Nkx2-1 Cas9-CKO *Strategy*

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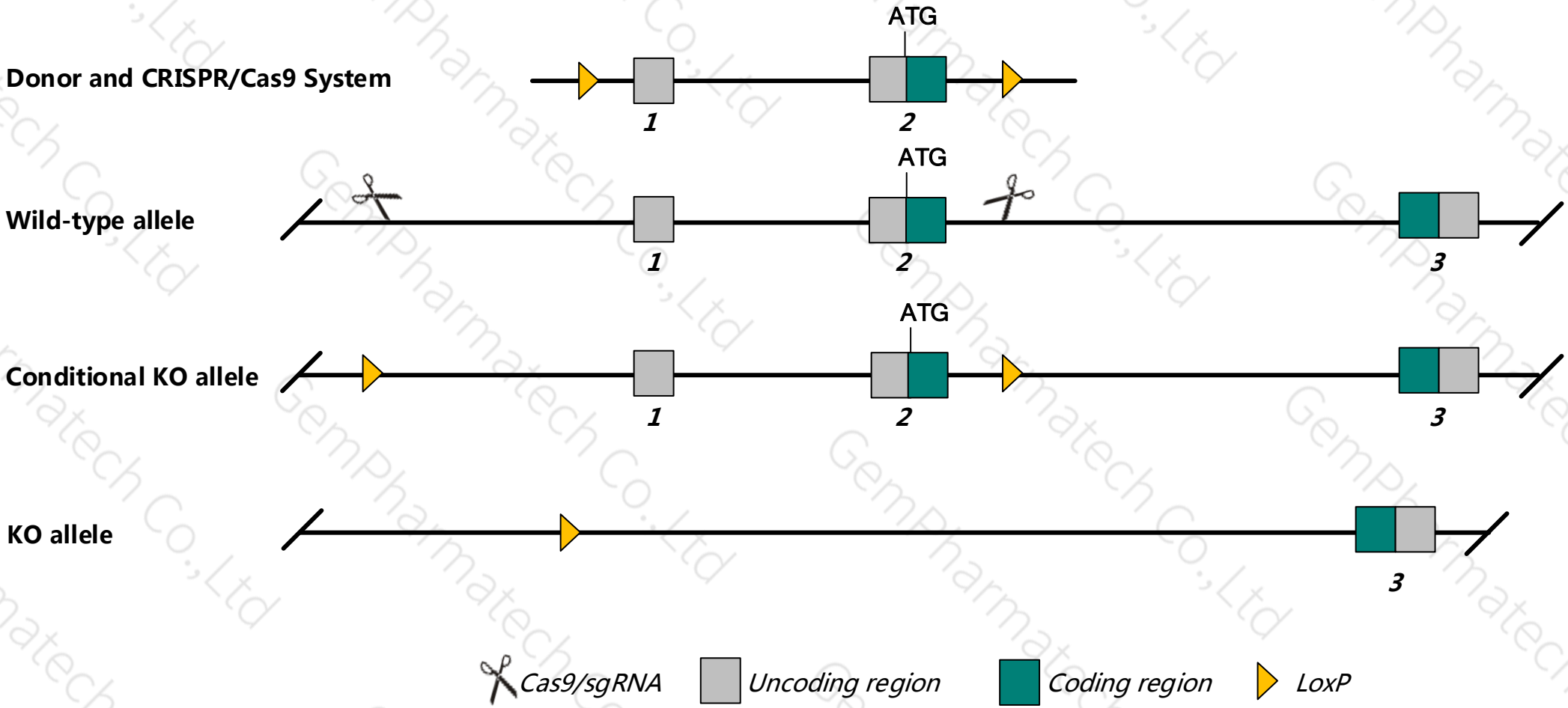
Project Overview

Project Name	***Nkx2-1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nkx2-1* gene. The schematic diagram is as follows:



Technical routes

- The *Nkx2-1* gene has 2 transcripts.According to the structure of *Nkx2-1* gene, exon1-exon2 of *Nkx2-1*-201 transcript is recommended as the knockout region.The region contains start codon ATG coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Nkx2-1* 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/6JGpt 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，Homozygotes for a targeted mutation have profoundly abnormal lungs and ventral forebrain defects, lack thyroids, pituitary gland, and tracheoesophageal septation, and die at birth from respiratory failure. Carriers show incoordination and high TSH.

- The KO region contains the functional region of the *Gm26973* gene.Knockout the region may affect its function of *Gm26973* gene.

- The *Nkx2-1* gene is located on the Chr12. 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information （NCBI）

Nkx2-1 NK2 homeobox 1 [*Mus musculus* (house mouse)]

Gene ID: 21869, updated on 28-May-2019

Summary

Official Symbol	Nkx2-1 provided by MGI
Official Full Name	NK2 homeobox 1 provided by MGI
Primary source	MGI:MGI:108067
See related	Ensembl:ENSMUSG00000001496
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	T/EBP; Titf1; Ttf-1; Nkx2.1; AV026640
Annotation information	Note: Ttf1 (GeneID 22130) and Nkx2-1 (GeneID 21869) loci share the Ttf1 symbol/alias in common. Ttf1 is a widely used alternative name for thyroid transcription factor 1 (Nkx2-1) conflicting with the official symbol for transcription termination factor, RNA polymerase I (Ttf1). [13 Feb 2013]
Expression	Biased expression in lung adult (RPKM 102.7), whole brain E14.5 (RPKM 11.8) and 1 other tissue See more
Orthologs	human all

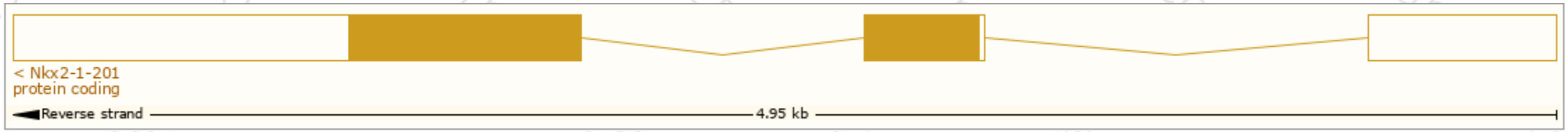
Transcript information（ Ensembl ）

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

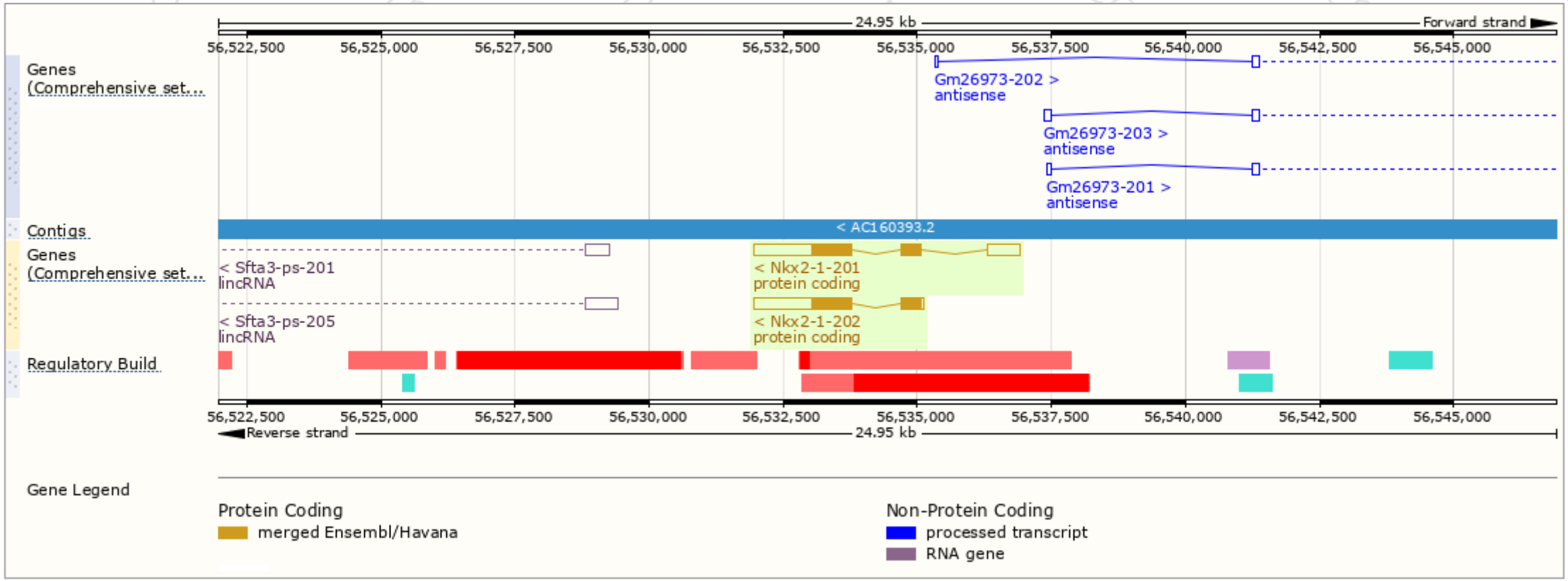
Show/hide columns (1 hidden) Filter

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nkx2-1-201	ENSMUST00000001536.8	2809	372aa	Protein coding	CCDS25922	P50220	TSL:1 GENCODE basic APPRIS P1
Nkx2-1-202	ENSMUST00000178477.8	2242	372aa	Protein coding	CCDS25922	P50220	TSL:1 GENCODE basic APPRIS P1

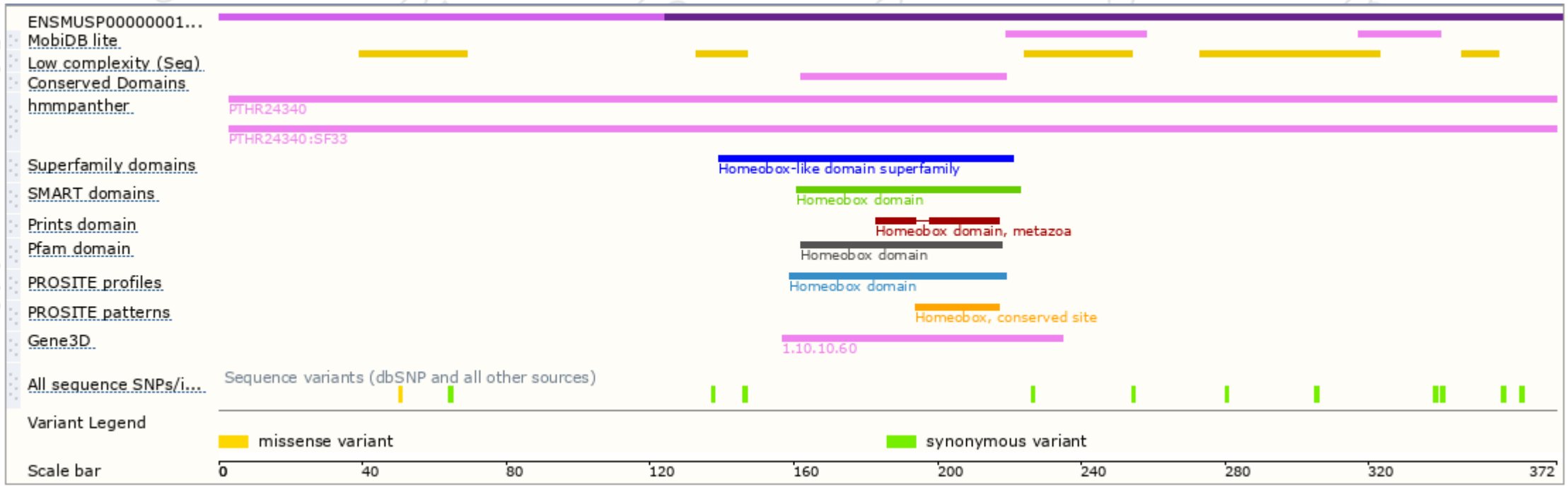
The strategy is based on the design of *Nkx2-1*-201 transcript,The transcription is shown below



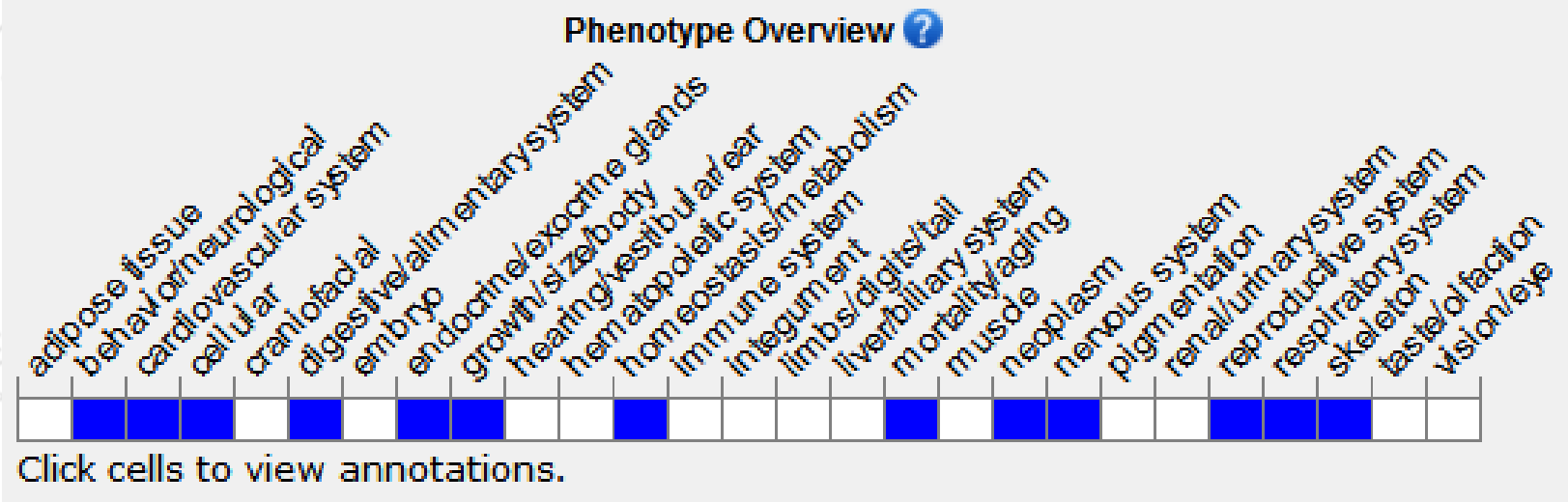
Genomic location distribution



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 a targeted mutation have profoundly abnormal lungs and ventral forebrain defects, lack thyroids, pituitary gland, and tracheoesophageal septation, and die at birth from respiratory failure. Carriers show incoordination and high TSH.

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

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