

# ***Tbc1d32* Cas9-CKO Strategy**

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**Design Date: 2020-8-17**

# Project Overview

**Project Name**

***Tbc1d32***

**Project type**

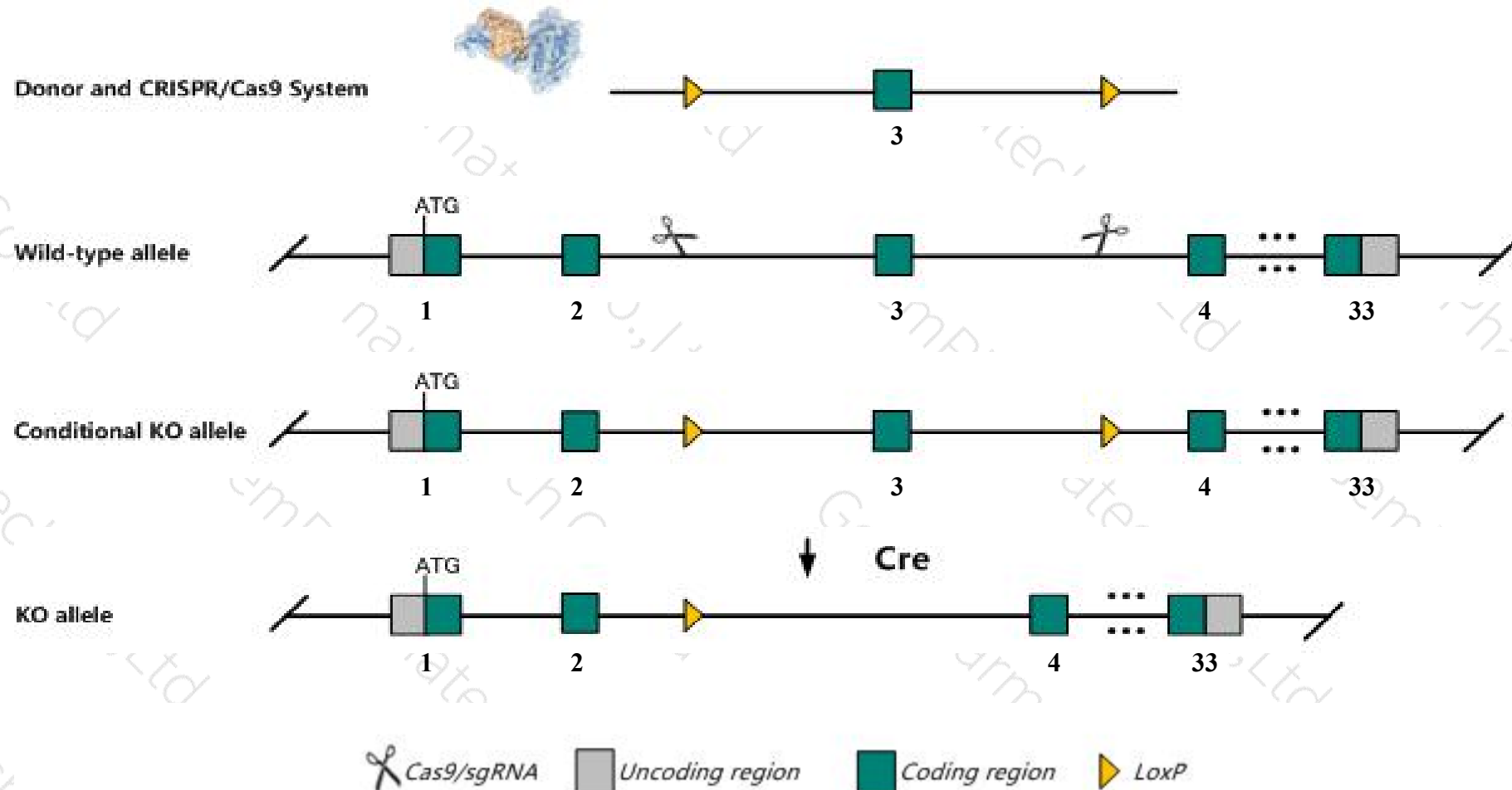
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tbc1d32* gene. The schematic diagram is as follows:



- The *Tbc1d32* gene has 3 transcripts. According to the structure of *Tbc1d32* gene, exon3 of *Tbc1d32*-201(ENSMUST00000099739.4) transcript is recommended as the knockout region. The region contains 169bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Tbc1d32* 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.
- The flox mice was 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 gene trap allele or ENU induced mutation exhibit exencephaly and poor eye development.
- The *Tbc1d32* gene is located on the Chr10. 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)

## Tbc1d32 TBC1 domain family, member 32 [Mus musculus (house mouse)]

Gene ID: 544696, updated on 13-Mar-2020

### Summary



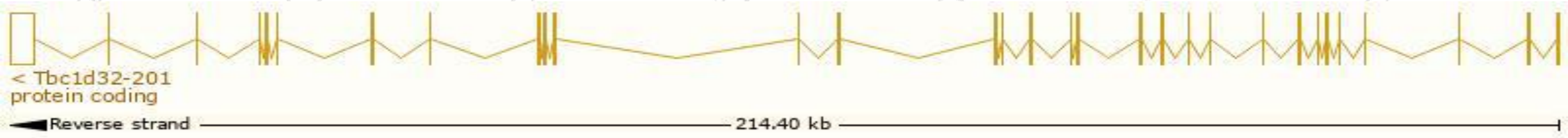
<b>Official Symbol</b>	Tbc1d32 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	TBC1 domain family, member 32 provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:2442827</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000038122</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	VALIDATED
<b>Organism</b>	<a href="#">Mus musculus</a>
<b>Lineage</b>	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
<b>Also known as</b>	Bromi, C6orf170, D630037F22Rik, b2b2284Clo
<b>Expression</b>	Ubiquitous expression in bladder adult (RPKM 1.5), CNS E18 (RPKM 1.1) and 26 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

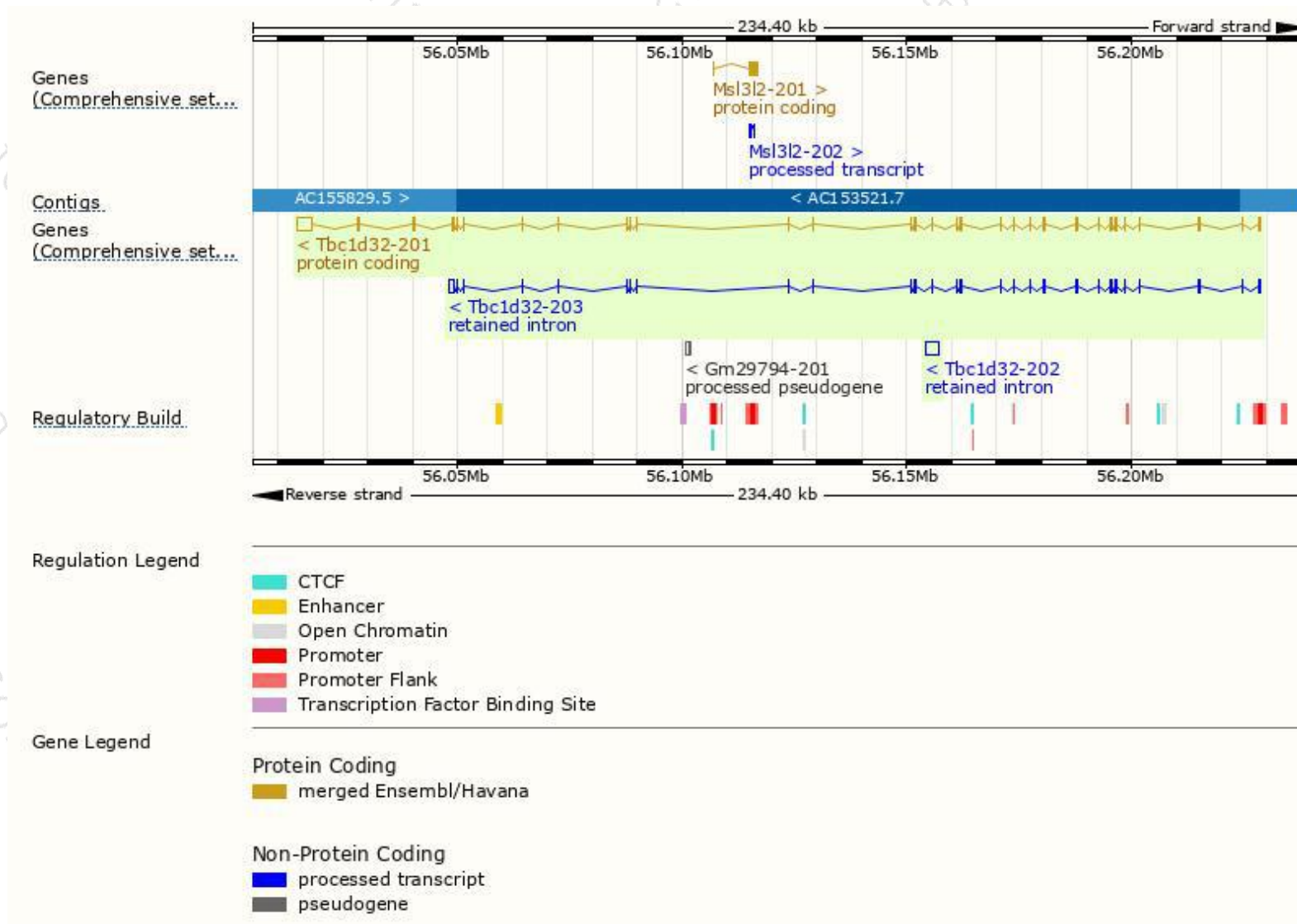
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tbc1d32-201	<a href="#">ENSMUST00000099739.4</a>	7240	<a href="#">1296aa</a>	Protein coding	<a href="#">CCDS48562</a>	<a href="#">Q3URV1</a>	TSL:5 GENCODE basic APPRIS P1
Tbc1d32-203	<a href="#">ENSMUST00000219385.1</a>	4192	No protein	Retained intron	-	-	TSL:5
Tbc1d32-202	<a href="#">ENSMUST00000217792.1</a>	2761	No protein	Retained intron	-	-	TSL:NA

The strategy is based on the design of *Tbc1d32-201* transcript,the transcription is shown below:

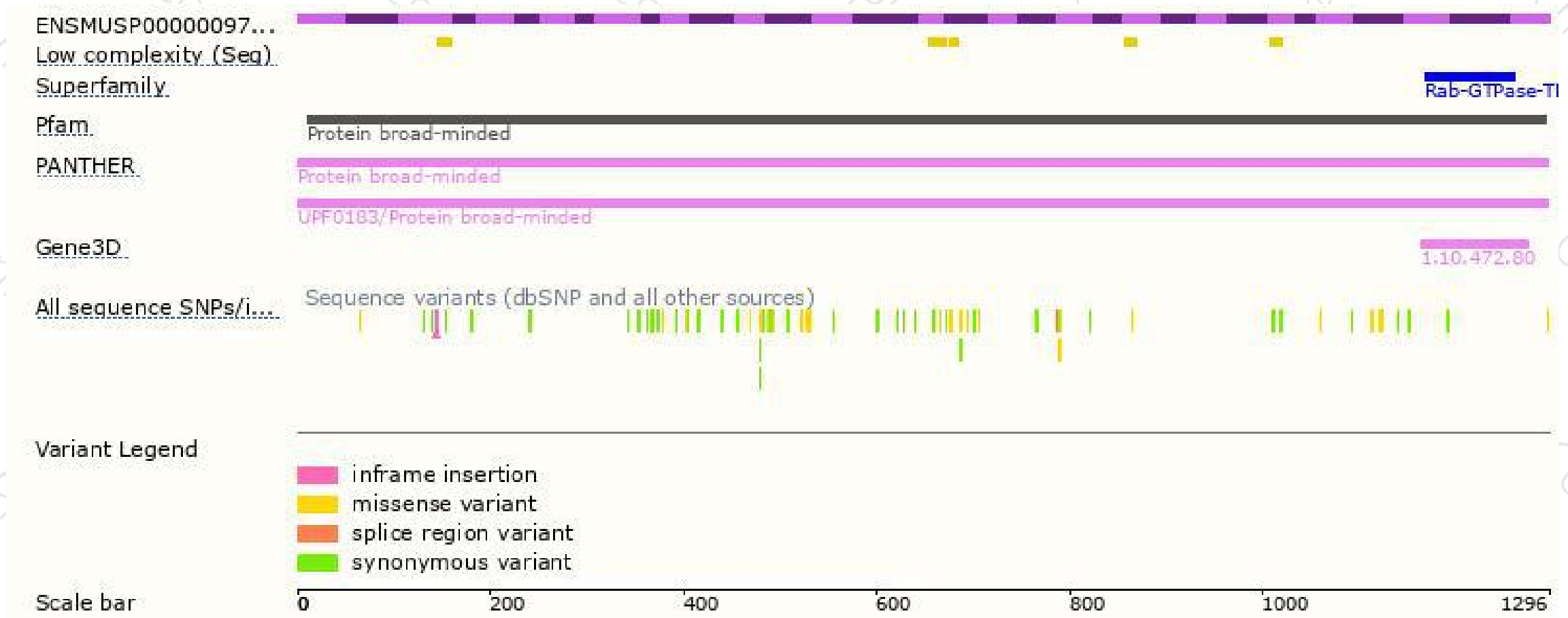


# Genomic location distribution

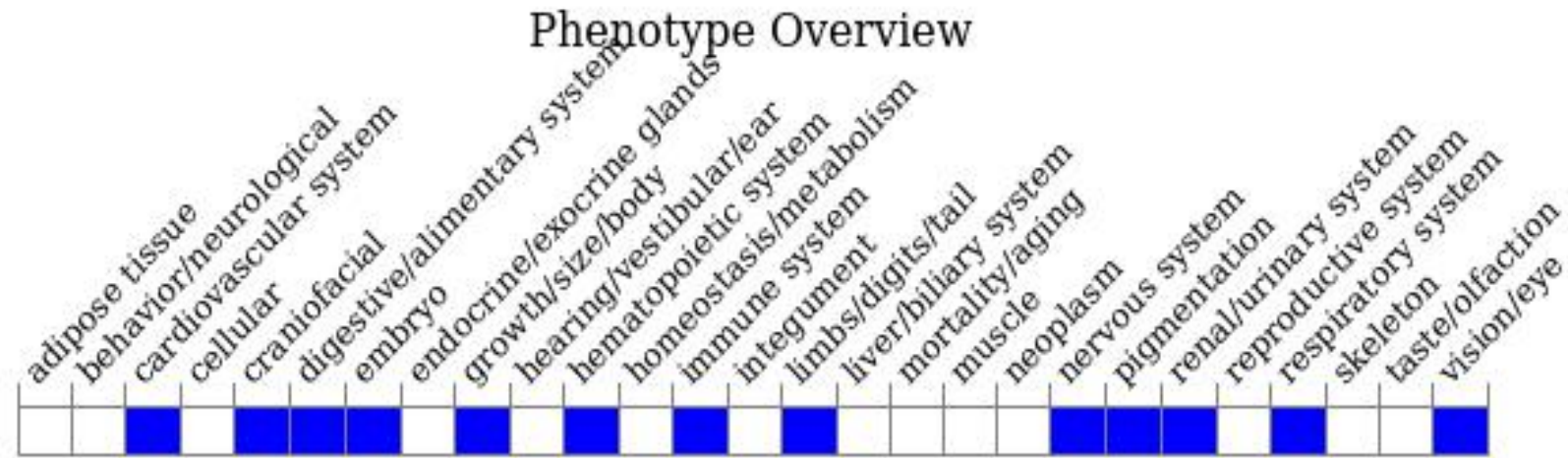




# Protein domain



# 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 gene trap allele or ENU induced mutation exhibit exencephaly and poor eye development.

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

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