

***Htr4* Cas9-CKO Strategy**

Designer: Yun Li

Reviewer: Longyun Hu

Design Date: 2019-10-31

Project Overview

Project Name

Htr4

Project type

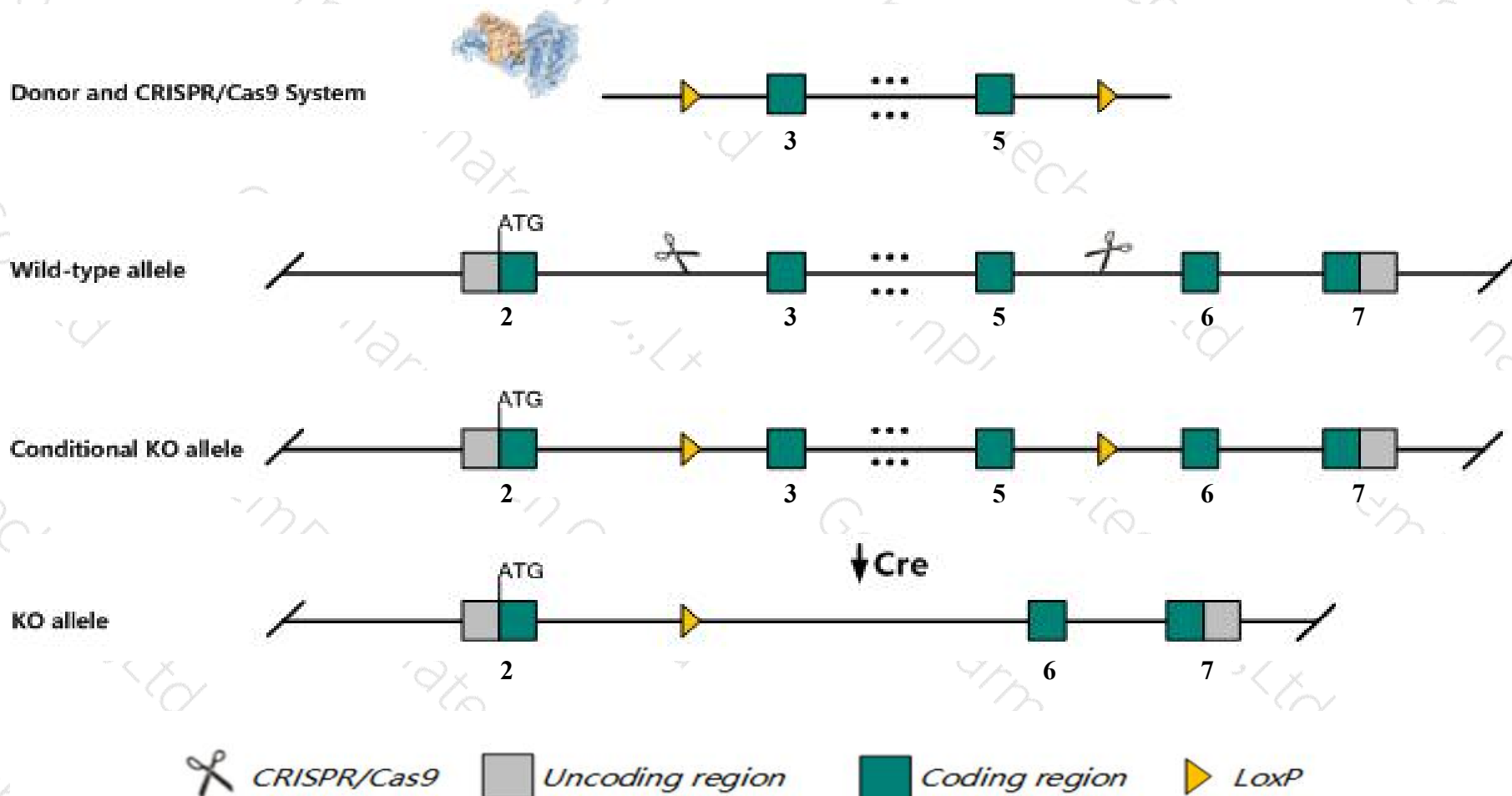
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Htr4* gene. The schematic diagram is as follows:



- The *Htr4* gene has 4 transcripts. According to the structure of *Htr4* gene, exon3-exon5 of *Htr4-201* (ENSMUST00000027560.7) transcript is recommended as the knockout region. The region contains 481bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Htr4* 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 mutant mice exhibit attenuated feeding behavior following stress and novelty and show a hypersensitivity to seizures.
- The *Htr4* gene is located on the Chr18. 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)

Htr4 5 hydroxytryptamine (serotonin) receptor 4 [Mus musculus (house mouse)]

Gene ID: 15562, updated on 31-Jan-2019

Summary



Official Symbol	Htr4 provided by MGI
Official Full Name	5 hydroxytryptamine (serotonin) receptor 4 provided by MGI
Primary source	MGI:MGI:109246
See related	Ensembl:ENSMUSG00000026322
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	5-HT-4, 5-HT4, 5-HT<4L>, 5HTR4
Expression	Biased expression in bladder adult (RPKM 1.5), colon adult (RPKM 1.5) and 6 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

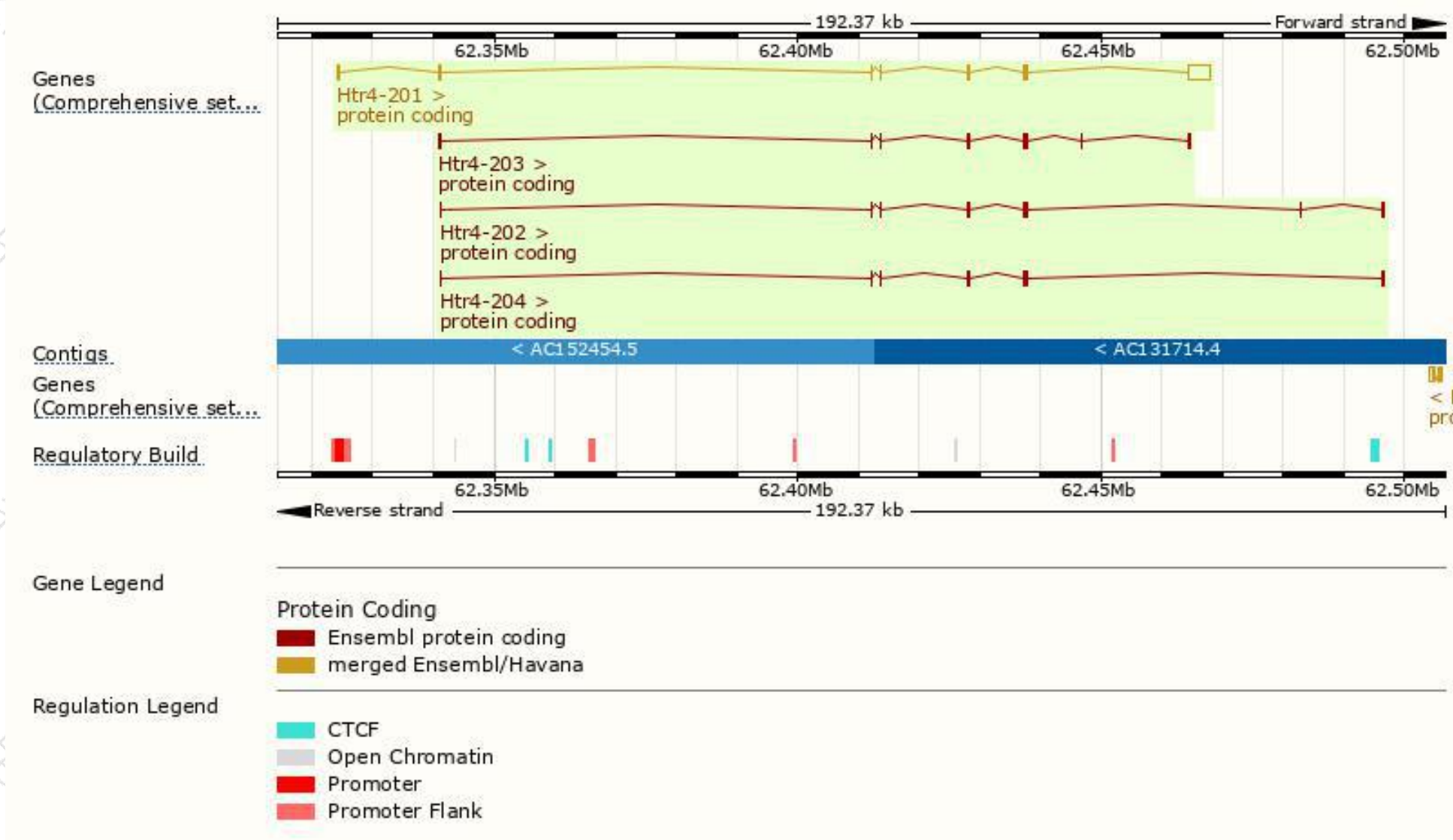
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Htr4-201	ENSMUST00000027560.7	4661	388aa	Protein coding	CCDS29291	P97288	TSL:1 GENCODE basic APPRIS P2
Htr4-203	ENSMUST00000237114.1	1389	377aa	Protein coding	-	-	GENCODE basic
Htr4-202	ENSMUST00000236691.1	1267	363aa	Protein coding	-	-	GENCODE basic
Htr4-204	ENSMUST00000238081.1	1203	387aa	Protein coding	-	-	GENCODE basic APPRIS ALT1

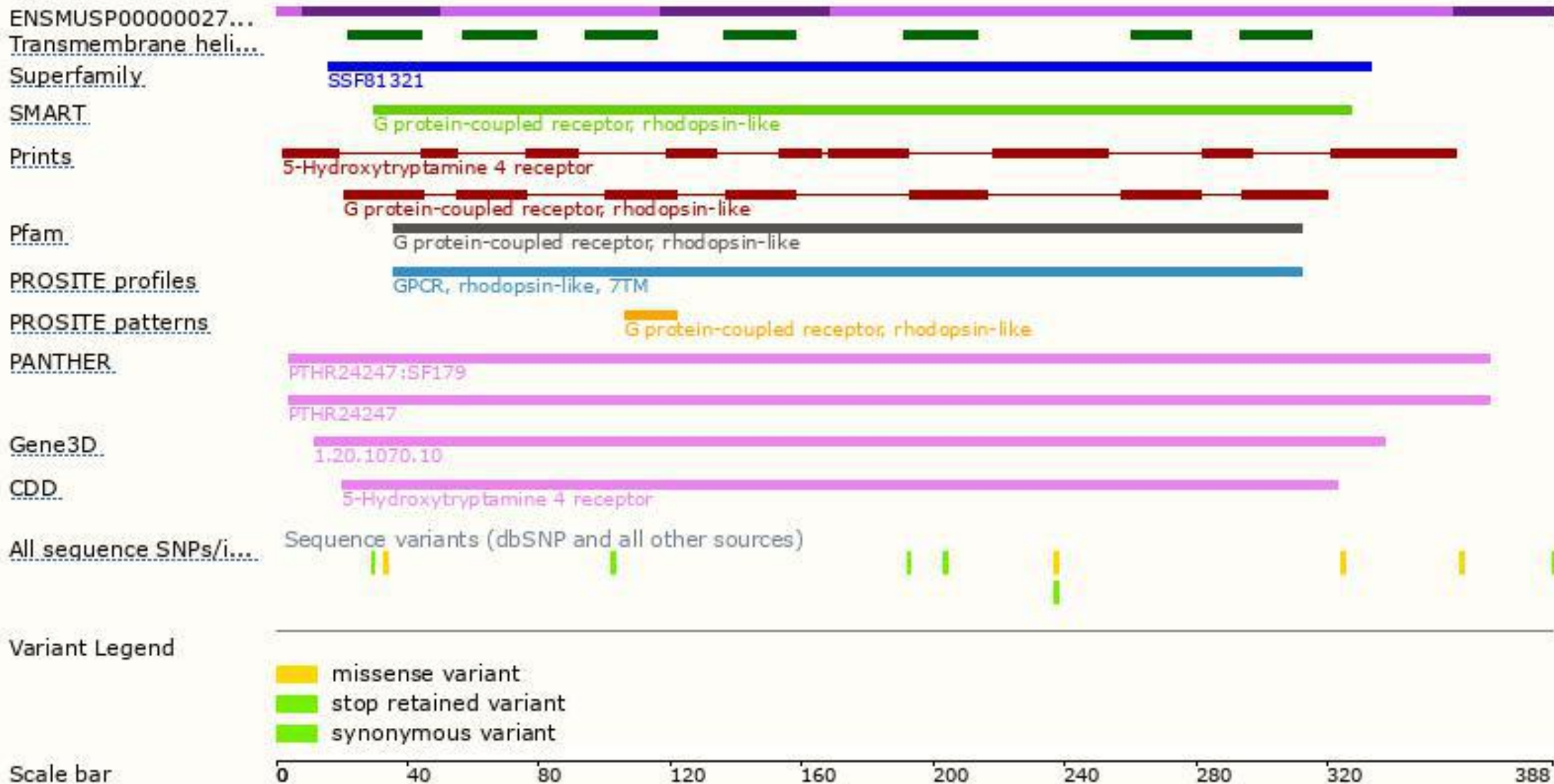
The strategy is based on the design of *Htr4-201* transcript,The transcription is shown below



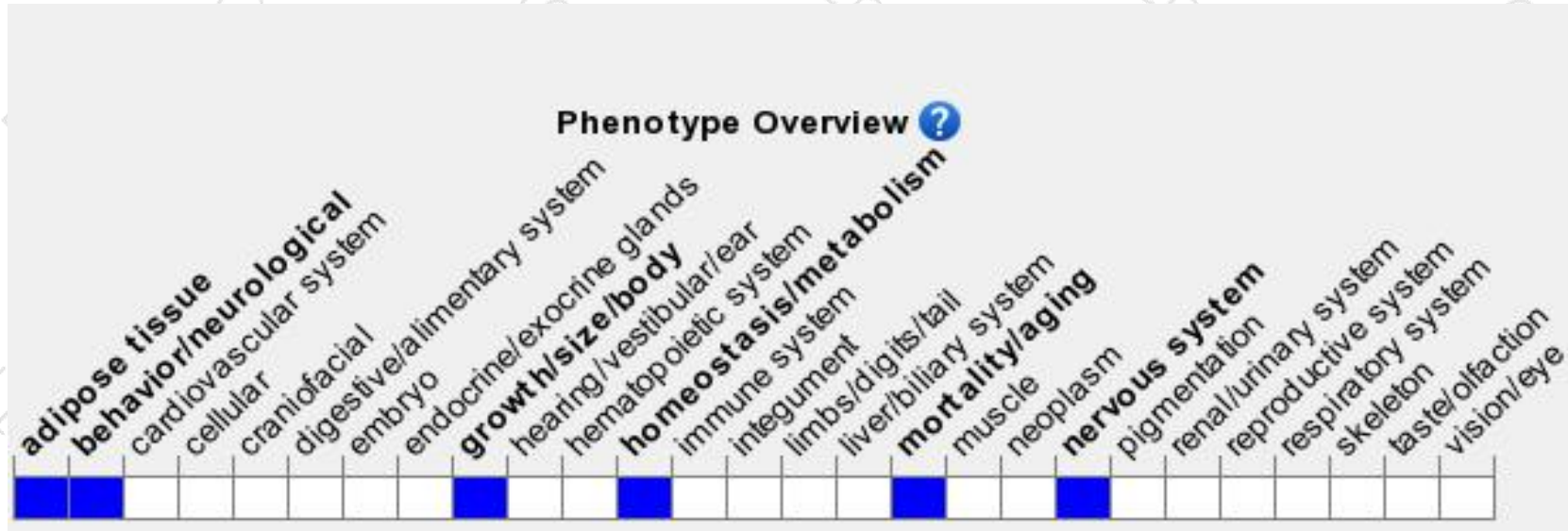
Genomic location distribution



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 mutant mice exhibit attenuated feeding behavior following stress and novelty and show a hypersensitivity to seizures.

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

Tel: 400-9660890

