

***Msh3* Cas9-CKO Strategy**

Designer: Xueting Zhang

Reviewer: Yanhua Shen

Date: 2020-1-21

Project Overview

Project Name

Msh3

Project type

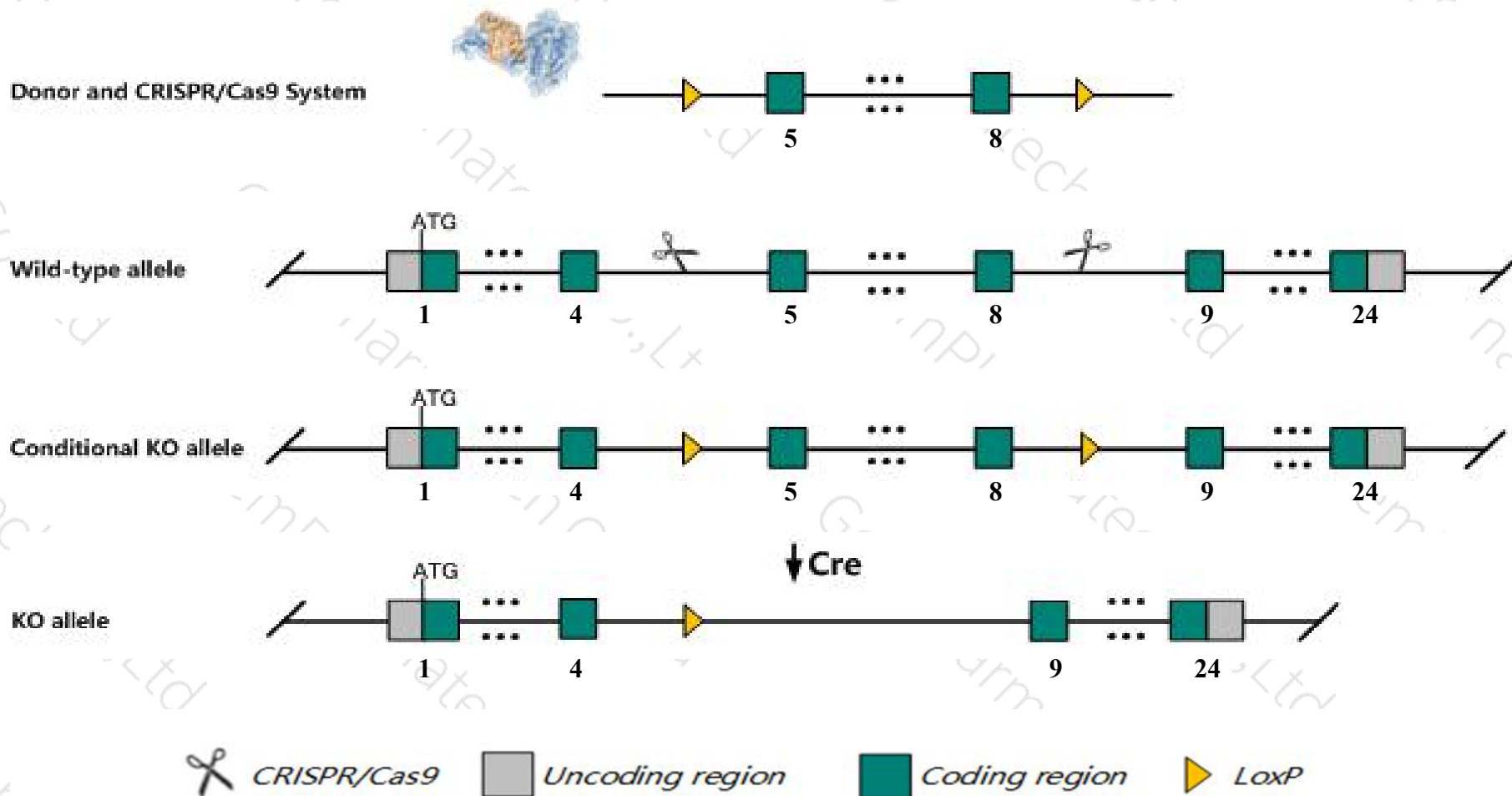
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Msh3* gene. The schematic diagram is as follows:



- The *Msh3* gene has 15 transcripts. According to the structure of *Msh3* gene, exon5-exon8 of *Msh3*-202 (ENSMUST00000185852.6) transcript is recommended as the knockout region. The region contains 548bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Msh3* 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 a partial defect mismatch repair and development of intestinal tumors.
- Transcript *Msh3*-209 may be destroyed directly.
- Transcript *Msh3*-203&207&210&212&213&215 may not be affected.
- The effect on transcript *Msh3*-205&211 is unknown.
- The floxed region is near to the N-terminal of *Dhfr* gene, this strategy may influence the regulatory function of the N-terminal of *Dhfr* gene.
- The N-terminal of *Msh3* gene will remain several amino acids, it may remain the partial function of *Msh3* gene.
- The *Msh3* 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)

Msh3 mutS homolog 3 [*Mus musculus* (house mouse)]

Gene ID: 17686, updated on 12-Aug-2019

Summary

- Official Symbol

Msh3 provided by MGI
- Official Full Name

mutS homolog 3 provided by MGI
- Primary source

MGI:MGI:109519
- See related

Ensembl:ENSMUSG00000014850
- 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

Rep3; Rep-3; D13Em1
- Expression

Ubiquitous expression in CNS E11.5 (RPKM 5.6), CNS E14 (RPKM 4.6) and 25 other tissues [See more](#)
- Orthologs

[human](#) [all](#)

Genomic context

Location: 13 C3; 13 47.63 cM

See Msh3 in [Genome Data Viewer](#)

Exon count: 24

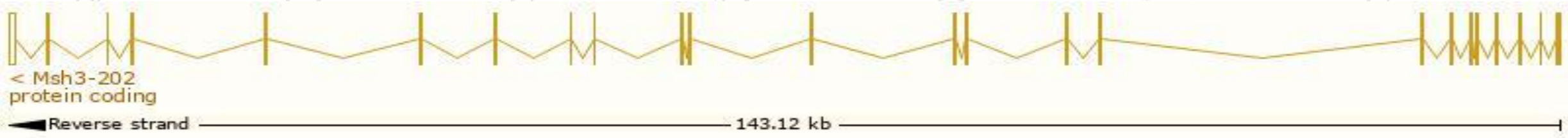
| Annotation release | Status | Assembly | Chr | Location |
|---------------------|-------------------|--|-----|--|
| 108 | current | GRCm38.p6 (GCF_000001635.26) | 13 | NC_000079.6 (92211878..92355004, complement) |
| Build 37.2 | previous assembly | MGSCv37 (GCF_000001635.18) | 13 | NC_000079.5 (92981836..93124958, complement) |

Transcript information (Ensembl)

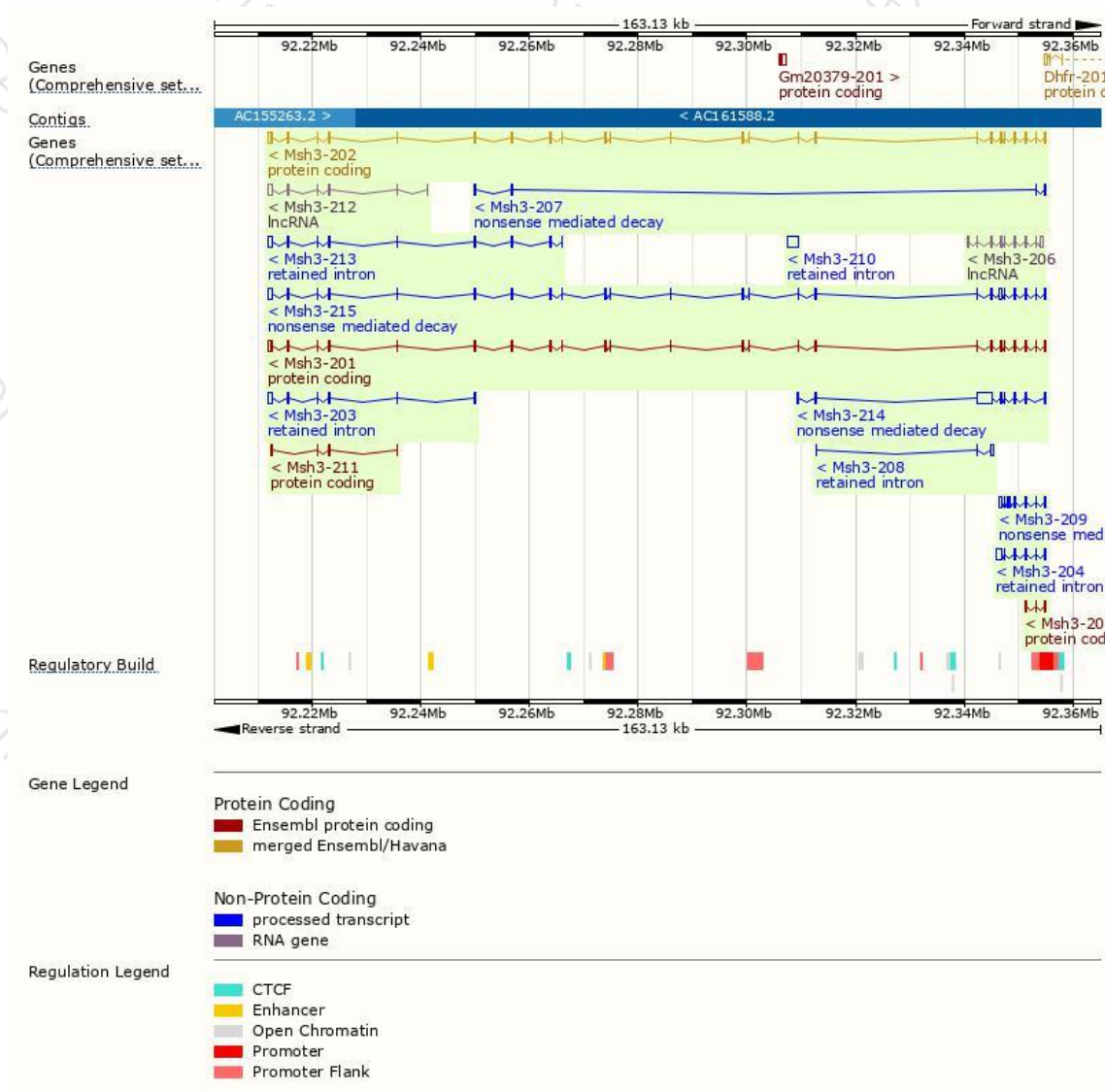
The gene has 15 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|----------|---------------------------------------|------|------------------------|-------------------------|---------------------------|----------------------------|---------------------------------|
| Msh3-202 | ENSMUST00000185852.6 | 3946 | 1095aa | Protein coding | CCDS49326 | A0A087WQ16 | TSL:1 GENCODE basic APPRIS P3 |
| Msh3-201 | ENSMUST00000022220.12 | 3937 | 1094aa | Protein coding | CCDS79222 | E9QPY6 | TSL:1 GENCODE basic APPRIS ALT2 |
| Msh3-205 | ENSMUST00000187424.1 | 666 | 167aa | Protein coding | - | A0A087WP45 | TSL:2 GENCODE basic |
| Msh3-211 | ENSMUST00000190761.1 | 633 | 129aa | Protein coding | - | A0A087WQZ2 | CDS 5' incomplete TSL:5 |
| Msh3-215 | ENSMUST00000191550.6 | 4254 | 159aa | Nonsense mediated decay | - | A0A087WRJ5 | TSL:1 |
| Msh3-214 | ENSMUST00000191509.6 | 4137 | 56aa | Nonsense mediated decay | - | A0A087WSR8 | TSL:1 |
| Msh3-209 | ENSMUST00000190393.6 | 1818 | 360aa | Nonsense mediated decay | - | A0A087WSS2 | TSL:1 |
| Msh3-207 | ENSMUST00000187874.6 | 534 | 101aa | Nonsense mediated decay | - | A0A087WP43 | TSL:5 |
| Msh3-210 | ENSMUST00000190462.1 | 1961 | No protein | Retained intron | - | - | TSL:NA |
| Msh3-204 | ENSMUST00000186878.6 | 1818 | No protein | Retained intron | - | - | TSL:1 |
| Msh3-213 | ENSMUST00000191304.6 | 1796 | No protein | Retained intron | - | - | TSL:1 |
| Msh3-203 | ENSMUST00000186392.1 | 1555 | No protein | Retained intron | - | - | TSL:1 |
| Msh3-208 | ENSMUST00000189376.6 | 625 | No protein | Retained intron | - | - | TSL:3 |
| Msh3-206 | ENSMUST00000187831.6 | 1753 | No protein | lncRNA | - | - | TSL:1 |
| Msh3-212 | ENSMUST00000191242.6 | 1363 | No protein | lncRNA | - | - | TSL:1 |

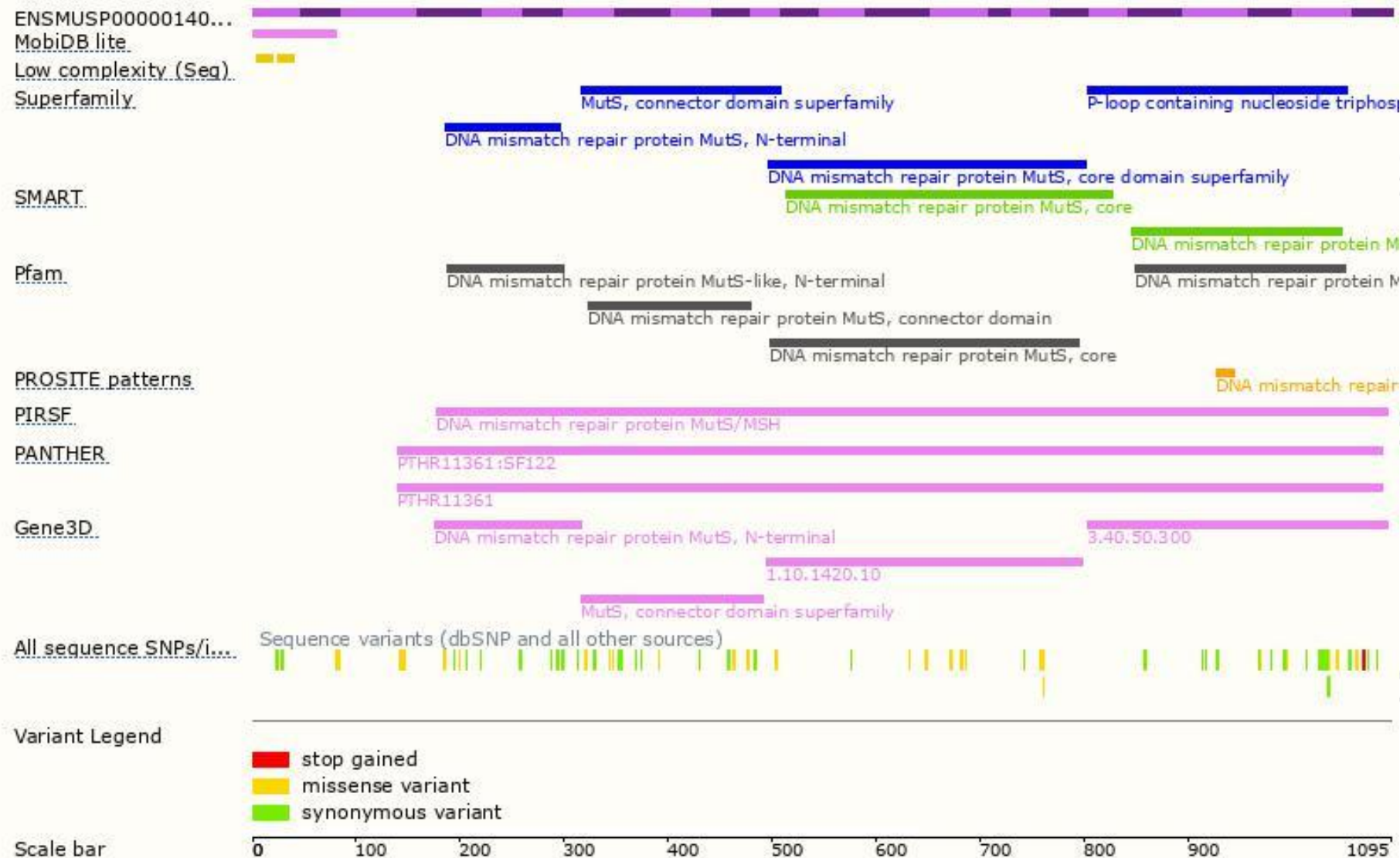
The strategy is based on the design of *Msh3-202* transcript,The transcription is shown below



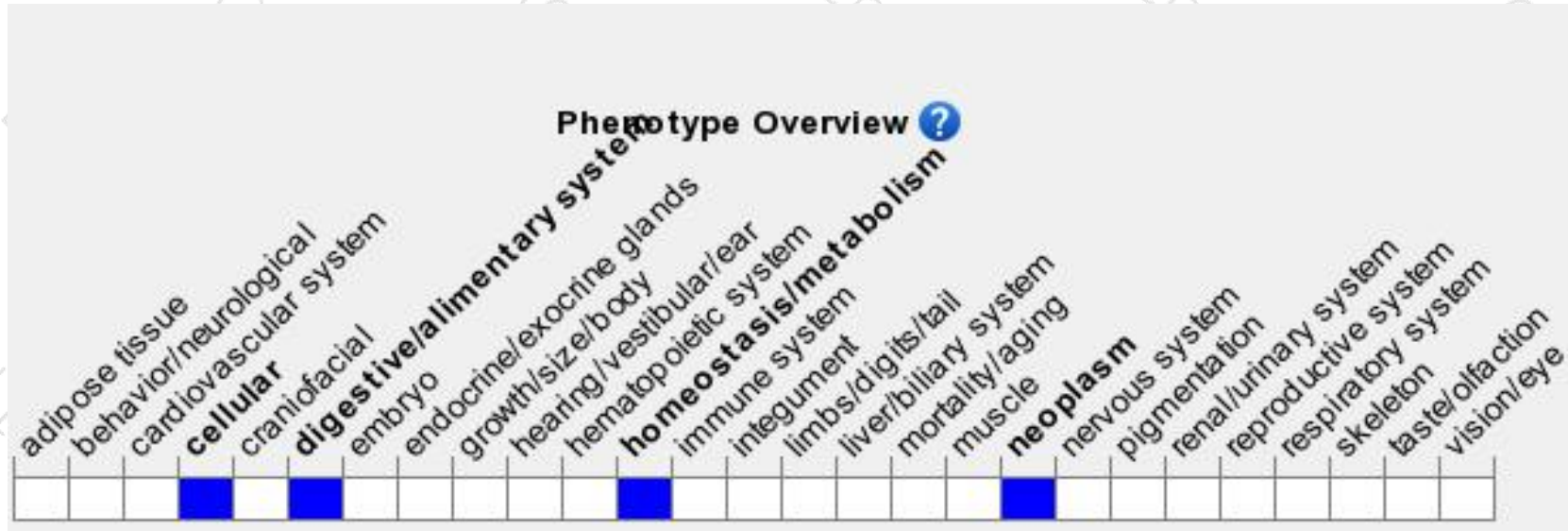
Genomic location distribution



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 a partial defect mismatch repair and development of intestinal tumors.

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

Tel: 400-9660890

