

***Sox1-P2A-iCre-P2A-EGFP* Cas9-KI Strategy**

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Reviewer

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Design Date:

2020-11-23

Project Overview

Project Name ***Sox1-P2A-iCre-P2A-EGFP***

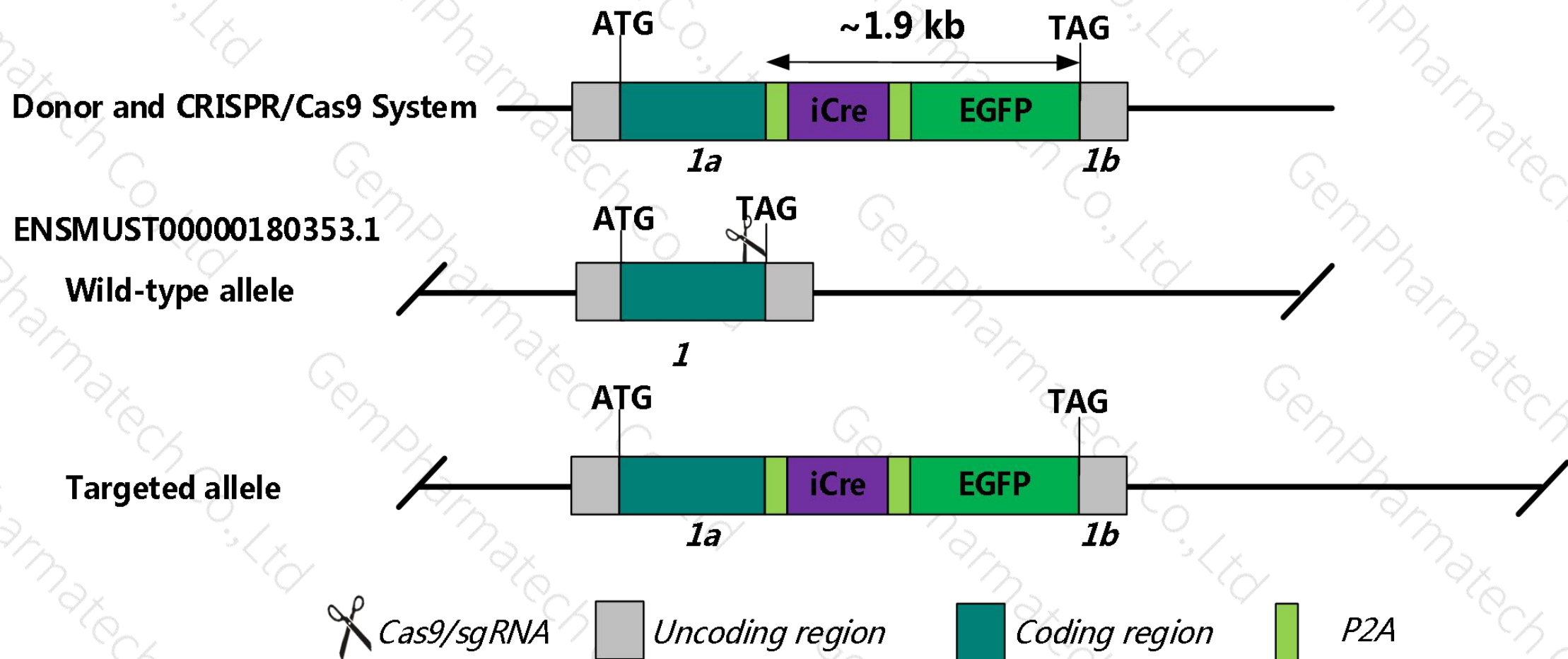
Project type **Cas9-KI**

Strain background **C57BL/6JGpt**

- The *Sox1* gene has 1 transcript. According to the structure of *Sox1* gene, *Sox1-201*(ENSMUST00000180353.1) is selected for presentation of the recommended strategy.
- *Sox1-201* gene has 1 exon, with the ATG start codon and TAG stop codon in exon1.
- We make *Sox1-P2A-iCre-P2A-EGFP* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near stop codon(TAG) of *Sox1* gene, and create a DSB(doublestrand break). Such breaks will be repaired, and result in *P2A-iCre-P2A-EGFP* before stop codon(TAG) of *Sox1* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Sox1* gene. The schematic diagram is as follows:



- According to the existing MGI data, homozygous null mutants exhibit lens opacity associated with a lack of gamma crystallin expression, microphthalmia, episodic seizures, sexual dysfunction, impaired maternal nurturing, and reduced lifespan.
- There will be 1 to 2 amino acid synonymous mutation in exon1 of *Sox1* gene in this strategy.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- Insertion of *P2A-iCre-P2A-EGFP* may destroy the expression of *Gm25239-201* and *Sox1ot* gene.
- The *Sox1* gene is located on the Chr8. If the knockin mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- 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 complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Coding Sequence of Codon-Optimized *Cre* Gene (*iCre*:1056 bp)

ATGGTGCCCAAGAAGAAGAGAGGAAAGTCTCCAACCTGCTGACTGTGCACCAAAACCTGCCTGCCCTCCCTGTGGATGCCA
CCTCTGATGAAGTCAGGAAGAACCTGATGGACATGTTTCAGGGACAGGCAGGCCTTCTCTGAACACACCTGGAAGATGCT
CCTGTCTGTGTGCAGATCCTGGGCTGCCTGGTGCAAGCTGAACAACAGGAAATGGTTCCCTGCTGAACCTGAGGATGTG
AGGGACTACCTCCTGTACCTGCAAGCCAGAGGCCTGGCTGTGAAGACCATCCAACAGCACCTGGGCCAGCTCAACATG
CTGCACAGGAGATCTGGCCTGCCTCGCCCTTCTGACTCCAATGCTGTGTCCCTGGTGATGAGGAGAATCAGAAAGGAGA
ATGTGGATGCTGGGGAGAGAGCCAAGCAGGCCCTGGCCTTTGAACGCACTGACTTTGACCAAGTCAGATCCCTGATGG
AGAACTCTGACAGATGCCAGGACATCAGGAACCTGGCCTTCCTGGGCATTGCCTACAACACCCTGCTGCGCATTGCCGA
AATTGCCAGAATCAGAGTGAAGGACATCTCCCGCACCGATGGTGGGAGAATGCTGATCCACATTGGCAGGACCAAGACC
CTGGTGTCCACAGCTGGTGTGGAGAAGGCCCTGTCCCTGGGGGTTACCAAGCTGGTGGAGAGATGGATCTCTGTGTCTG
GTGTGGCTGATGACCCCAACAACACTACCTGTTCTGCCGGGTCAGAAAGAATGGTGTGGCTGCCCCTTCTGCCACCTCCCAA
CTGTCCACCCGGGCCCTGGAAGGGATCTTTGAGGCCACCCACCGCCTGATCTATGGTGCCAAGGATGACTCTGGGCAGA
GATACCTGGCCTGGTCTGGCCACTCTGCCAGAGTGGGTGCTGCCAGGGACATGGCCAGGGCTGGTGTGTCCATCCCTGA
AATCATGCAGGCTGGTGGCTGGACCAATGTGAACATTGTGATGAACTACATCAGAAACCTGGACTCTGAGACTGGGGCC
ATGGTGAGGCTGCTCGAGGATGGGGACTGA

Shimshek DR, Kim J, Hübner MR, Spergel DJ. Codon-improved Cre recombinase (iCre) expression in the mouse.
Genesis.2002 Jan.32(1):19-26.

Gene information (NCBI)

Sox1 SRY (sex determining region Y)-box 1 [*Mus musculus* (house mouse)]

Gene ID: 20664, updated on 17-Nov-2020

Summary

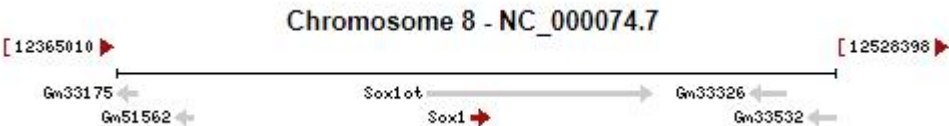
Official Symbol	Sox1 provided by MGI
Official Full Name	SRY (sex determining region Y)-box 1 provided by MGI
Primary source	MGI:MGI:98357
See related	Ensembl:ENSMUSG00000096014
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Sox; Sox-1; BB176347
Orthologs	human all

Genomic context

Location: 8 A1.1; 8 5.73 cM

See Sox1 in [Genome Data Viewer](#)

Exon count: 1

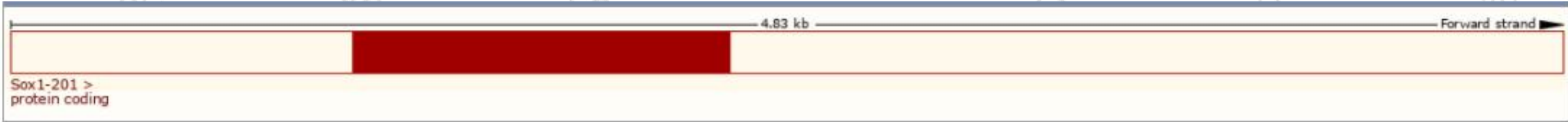


Transcript information (Ensembl)

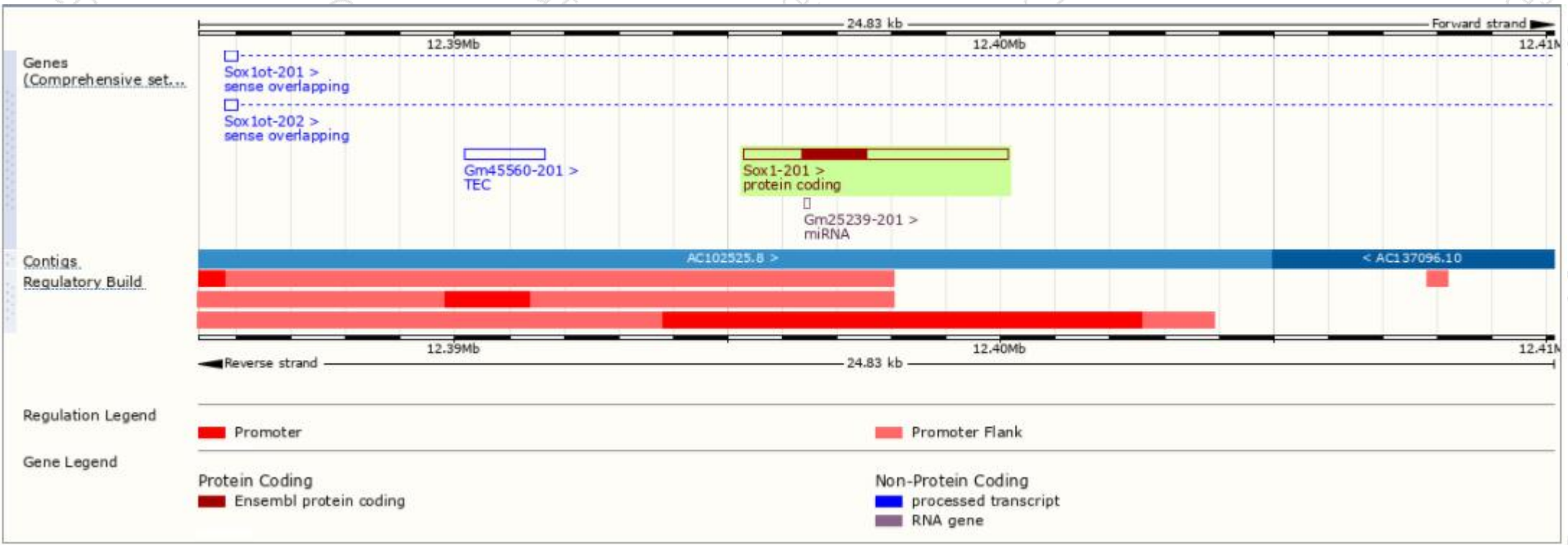
The gene has 1 transcript, and all transcript is shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags		
Sox1-201	ENSMUST00000180353.1	4832	391aa	Protein coding	CCDS57605	P53783	TSL:NA	GENCODE basic	APPRIS P1

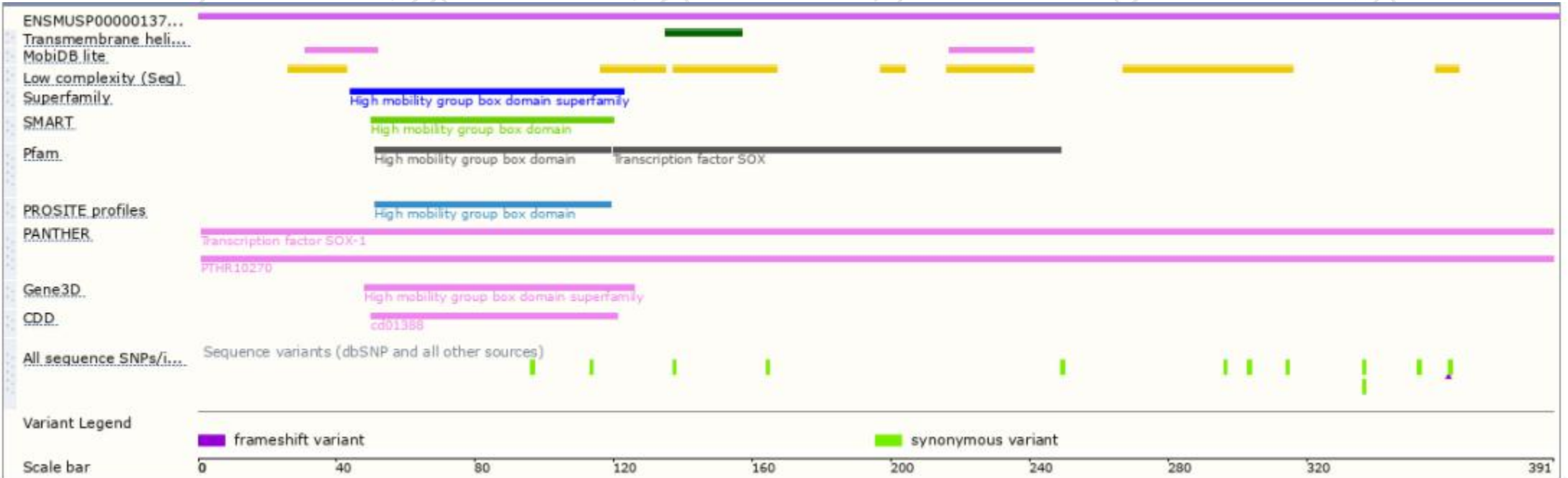
The strategy is based on the design of *Sox1-201* transcript,the transcription is shown below



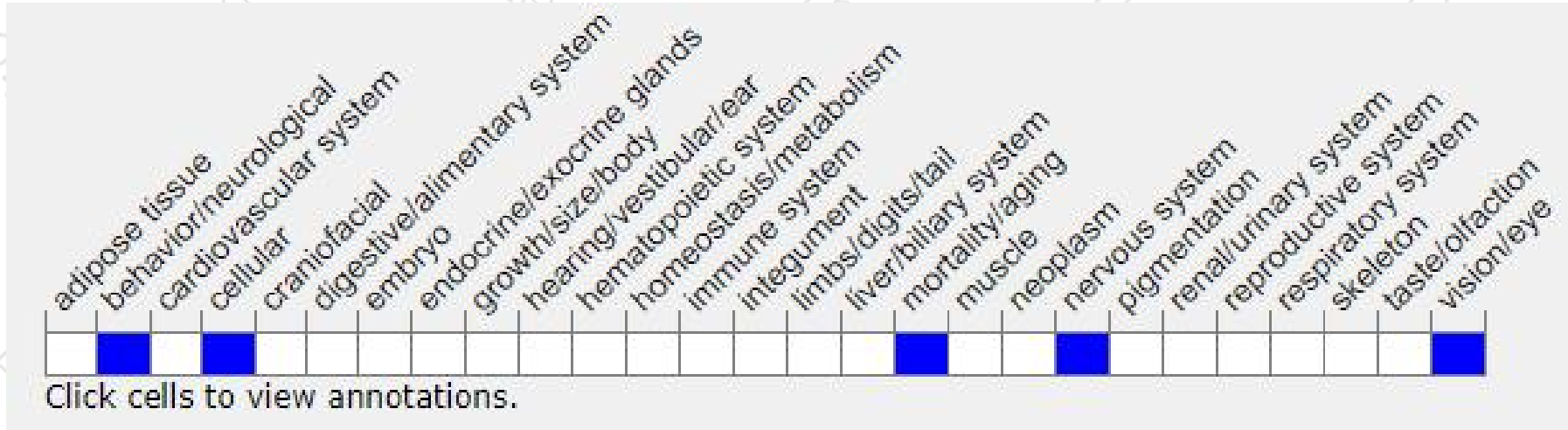
Genomic location distribution



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/marker/MGI:98357>) .

Homozygous null mutants exhibit lens opacity associated with a lack of gamma crystallin expression, microphthalmia, episodic seizures, sexual dysfunction, impaired maternal nurturing, and reduced lifespan.

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