Crygn Cas9-CKO *Strategy*

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Reviewer: **Ruirui Zhang**

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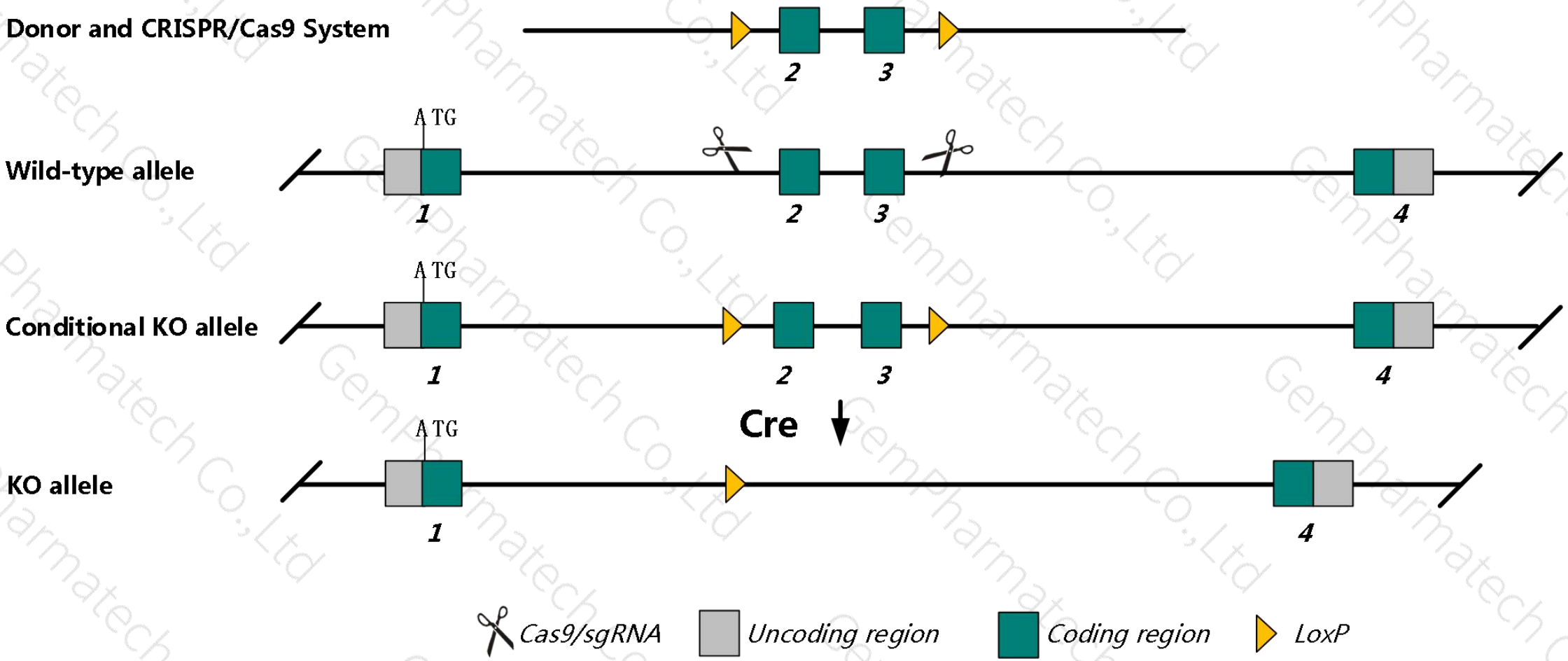
Project Overview

Project Name	***Crygn***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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



Technical routes

- The *Crygn* gene has 2 transcripts.According to the structure of *Crygn* gene, exon2 and exon3 of *Crygn*-201 (ENSMUST00000047119.4) transcript are recommended as the knockout region.The region contains 395bp(most of) coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Crygn* 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/6J Gpt 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 or cell types.

Notice

- According to the existing MGI data, mice homozygous for a conditional allele activated in rhombomeres 3 and 5 derived neurons exhibit reduced MNTB volumne between P4 and P25 with increase in the amplitude of wave IV ABR.
- The *Crygn* gene is located on the Chr5. 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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Crygn crystallin, gamma N [*Mus musculus* (house mouse)]

Gene ID: 214301, updated on 26-Jun-2020

Summary

Official Symbol	Crygn provided by MGI
Official Full Name	crystallin, gamma N provided by MGI
Primary source	MGI:MGI:2449167
See related	Ensembl:ENSMUSG00000038135
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
Expression	Low expression observed in reference dataset See more
Orthologs	human all

Transcript information（Ensembl）

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

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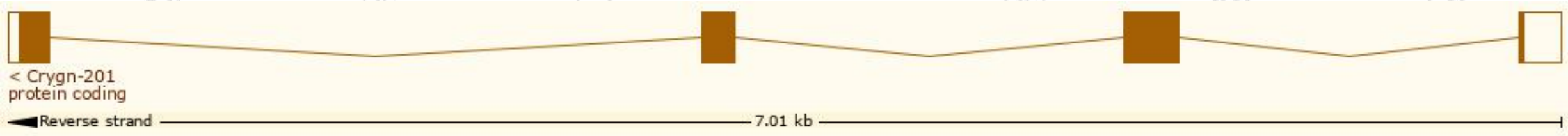
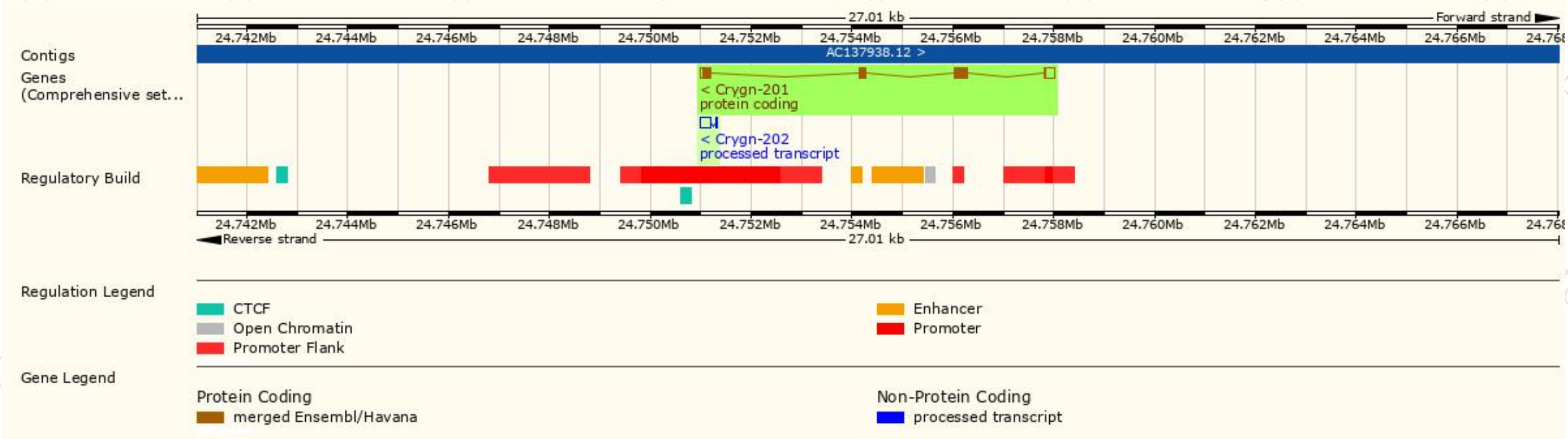
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Crygn-201	ENSMUST00000047119.4	768	183aa	Protein coding	CCDS19129	Q8VHL5	TSL:1 GENCODE basic APPRIS P1
Crygn-202	ENSMUST00000123386.1	221	No protein	Processed transcript	-	-	TSL:3

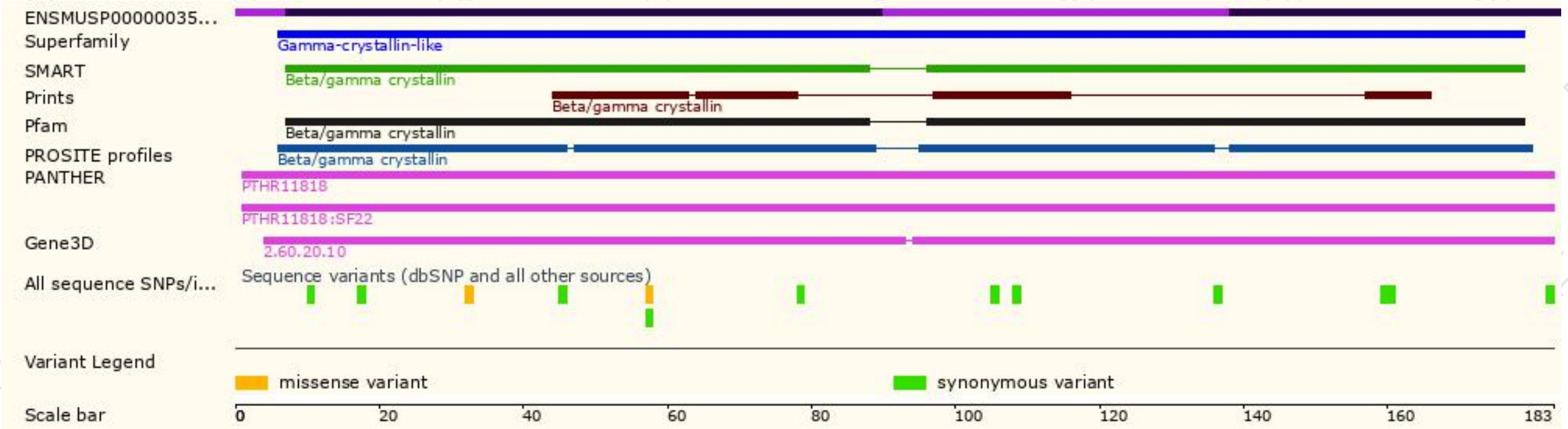
The strategy is based on the design of *Crygn*-201 transcript,The transcription is shown below:



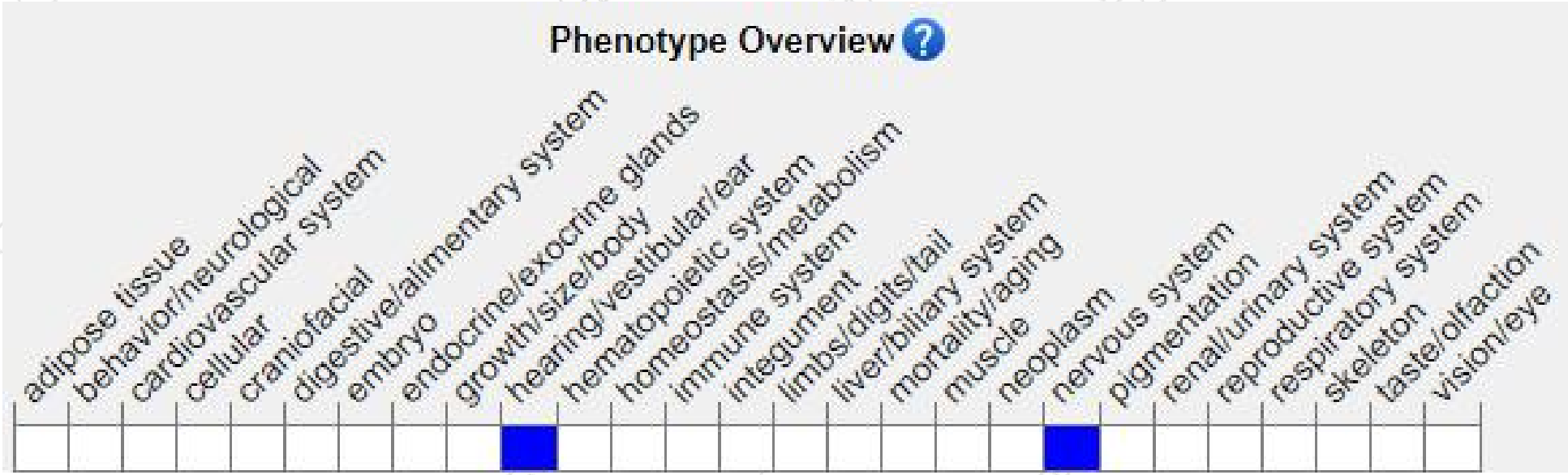
Genomic location distribution



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 conditional allele activated in rhombomeres 3 and 5 derived neurons exhibit reduced MNTB volumne between P4 and P25 with increase in the amplitude of wave IV ABR.

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

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