Sox2 Cas9-CKO *Strategy*

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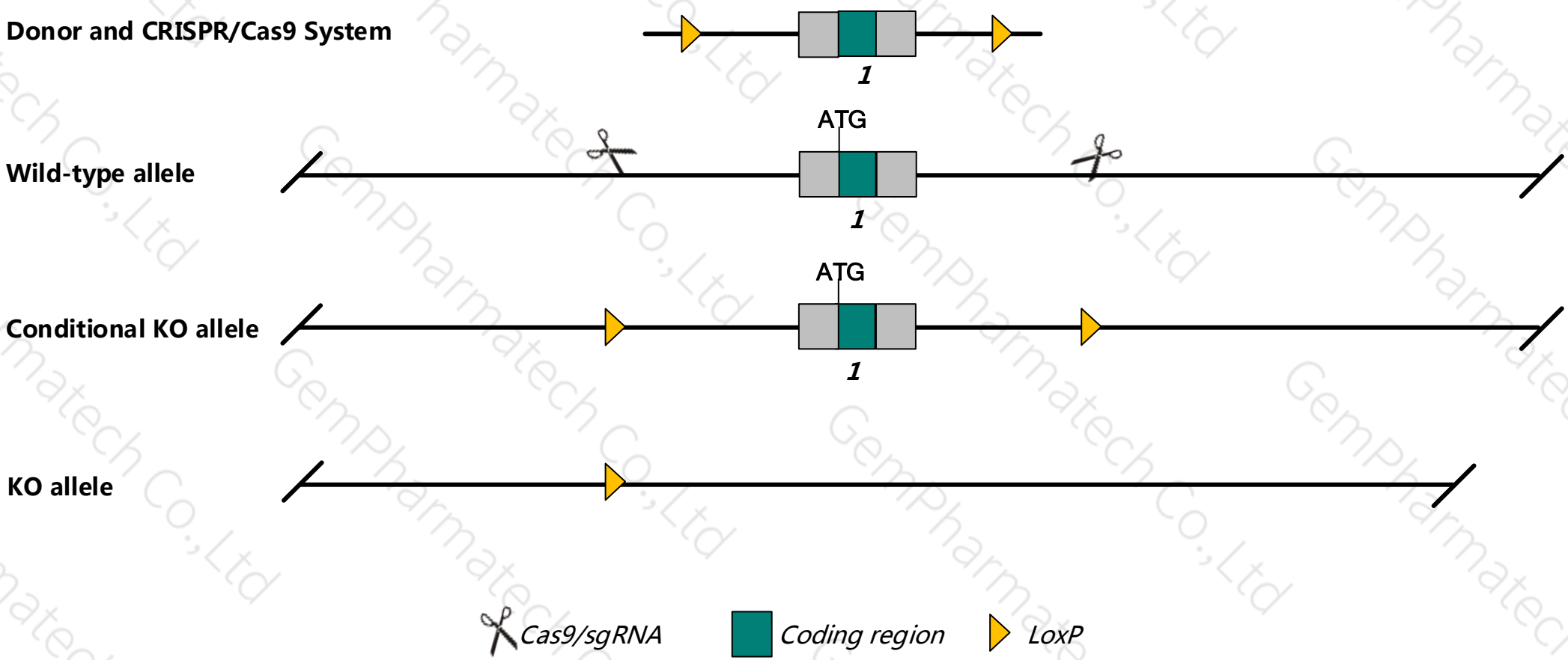
Project Overview

Project Name	*Sox2*
Project type	Cas9-CKO
Animal background	C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Sox2* gene. The schematic diagram is as follows:



Technical routes

- The *Sox2* gene has 1 transcript, According to the structure of *Sox2* gene, exon1 of *Sox2-201* transcript is recommended as the knockout region.The region contains the all of coding seqence.Knock out the region,result in destruction of protein.

- This project uses CRISPR/Cas9 technology to modify *Sox2* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed, Cas9, sgRNA and donor were microinjected into fertilized eggs of C57BL/6JGpt mice and homologous recombination was carried out to obtain F0 mice. A stable and hereditary F1 generation mouse model was obtained by mating F0 generation mice with C57BL/6JGpt mice which were confirmed positive by PCR-sequencing.

- 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.

Notice

- According to the existing MGI data，Homozygotes for targeted null mutations implant but fail to develop an egg cylinder or epiblast, and die shortly thereafter. Other mutations that affect only regulatory elements show circling behavior and deafness, inner ear defects, and a yellow coat color.
- Insertion of the 3-terminal loxp may affect the 5-terminal regulation of the Gm27611 gene.
- Knocking out the gene of interest also knocks out part of the intron of the Sox2ot gene.
- The *Sox2* gene is located in the Chr3. If the knockout mice are mixed with other mice, two target genes are avoided on the same chromosome as possible, otherwise the offspring of mice with double gene positive and homozygous gene knockout can not be obtained.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information （NCBI）

Sox2 SRY (sex determining region Y)-box 2 [*Mus musculus* (house mouse)]

Gene ID: 20674, updated on 9-Apr-2019

Summary

Official Symbol	Sox2 provided by MGI
Official Full Name	SRY (sex determining region Y)-box 2 provided by MGI
Primary source	MGI:MGI:98364
See related	Ensembl:ENSMUSG00000074637
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	Icc; ysb; Sox-2
Summary	This intronless gene encodes a member of the SRY-related HMG-box (SOX) family of transcription factors involved in the regulation of embryonic development and in the determination of cell fate. The product of this gene is required for stem-cell maintenance in the central nervous system, and also regulates gene expression in the stomach. Mutations in a similar gene in human have been associated with optic nerve hypoplasia and with syndromic microphthalmia, a severe form of structural eye malformation. This gene lies within an intron of another gene called SOX2 overlapping transcript (Sox2ot). [provided by RefSeq, Sep 2015]
Orthologs	human all

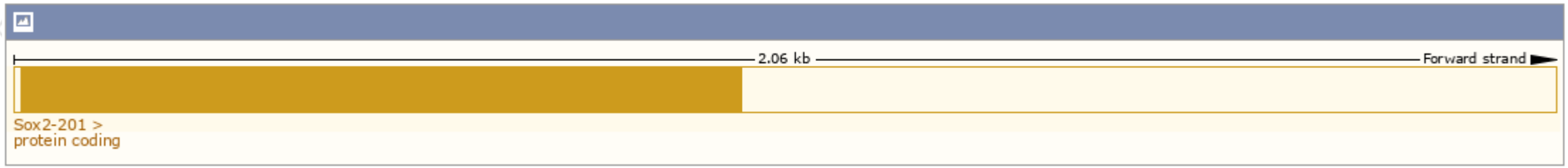
Transcript information （Ensembl）

The gene has 1 transcript, and all transcripts are shown below ：

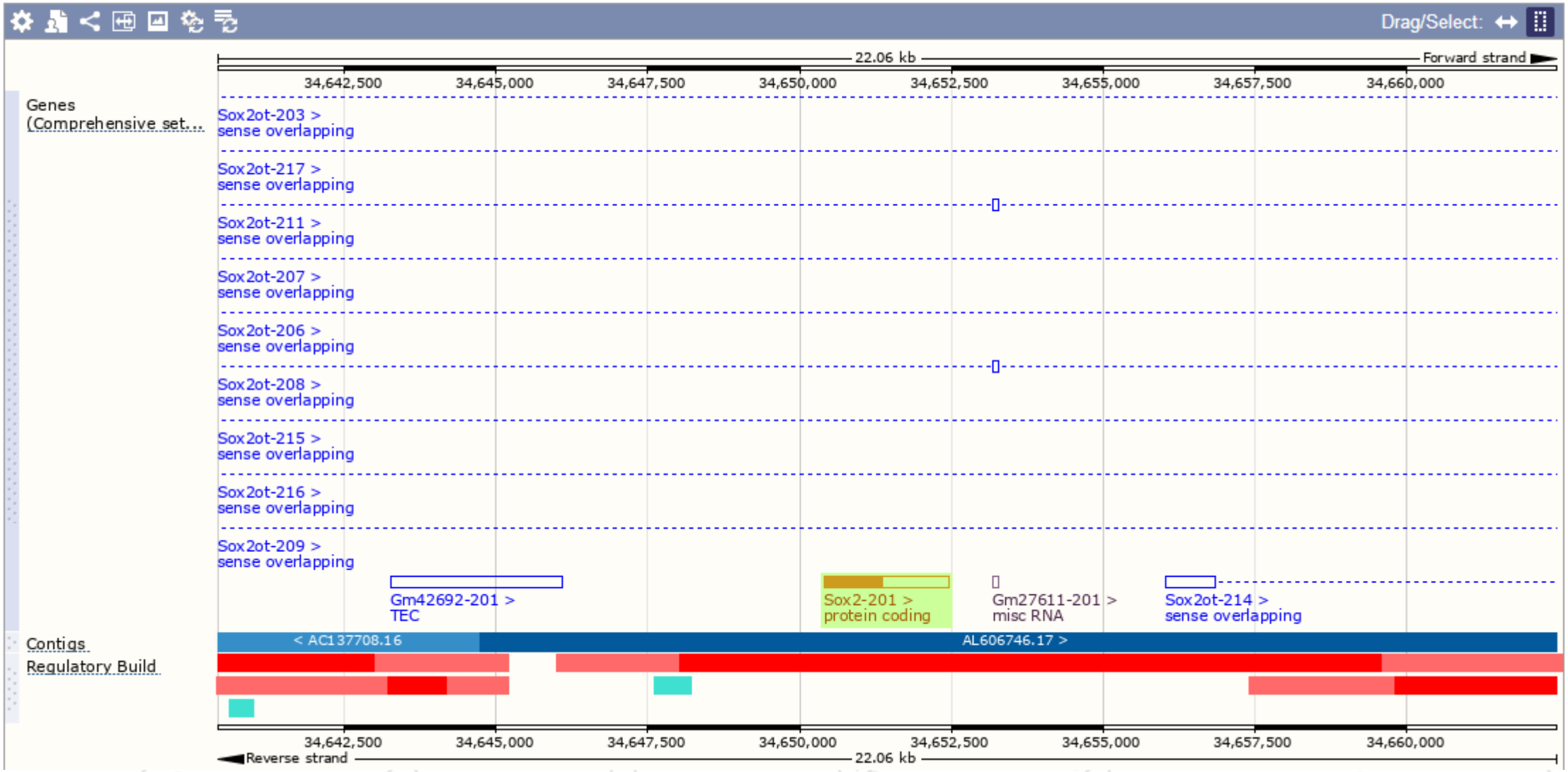
Show/hide columns (1 hidden) Filter

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sox2-201	ENSMUST00000099151.5	2057	319aa	Protein coding	CCDS38413	Q60I23	TSL:NA GENCODE basic APPRIS P1

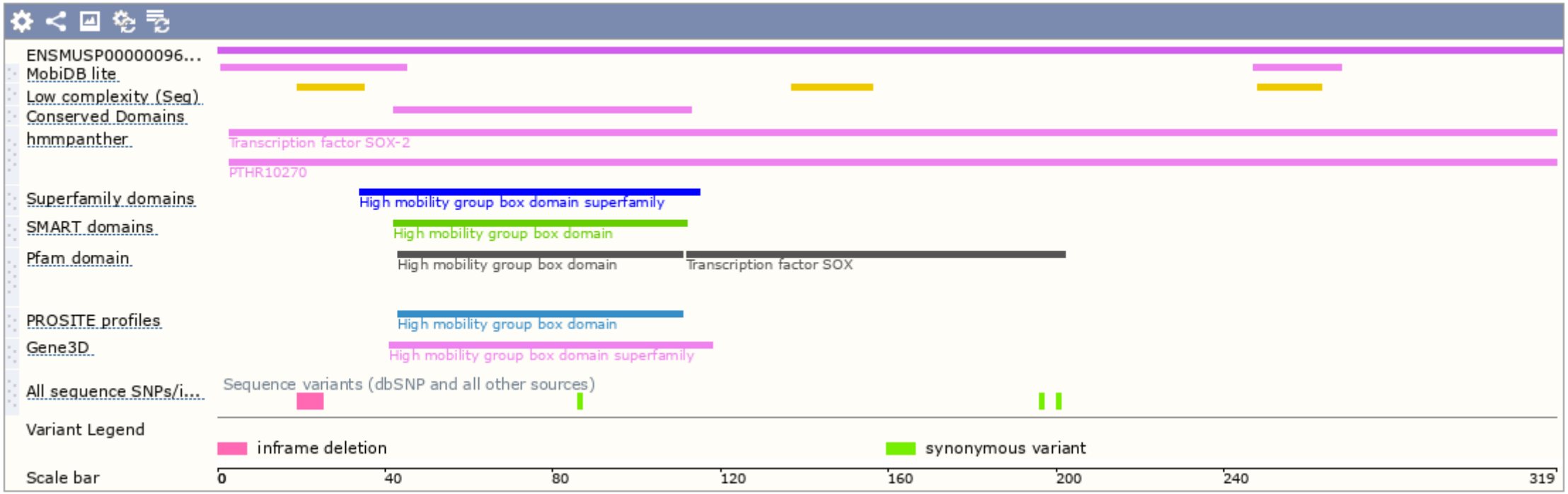
The strategy is based on the design of *Sox2-201* transcript,The transcription is shown below ：



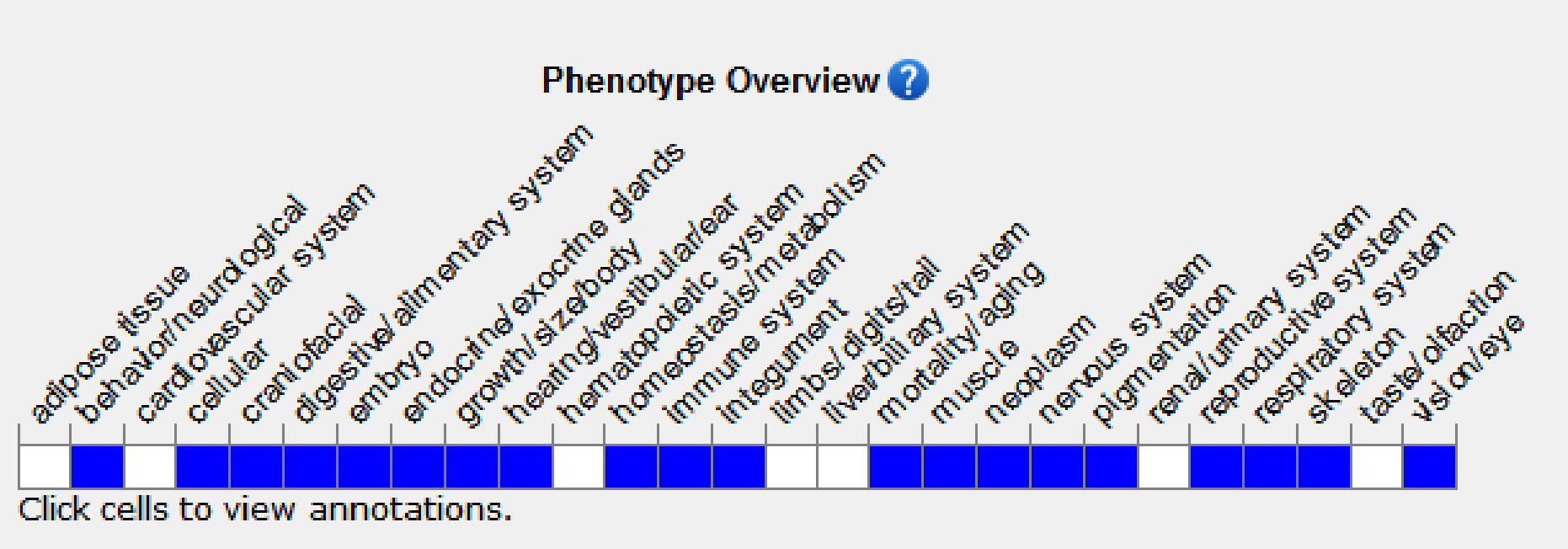
Genomic location distribution



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,Homozygotes for targeted null mutations implant but fail to develop an egg cylinder or epiblast, and die shortly thereafter. Other mutations that affect only regulatory elements show circling behavior and deafness, inner ear defects, and a yellow coat color.

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

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