

Pappa Cas9-CKO Strategy

Designer:

Yang Zeng

Reviewer:

Jia Yu

Design Date:

2019-12-16

Project Overview

Project Name

Pappa

Project type

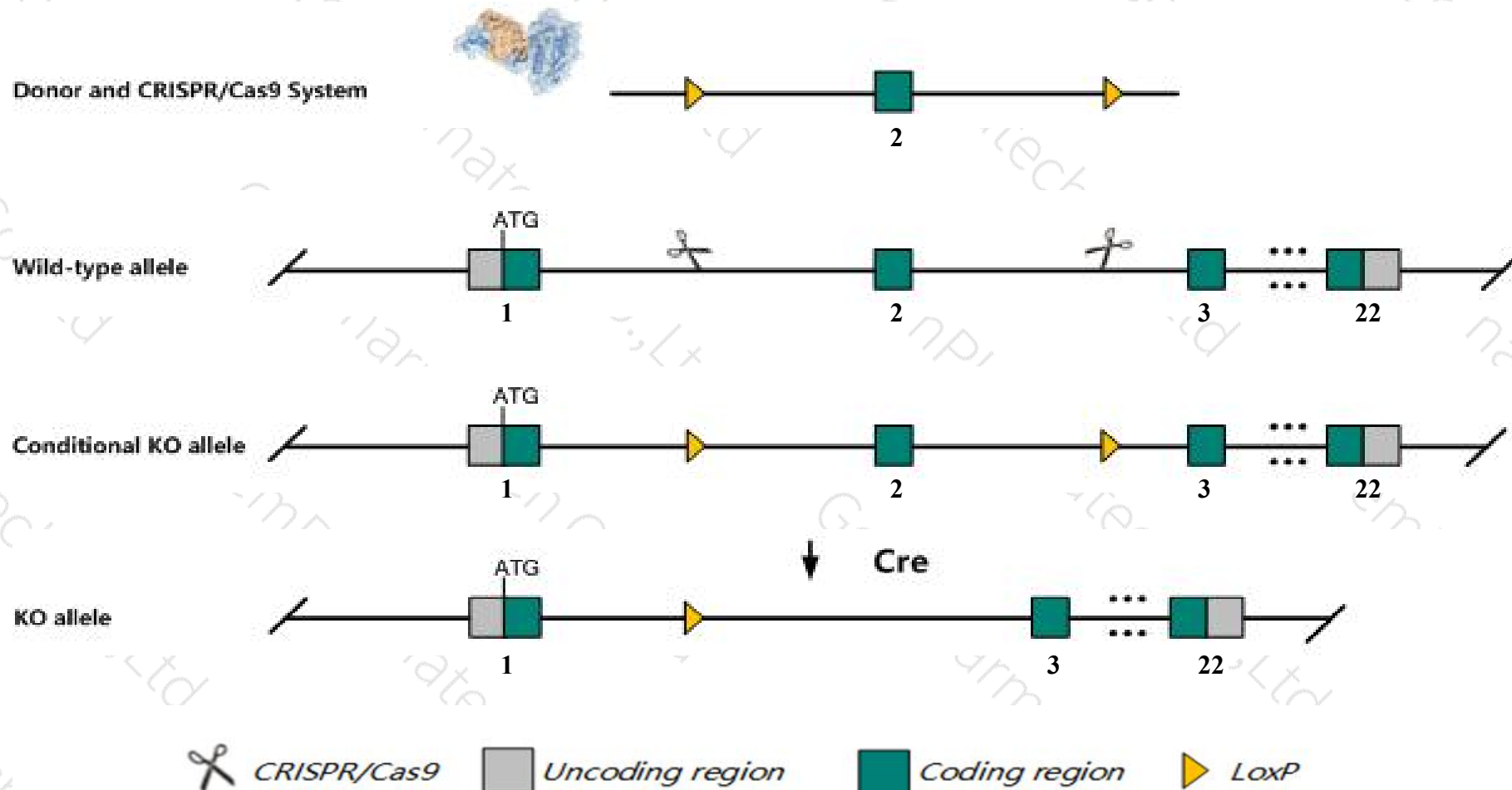
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



Technical routes

- The *Pappa* gene has 1 transcript. According to the structure of *Pappa* gene, exon2 of *Pappa-201* (ENSMUST00000084501.3) transcript is recommended as the knockout region. The region contains 1063bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Pappa* 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, Homozygous null mutants are smaller than normal with delayed ossification, but are otherwise normal and fertile.
- The *Pappa* 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.

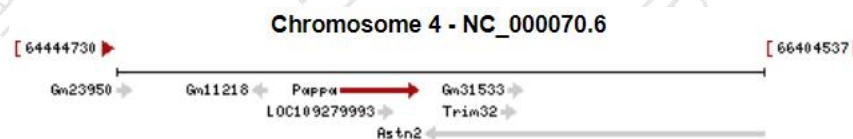
Gene information (NCBI)

Pappa pregnancy-associated plasma protein A [*Mus musculus* (house mouse)]

Gene ID: 18491, updated on 12-Aug-2019

Summary

Official Symbol	Pappa provided by MGI
Official Full Name	pregnancy-associated plasma protein A provided by MGI
Primary source	MGI:MGI:97479
See related	Ensembl:ENSMUSG00000028370
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	PAG1; PAPP-A; IGFBP-4ase; 8430414N03Rik
Expression	Broad expression in testis adult (RPKM 2.1), subcutaneous fat pad adult (RPKM 0.8) and 18 other tissues See more
Orthologs	human all

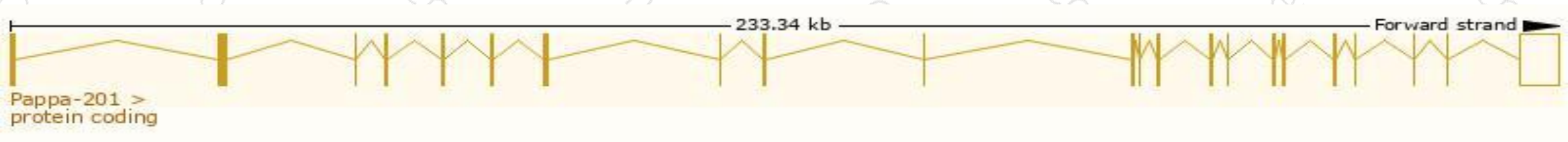


Transcript information (Ensembl)

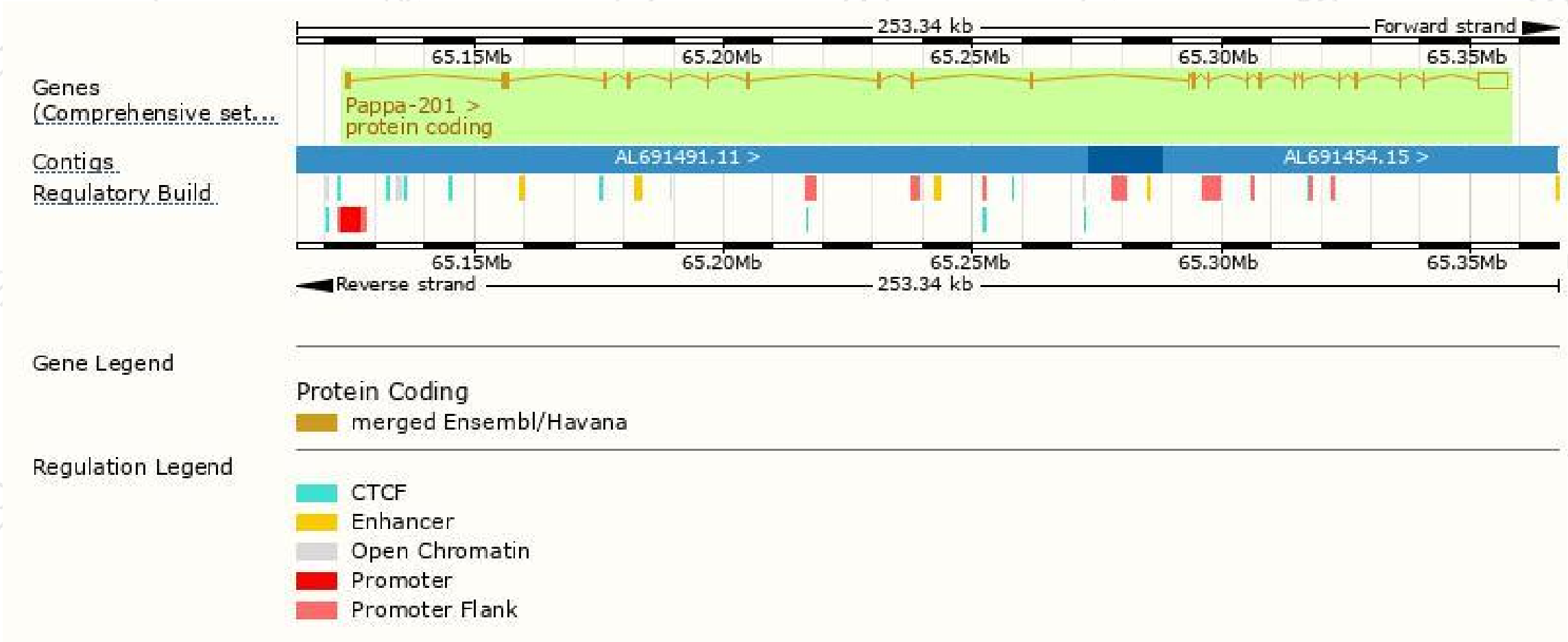
The gene has 1 transcript, and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pappa-201	ENSMUST00000084501.3	11027	1624aa	Protein coding	CCDS18267	Q8R4K8	TSL:1 GENCODE basic APPRIS P1

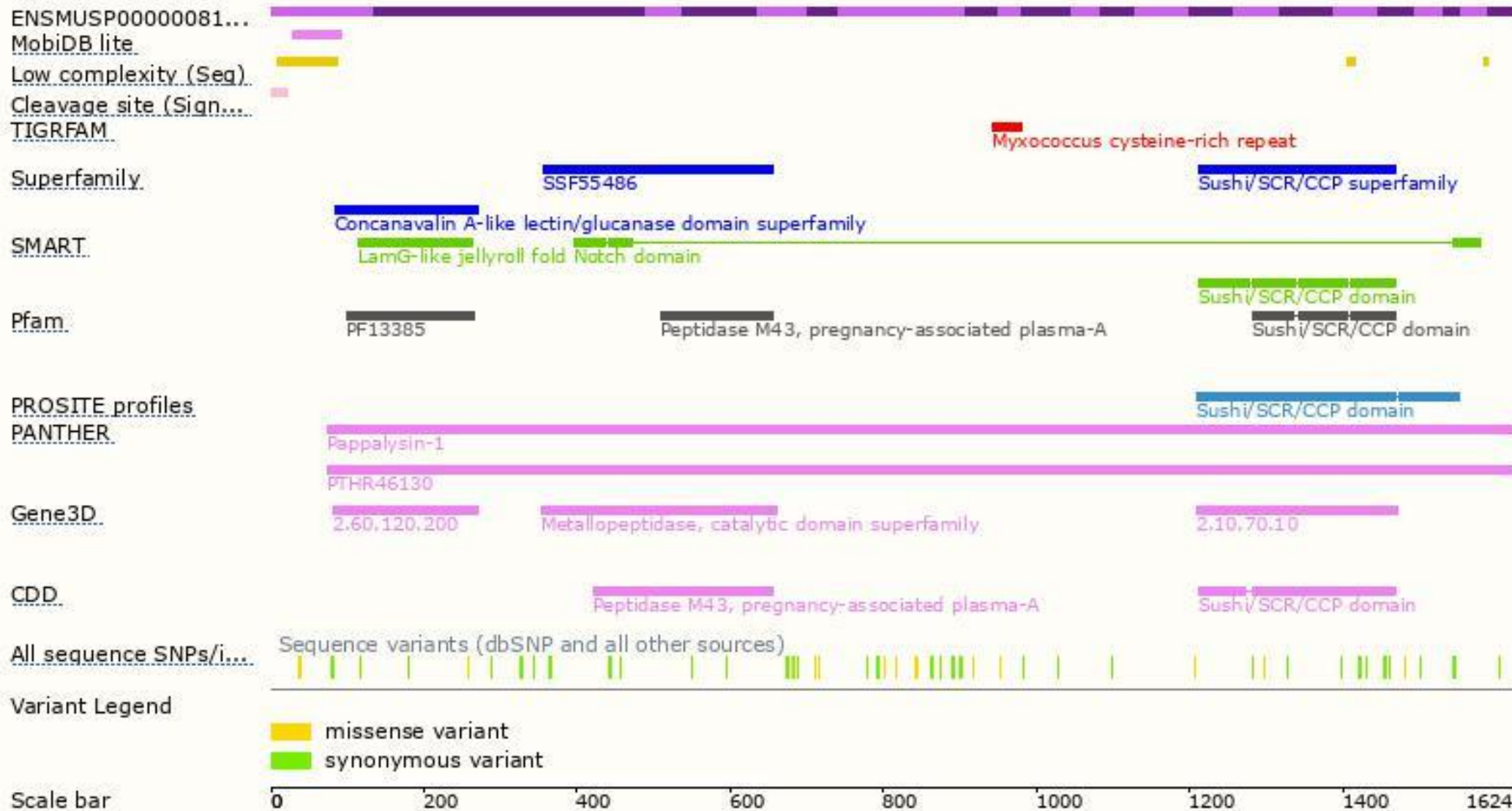
The strategy is based on the design of *Pappa-201* transcript, The transcription is shown below



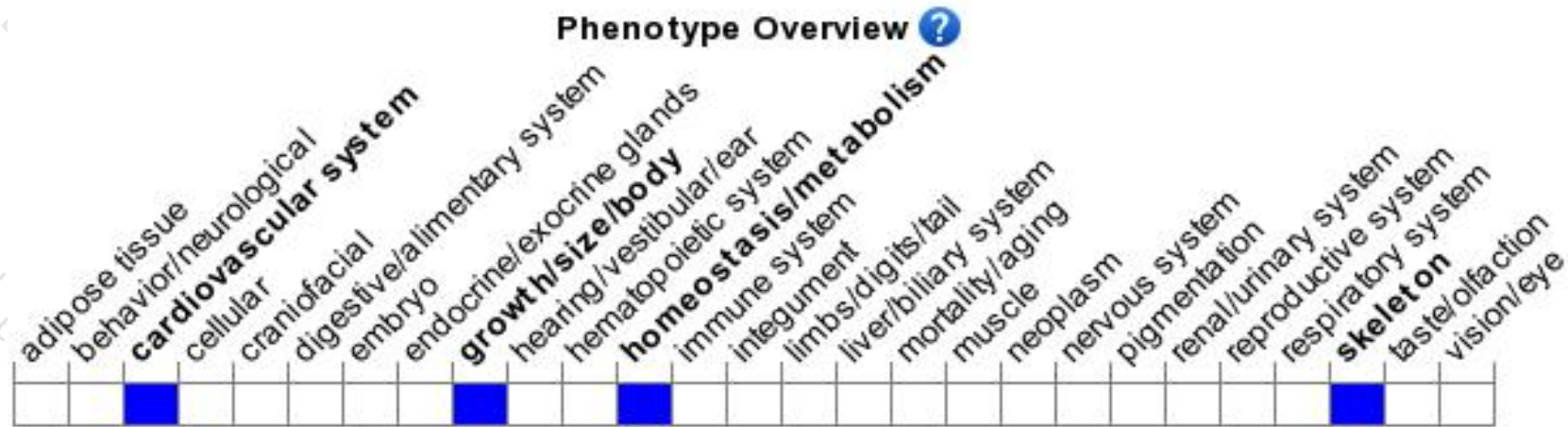
Genomic location distribution



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, Homozygous null mutants are smaller than normal with delayed ossification, but are otherwise normal and fertile.

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

Tel: 400-9660890

