

EGFP-P2A-Camk2a-cas9-ki Mouse Model Strategy -CRISPR/Cas9 technology

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Project Overview

Project Name	EGFP-P2A-Camk2a
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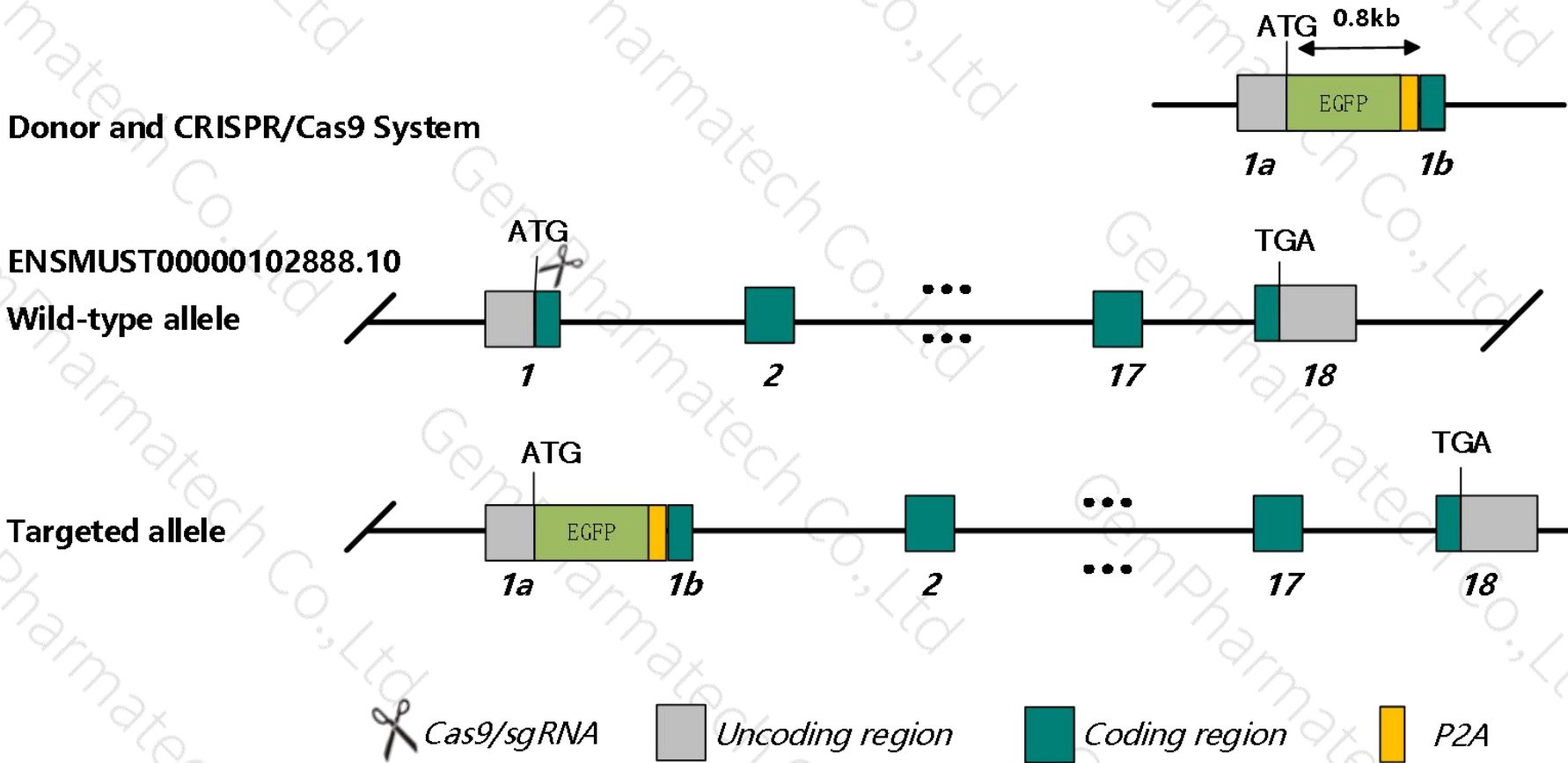
Project type	Cas9-KI
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Strain background	C57BL/6JGpt
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Technical routes

- The mouse *Camk2a* gene has 7 transcripts.
- According to the structure of *Camk2a* gene, the element EGFP-P2A will be inserted at after the translation start site of *Camk2a*-203(ENSMUST00000102888.10), the length of inserted fragment is about 0.8kb.
- In this project, *Camk2a* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: In vitro, sgRNA and donor vectors were constructed. Cas9, sgRNA and donor were injected into the fertilized eggs of C57BL/6JGpt mice for homologous recombination, and obtained positive F0 mice identified by PCR, sequencing analysis. The stable inheritable positive F1 mice model was obtained by mating F0 mice with C57BL/6JGpt mice.

This model uses CRISPR/Cas9 technology to edit the *Camk2a* gene and the schematic diagram is as follow:



- According to the existing MGI data, Homozygous targeted mutants display deficient long-term hippocampal potentiation (LTP) and specific impairment in spatial learning; heterozygotes show decreased fear response and increased defensive aggression, which is more pronounced in homozygotes.
- It is necessary to introduce 1-2 synonymous mutation in exon1.
- The effect of this strategy on *Camk2a*-202、204、207 transcripts are unknown.
- The P2A-linked genes will be transcribed together and then be translated two protein separately, the front protein will carry a polypeptide translated by P2A sequence (21 aa), while the back gene will carry a proline translated by P2A (Pro)..
- Mouse *Camk2a* gene is located on Chr18. 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 Chr18, 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 5'UTR and the gene coding region may affect the expression of endogenous and foreign genes. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Gene information (NCBI)

Camk2a calcium/calmodulin-dependent protein kinase II alpha [*Mus musculus* (house mouse)]

Gene ID: 12322, updated on 26-Oct-2020

Summary



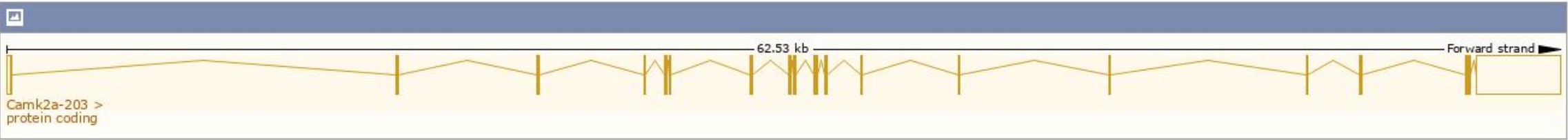
Official Symbol	Camk2a provided by MGI
Official Full Name	calcium/calmodulin-dependent protein kinase II alpha provided by MGI
Primary source	MGI:MGI:88256
See related	Ensembl:ENSMUSG00000024617
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CaMKII; R74975; mKIAA0968
Expression	Biased expression in cortex adult (RPKM 365.9) and frontal lobe adult (RPKM 310.4) See more
Orthologs	human all

Transcript information (Ensembl)

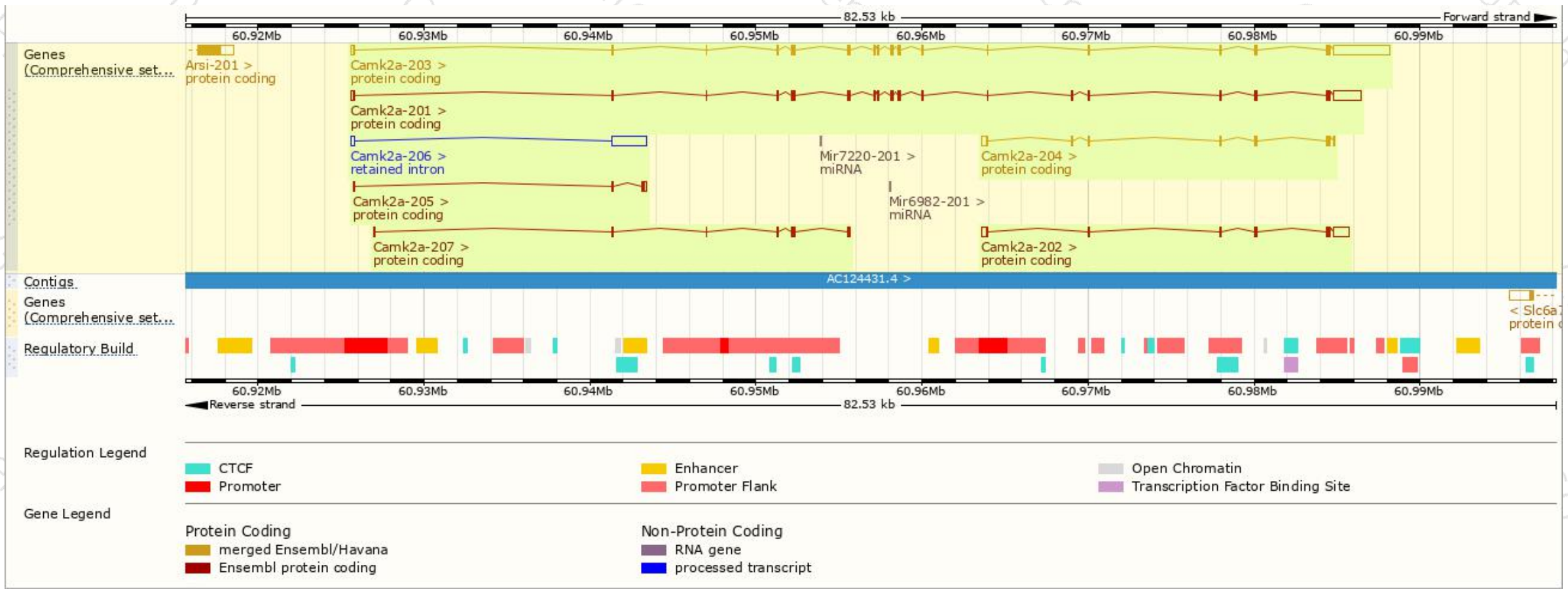
The gene has 7 transcripts,all transcripts are shown below:

Show/hide columns (1 hidden)							Filter	
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Camk2a-203	ENSMUST00000102888.9	4970	478aa	Protein coding	CCDS29276	P11798	TSL:1	GENCODE basic APPRIS P2
Camk2a-202	ENSMUST00000039904.6	1734	189aa	Protein coding	CCDS70898	F8WHB5	TSL:5	GENCODE basic
Camk2a-204	ENSMUST00000115295.8	979	200aa	Protein coding	CCDS29277	P11798	TSL:1	GENCODE basic
Camk2a-201	ENSMUST00000025519.10	3244	489aa	Protein coding	-	F8WIS9	TSL:5	GENCODE basic APPRIS ALT1
Camk2a-207	ENSMUST00000137805.2	530	177aa	Protein coding	-	F6WHR9	CDS 5' and 3' incomplete	TSL:3
Camk2a-205	ENSMUST00000115297.7	499	108aa	Protein coding	-	D3Z7K9	TSL:2	GENCODE basic
Camk2a-206	ENSMUST00000134496.1	2301	No protein	Retained intron	-	-	TSL:2	

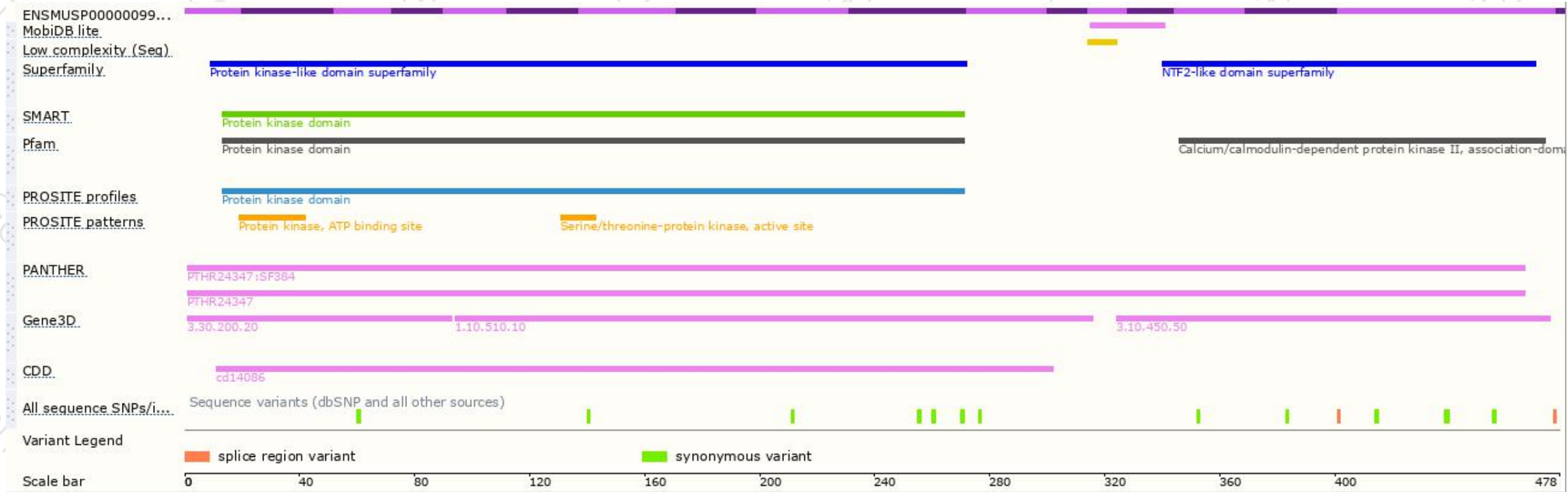
The strategy is based on the design of *Camk2a-203* transcript,the transcription is shown below:



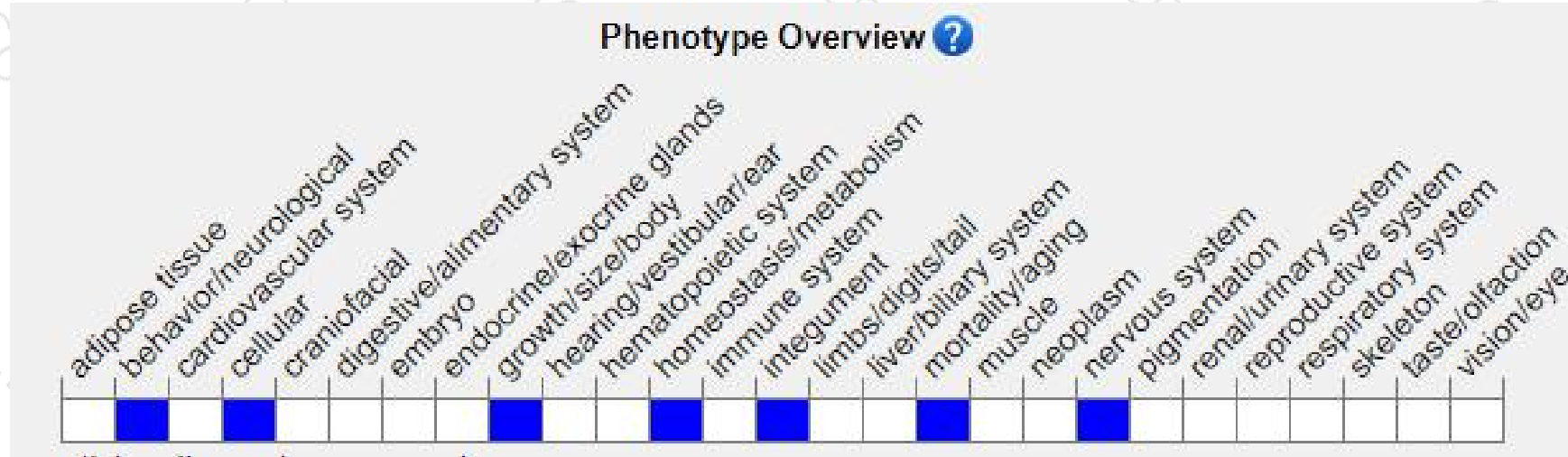
Genomic location distribution



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/>).

Homozygous targeted mutants display deficient long-term hippocampal potentiation (LTP) and specific impairment in spatial learning; heterozygotes show decreased fear response and increased defensive aggression, which is more pronounced in homozygotes.

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

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