Ntng2 Cas9-CKO Strategy

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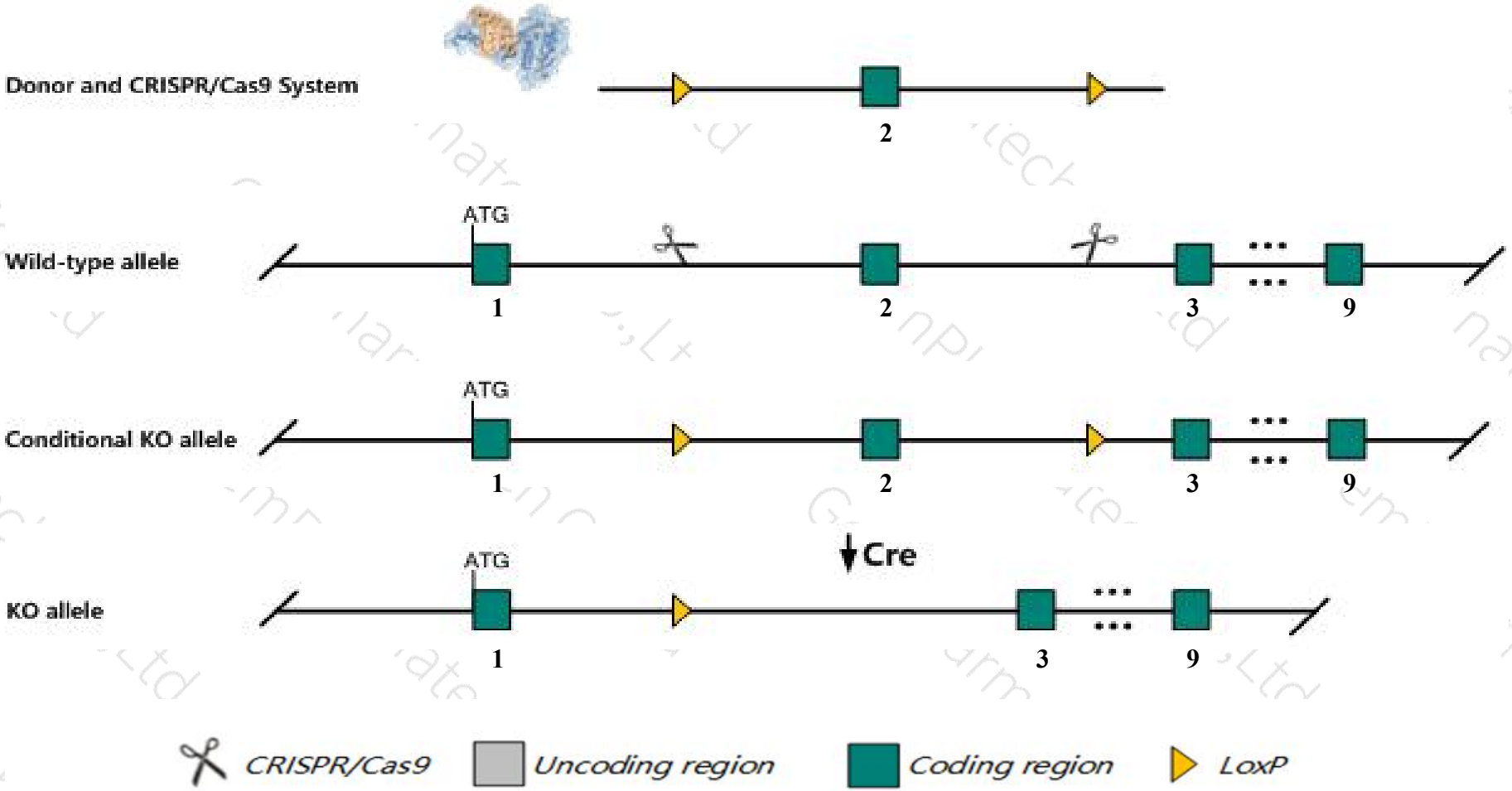
Project Overview

Project Name	***Ntng2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ntng2* gene. The schematic diagram is as follows:



Technical routes

- The *Ntng2* gene has 11 transcripts. According to the structure of *Ntng2* gene, exon2 of *Ntng2-201* (ENSMUST00000048455.8) transcript is recommended as the knockout region.The region contains 644bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Ntng2* 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.

Notice

- According to the existing MGI data,Mice homozygous for a null allele exhibit an absence of startle reflex and abnormal ABR amplitude.

- The *Ntng2* gene is located on the Chr2. 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）

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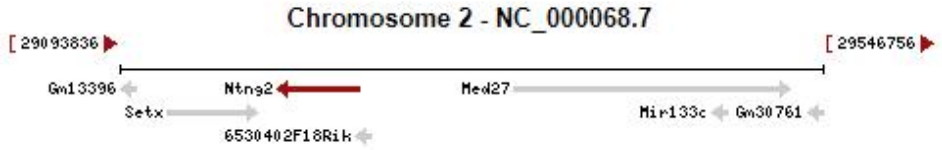
Ntng2 netrin G2 [*Mus musculus* (house mouse)]

Gene ID: 171171, updated on 10-Oct-2019

Summary

Official Symbol	Ntng2 provided by MGI
Official Full Name	netrin G2 provided by MGI
Primary source	MGI:MGI:2159341
See related	Ensembl:ENSMUSG00000035513
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Lmnt2; 2610016D08Rik
Summary	The protein encoded by this gene belongs to a subclass of the netrin family called netrin-G proteins. Unlike classic netrins, which act as diffusible chemoattractants, netrin-Gs are glycosylphosphatidylinositol-anchored membrane proteins that interact with specific transmembrane proteins. In mouse, this gene is preferentially expressed in the cerebral cortex, habenular nucleus and superior colliculus. Knockout mutant mice display a lack of behavioral startle in response to acoustic stimuli. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Apr 2015]
Expression	Broad expression in adrenal adult (RPKM 73.1), ovary adult (RPKM 25.0) and 20 other tissues See more
Orthologs	human all

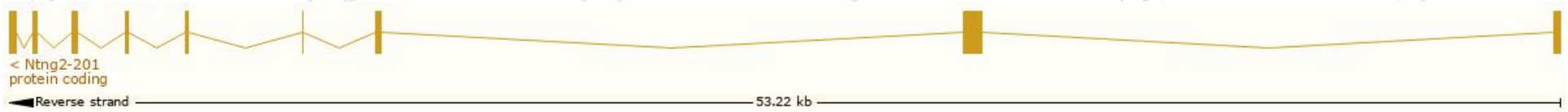


Transcript information（Ensembl）

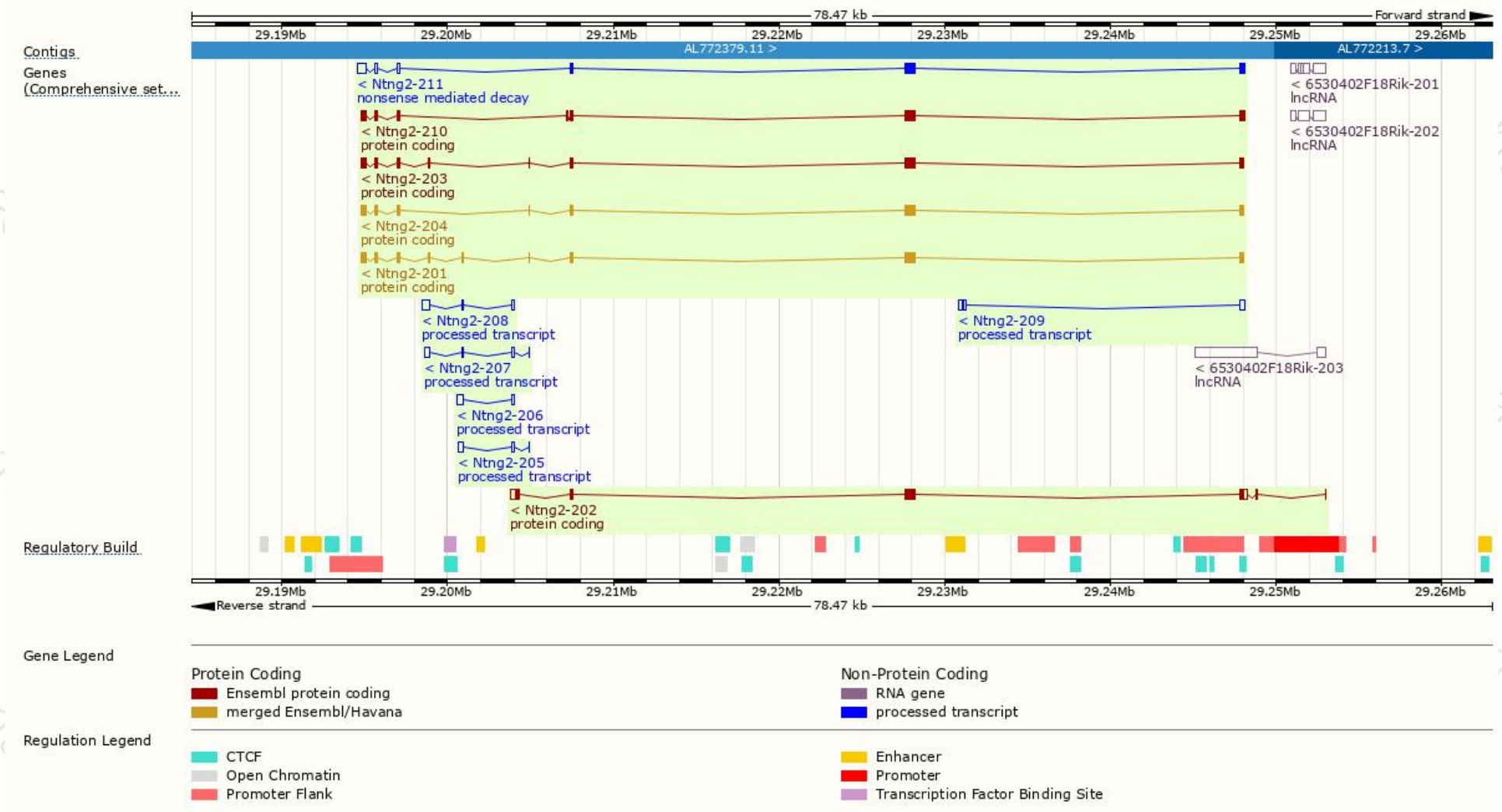
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ntng2-201	ENSMUST00000048455.8	1770	589aa	Protein coding	CCDS15852	Q8R4F1	TSL:1 GENCODE basic APPRIS P4
Ntng2-203	ENSMUST00000091153.8	1695	564aa	Protein coding	CCDS84499	Q8R4F1	TSL:1 GENCODE basic APPRIS ALT2
Ntng2-204	ENSMUST00000102873.7	1593	530aa	Protein coding	CCDS15851	A2AKW8 Q8R4F1	TSL:1 GENCODE basic APPRIS ALT2
Ntng2-202	ENSMUST00000071201.4	1881	430aa	Protein coding	-	A2AKX2	TSL:5 GENCODE basic
Ntng2-210	ENSMUST00000177689.7	1679	530aa	Protein coding	-	J3QNA0	TSL:5 GENCODE basic APPRIS ALT2
Ntng2-211	ENSMUST00000183583.7	1957	368aa	Nonsense mediated decay	-	V9GX85	TSL:5
Ntng2-209	ENSMUST00000149096.1	690	No protein	Processed transcript	-	-	TSL:3
Ntng2-208	ENSMUST00000147897.7	678	No protein	Processed transcript	-	-	TSL:5
Ntng2-206	ENSMUST00000143194.1	541	No protein	Processed transcript	-	-	TSL:3
Ntng2-207	ENSMUST00000147274.7	513	No protein	Processed transcript	-	-	TSL:3
Ntng2-205	ENSMUST00000125765.1	436	No protein	Processed transcript	-	-	TSL:2

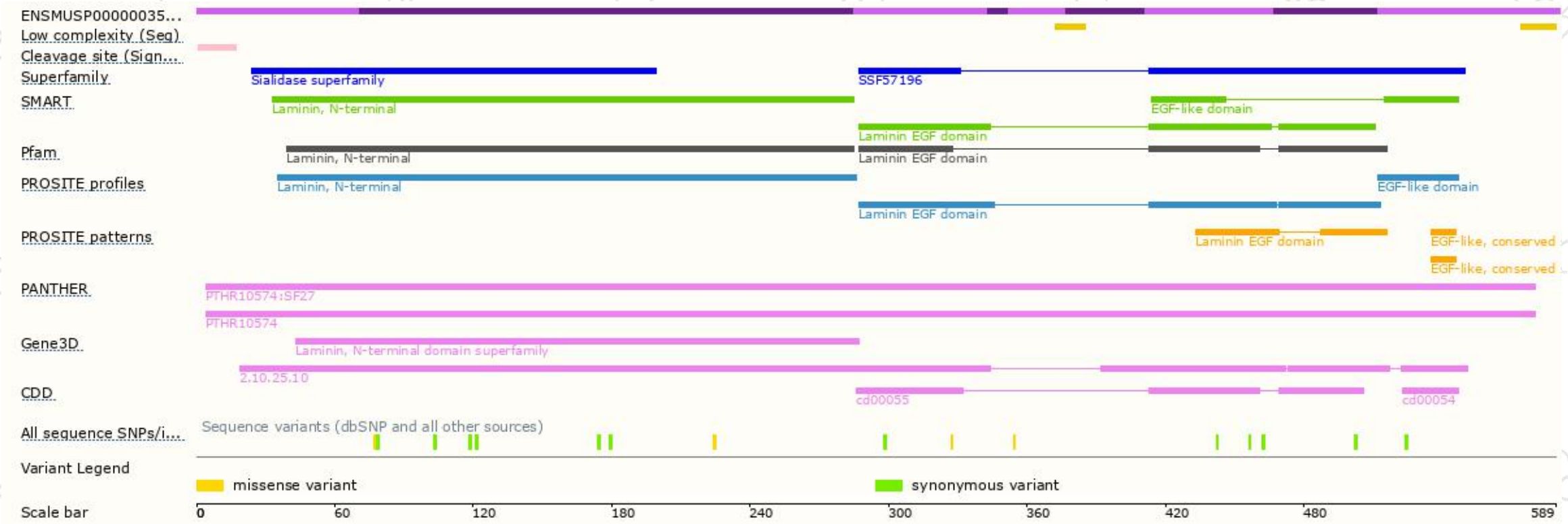
The strategy is based on the design of *Ntng2-201* transcript,The transcription is shown below



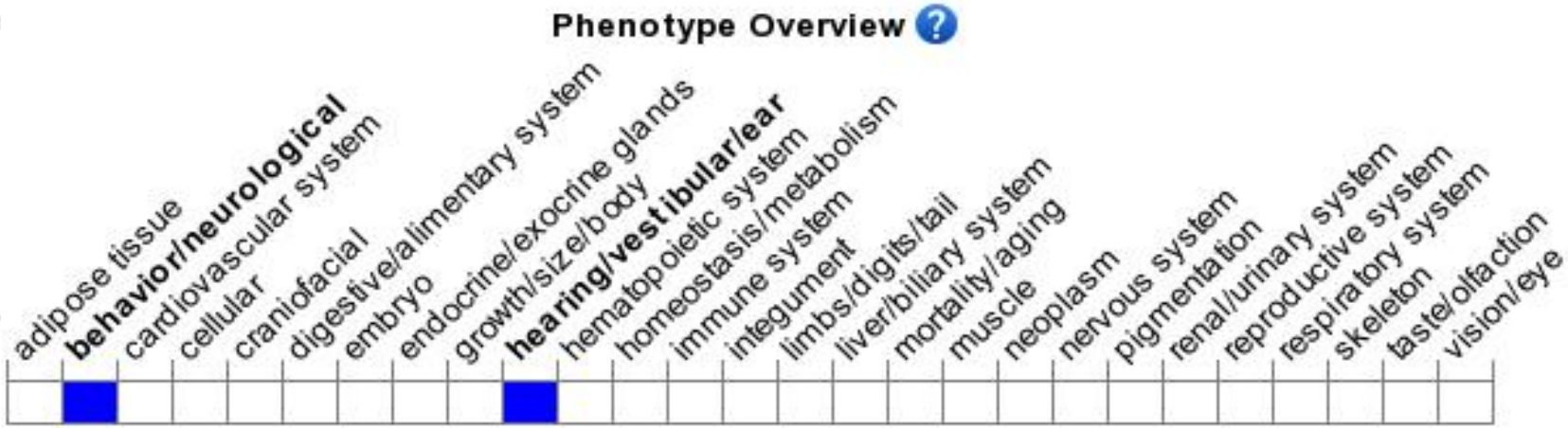
Genomic location distribution



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 null allele exhibit an absence of startle reflex and abnormal ABR amplitude.

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

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