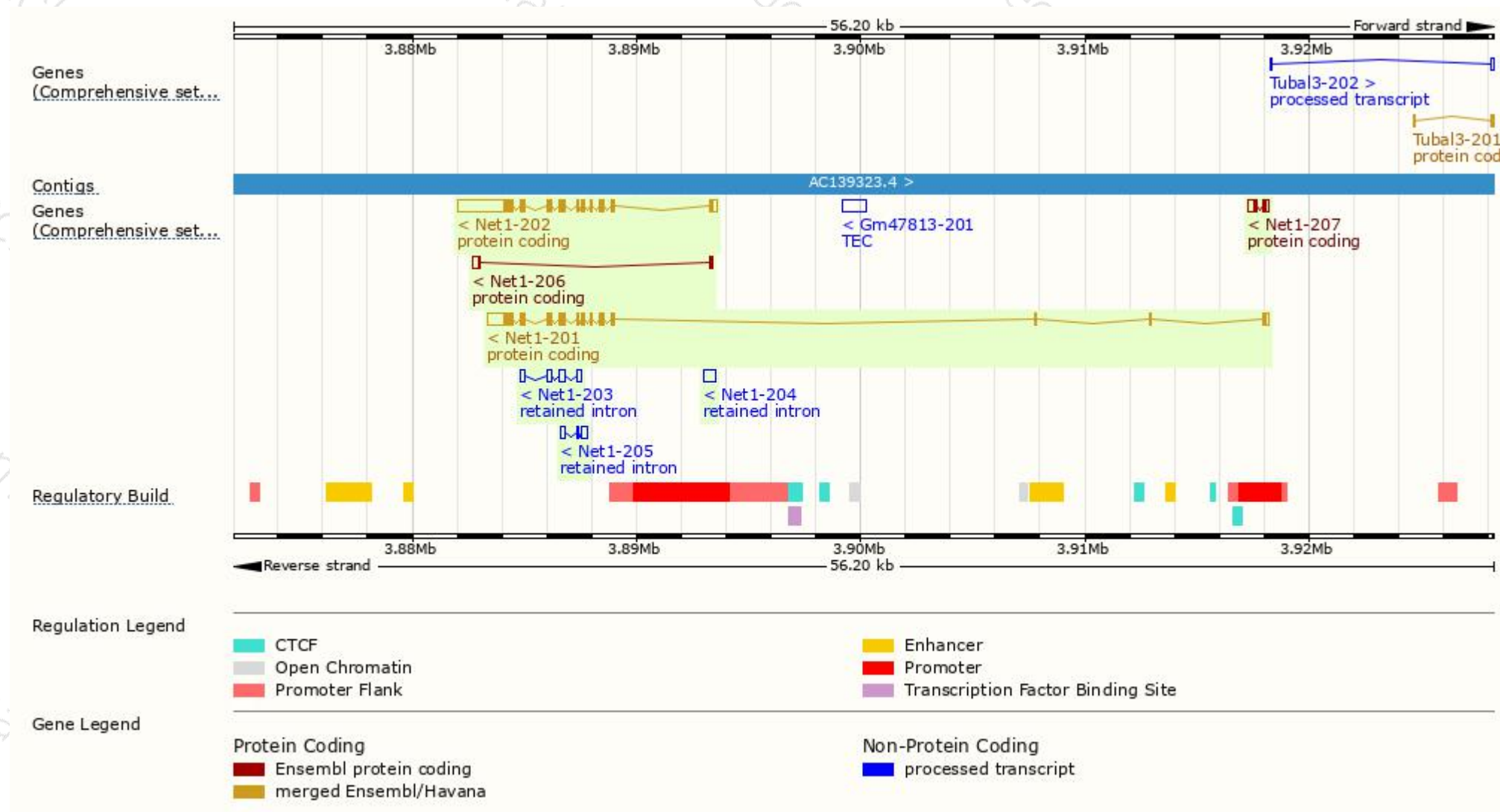
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Net1-202	ENSMUST00000099946.5	3871	541aa	Protein coding	CCDS36583	Q3USZ7 Q9Z206	TSL:1 GENCODE basic APPRIS ALT2
Net1-201	ENSMUST00000091853.11	2680	595aa	Protein coding	CCDS26216	Q9Z206	TSL:1 GENCODE basic APPRIS P3
Net1-207	ENSMUST00000223258.1	597	72aa	Protein coding	-	A0A1Y7VJ80	TSL:1 GENCODE basic
Net1-206	ENSMUST00000222504.1	447	44aa	Protein coding	-	A0A1Y7VKV6	TSL:3 GENCODE basic
Net1-203	ENSMUST00000220887.1	763	No protein	Retained intron	-	-	TSL:5
Net1-204	ENSMUST00000222017.1	569	No protein	Retained intron	-	-	TSL:NA
Net1-205	ENSMUST00000222442.1	519	No protein	Retained intron	-	-	TSL:3

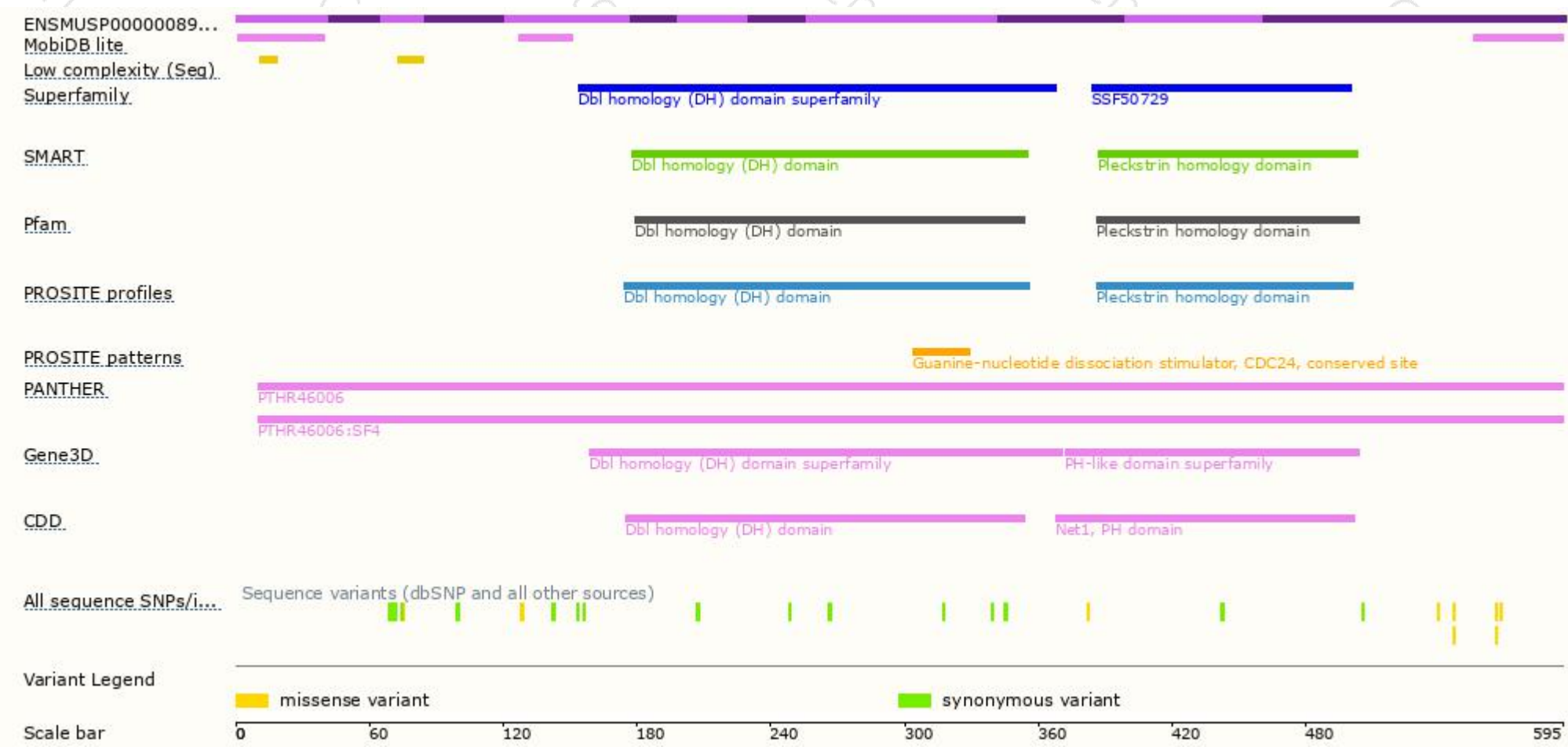
The strategy is based on the design of *Net1-201* transcript,The transcription is shown below



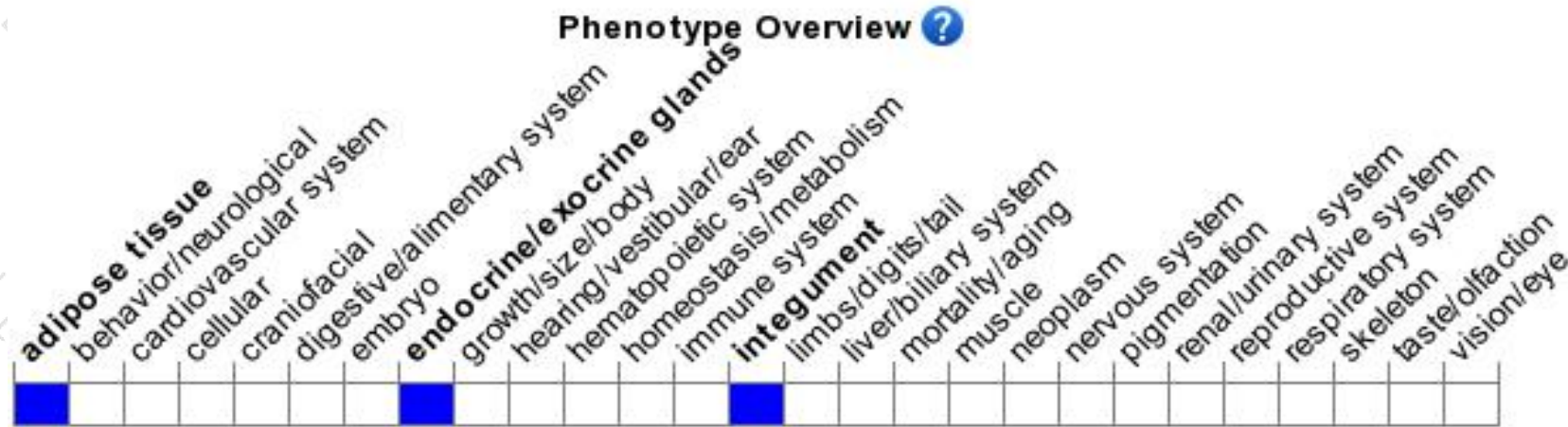
Genomic location distribution



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 delayed mammary gland development during puberty associated with slower ductal extension, reduced ductal branching and epithelial cell proliferation, disorganized myoepithelial and ductal epithelial cells, and increased collagen deposition.

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

Tel: 400-9660890

