

***Tph2-IRES-EGFP* Cas9-KI Strategy**

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Project Overview

Project Name

Tph2-IRES-EGFP

Project type

Cas9-KI

Strain background

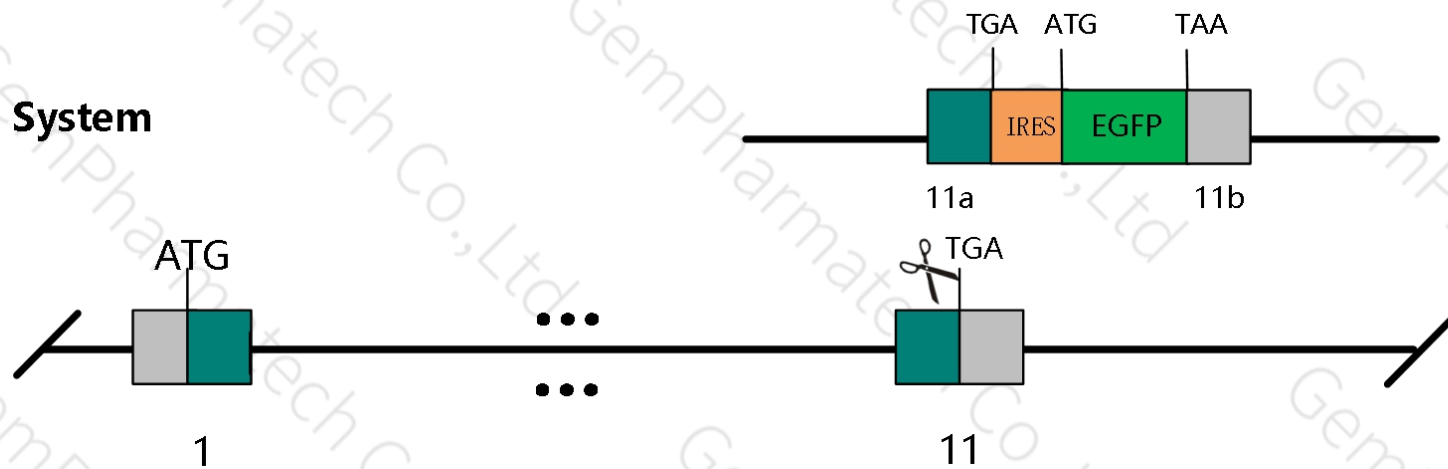
C57BL/6JGpt

Conditional Knockout strategy

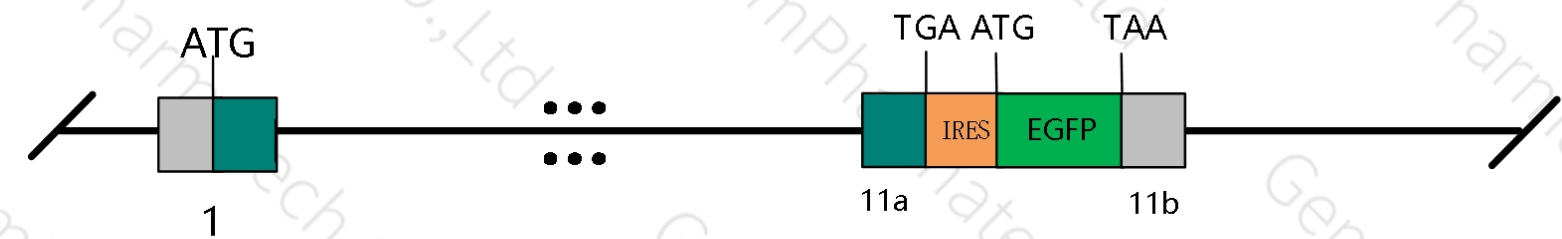
This model will use CRISPR/Cas9 technology to edit the *Tph2* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Wildtype allele
ENSMUST0000006949.8



Targeted allele



Cas9/sgRNA

Coding region

Uncoding region

Technical routes

- The *Tph2* gene has 2 transcripts.
- According to the structure of *Tph2* gene, the element IRES-EGFP will be inserted at the translation stop codon of *Tph2*-201(ENSMUST00000006949.8), the length of inserted fragment is about 1.3kb.
- In this project we use CRISPR/Cas9 technology to modify *Tph2* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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.

- According to the existing MGI data, Mutations in this locus result in abnormal serotonin levels in the brain. Whether an increase or decrease in serotonin levels is seen depends on the specific nucleotide substitution/point mutation.
- It is necessary to introduce 1-2 synonymous mutation in exon11.
- The IERS-linked genes will be transcribed together and then be translated two protein separately, but the downstream protein is lower than the upstream protein.
- The *Tph2* gene is located on the Chr10. Please take the loci in consideration when breeding this knockin mice with other gene modified (e.g., Tg, iCre) strains, if the other gene is also on Chr10, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene between the 3'UTR and the gene coding region may affect the expression of endogenous and foreign genes. Due to the complexity of biological processes, it cannot be predicted completely at the present technology level.

Gene information (NCBI)

Tph2 tryptophan hydroxylase 2 [Mus musculus (house mouse)]

Gene ID: 216343, updated on 19-Mar-2019

Summary



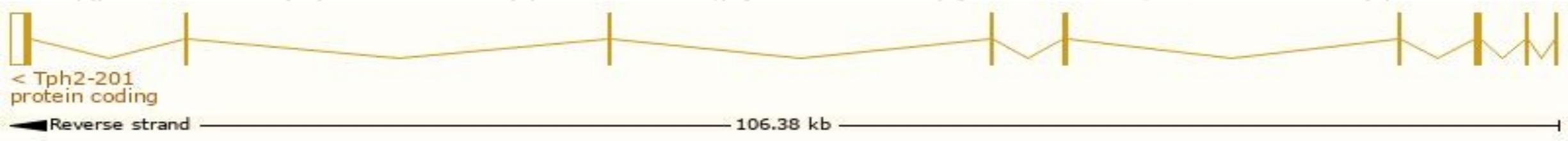
Official Symbol	Tph2 provided by MGI
Official Full Name	tryptophan hydroxylase 2 provided by MGI
Primary source	MGI:MGI:2651811
See related	Ensembl:ENSMUSG00000006764
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AU043594, Ntph
Expression	Biased expression in cerebellum adult (RPKM 1.7), CNS E18 (RPKM 1.0) and 8 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

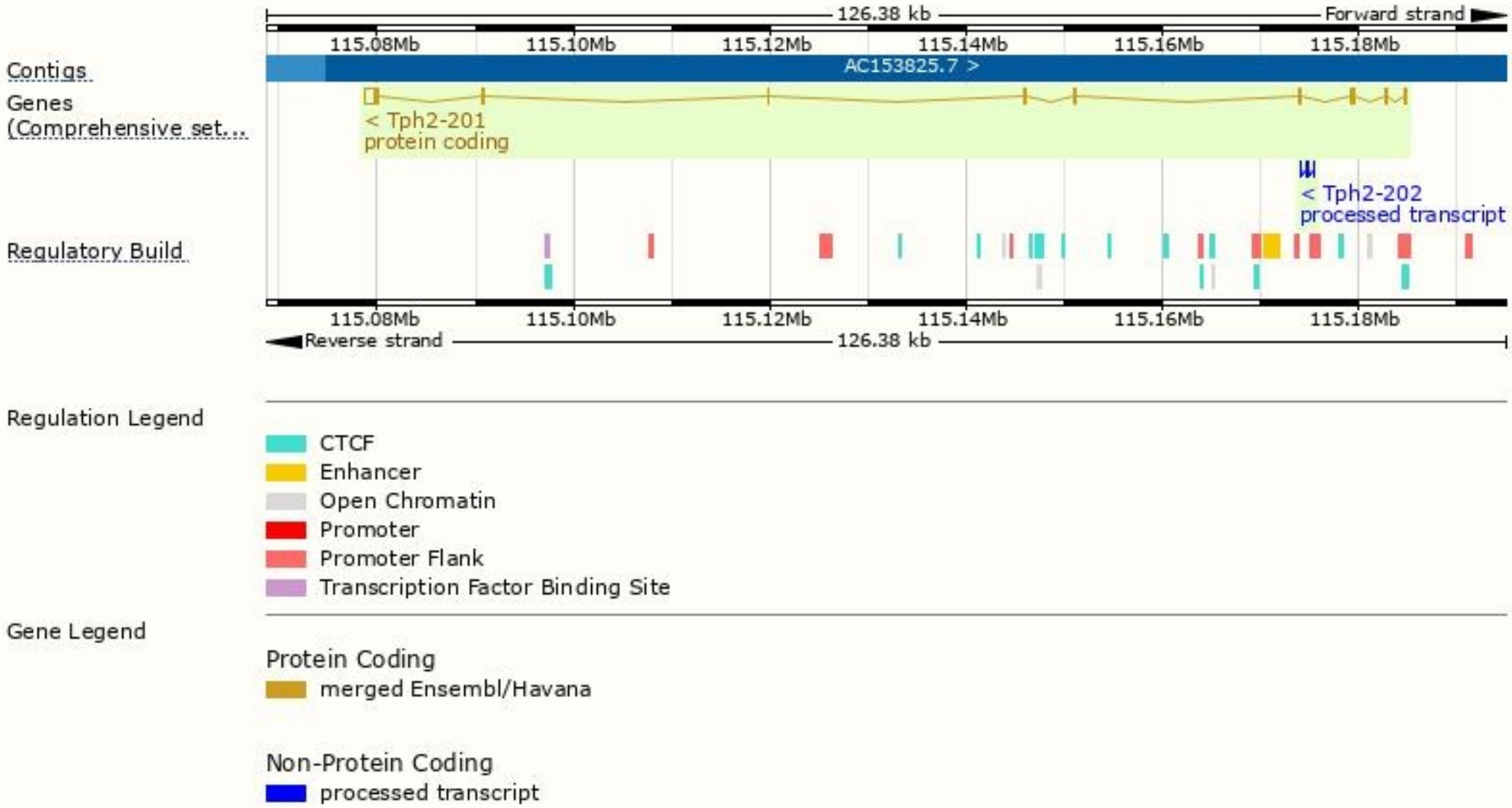
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tph2-201	ENSMUST00000006949.8	2626	488aa	Protein coding	CCDS36061	Q8CGV2	TSL:1 GENCODE basic APPRIS P1
Tph2-202	ENSMUST00000155794.1	293	No protein	lncRNA	-	-	TSL:3

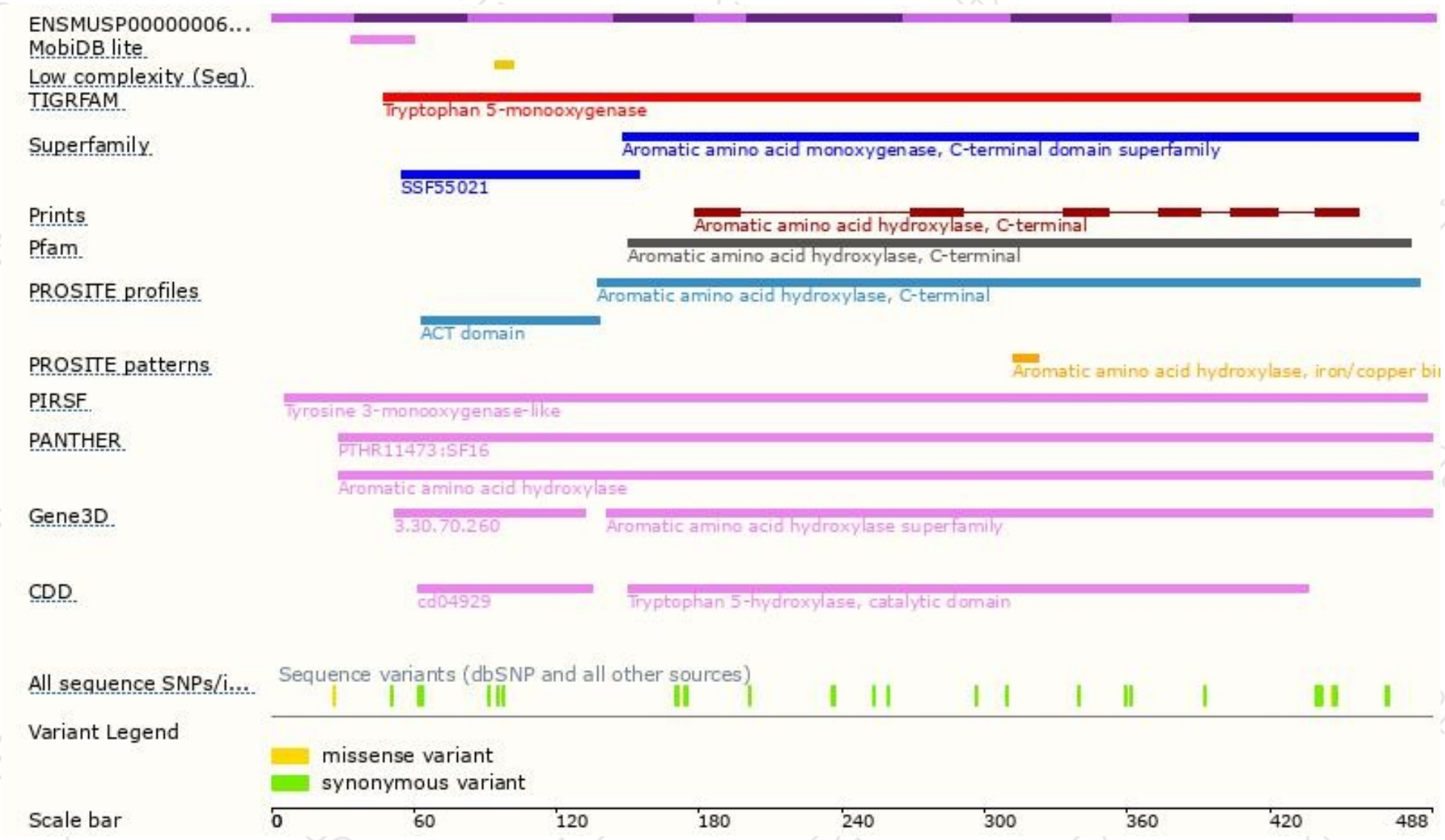
The strategy is based on the design of *Tph2-201* transcript,the transcription is shown below:



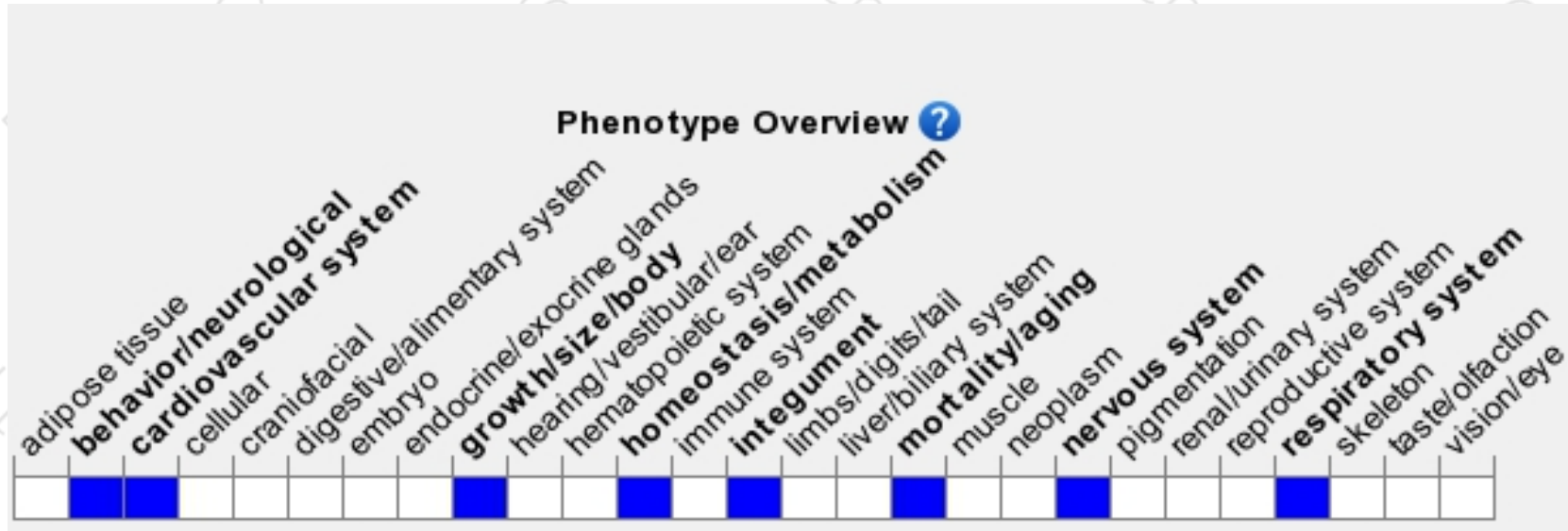
Genomic location distribution



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, mutations in this locus result in abnormal serotonin levels in the brain. Whether an increase or decrease in serotonin levels is seen depends on the specific nucleotide substitution/point mutation.

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

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