Dcx Cas9-CKO Strategy

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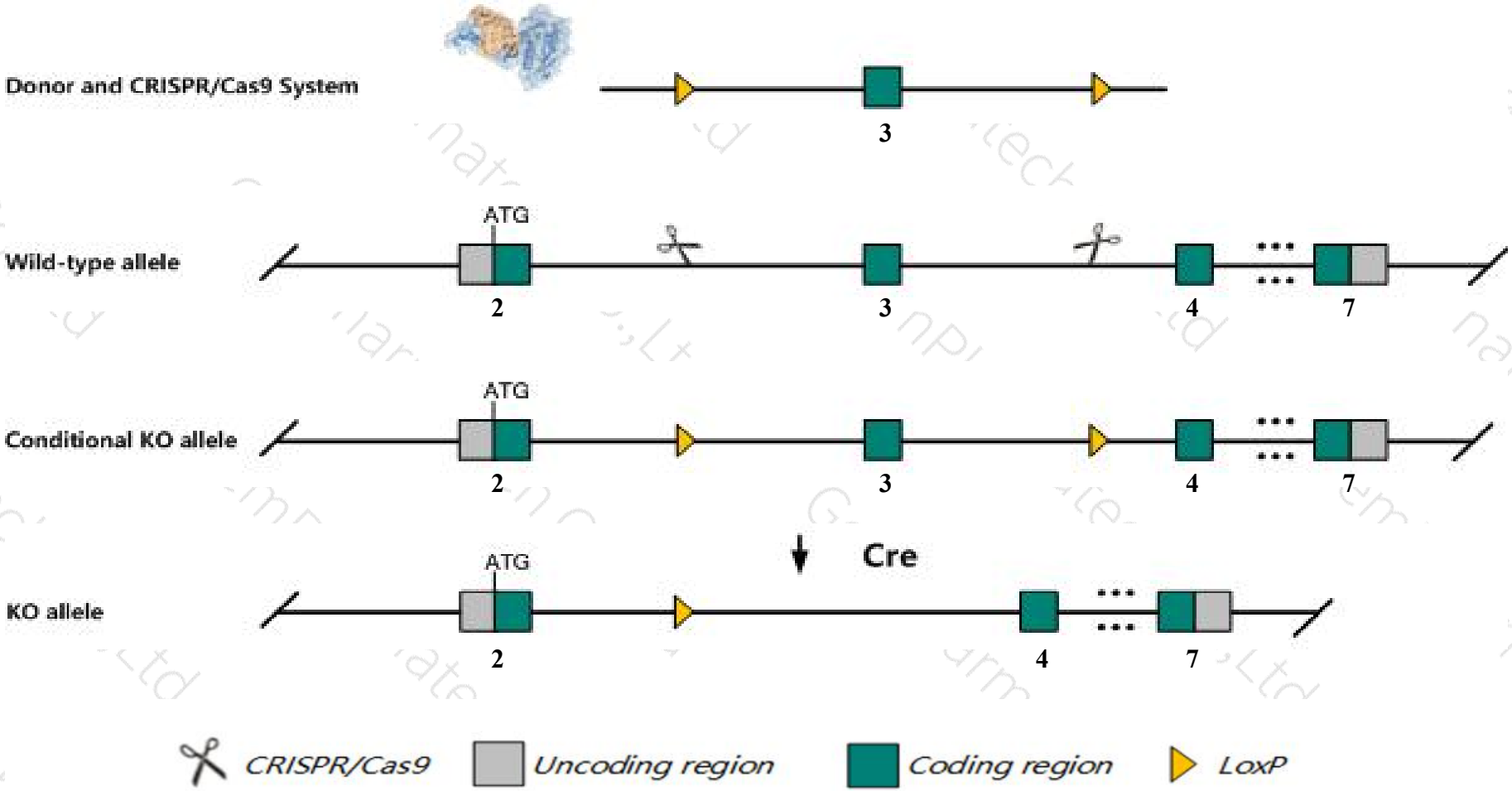
Project Overview

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

Conditional Knockout strategy

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



Technical routes

➢ The *Dcx* gene has 6 transcripts. According to the structure of *Dcx* gene, exon3 of *Dcx-201* (ENSMUST00000033642.9) transcript is recommended as the knockout region.The region contains 341bp coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Dcx* 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,Males hemizygous for a null allele are fertile but show branching and nucleokinesis defects in migrating interneurons. Males hemizygous for a reporter allele show severe postnatal lethality and variable fertility; both female and male mutants display hippocampal dyslamination and behavioral defects.

- The *Dcx* gene is located on the ChrX. 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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Dcx doublecortin [*Mus musculus* (house mouse)]

Gene ID: 13193, updated on 15-Oct-2019

Summary

Official Symbol	Dcx provided by MGI
Official Full Name	doublecortin provided by MGI
Primary source	MGI:MGI:1277171
See related	Ensembl:ENSMUSG00000031285
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	Dbct
Summary	This gene encodes a member of the doublecortin family. The protein encoded by this gene is a cytoplasmic protein and contains two doublecortin domains, which bind microtubules. In the developing cortex, cortical neurons must migrate over long distances to reach the site of their final differentiation. The encoded protein appears to direct neuronal migration by regulating the organization and stability of microtubules. In addition, the encoded protein interacts with LIS1, the regulatory gamma subunit of platelet activating factor acetylhydrolase. Studies in knockout mice lacking this gene and the LIS1 gene suggest that the molecular interaction of these two genes is important in both in neuronal migration and neurogenesis, and there is a cortical role of this gene in nuclear translocation and positioning of the mitotic spindle in radial glial mitotic division. Multiple transcript variants encoding three different isoforms have been found for this gene. [provided by RefSeq, Sep 2010]
Expression	Biased expression in whole brain E14.5 (RPKM 80.1), CNS E18 (RPKM 75.8) and 3 other tissues See more
Orthologs	human all

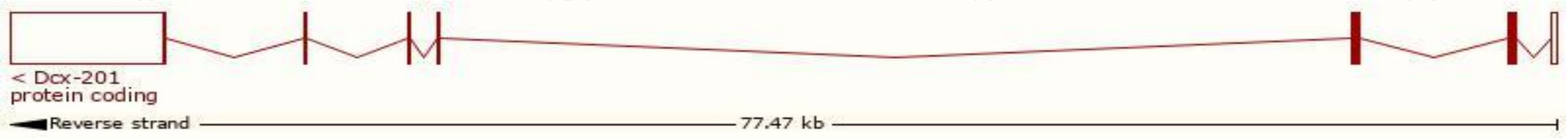
Chromosome X - NC_000086.7

Transcript information（Ensembl）

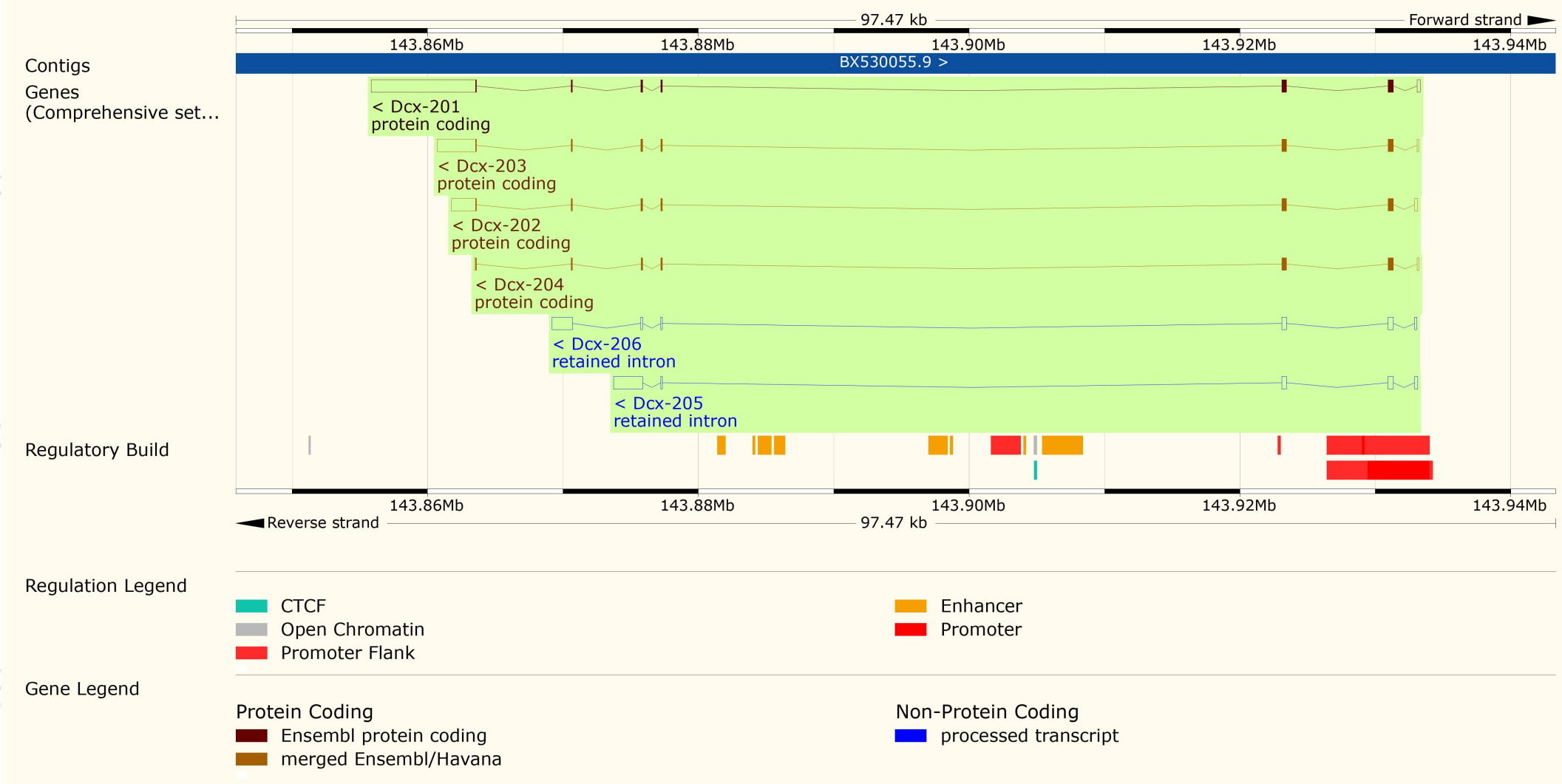
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Dcx-201	ENSMUST00000033642.9	9074	366aa	ENSMUSP00000033642.3	Protein coding	CCDS53209	O88809	TSL:1 GENCODE basic APPRIS ALT1
Dcx-203	ENSMUST00000112851.7	4095	365aa	ENSMUSP00000108472.1	Protein coding	CCDS53210	Q6PGI2	TSL:1 GENCODE basic APPRIS ALT1
Dcx-202	ENSMUST00000087313.9	3120	366aa	ENSMUSP00000084570.3	Protein coding	CCDS53209	O88809	TSL:1 GENCODE basic APPRIS ALT1
Dcx-204	ENSMUST00000112856.2	1333	360aa	ENSMUSP00000108477.2	Protein coding	CCDS41157	Q9CXL6	TSL:1 GENCODE basic APPRIS P3
Dcx-205	ENSMUST00000125768.1	3194	No protein	-	Retained intron	-	-	TSL:2
Dcx-206	ENSMUST00000139920.7	2617	No protein	-	Retained intron	-	-	TSL:2

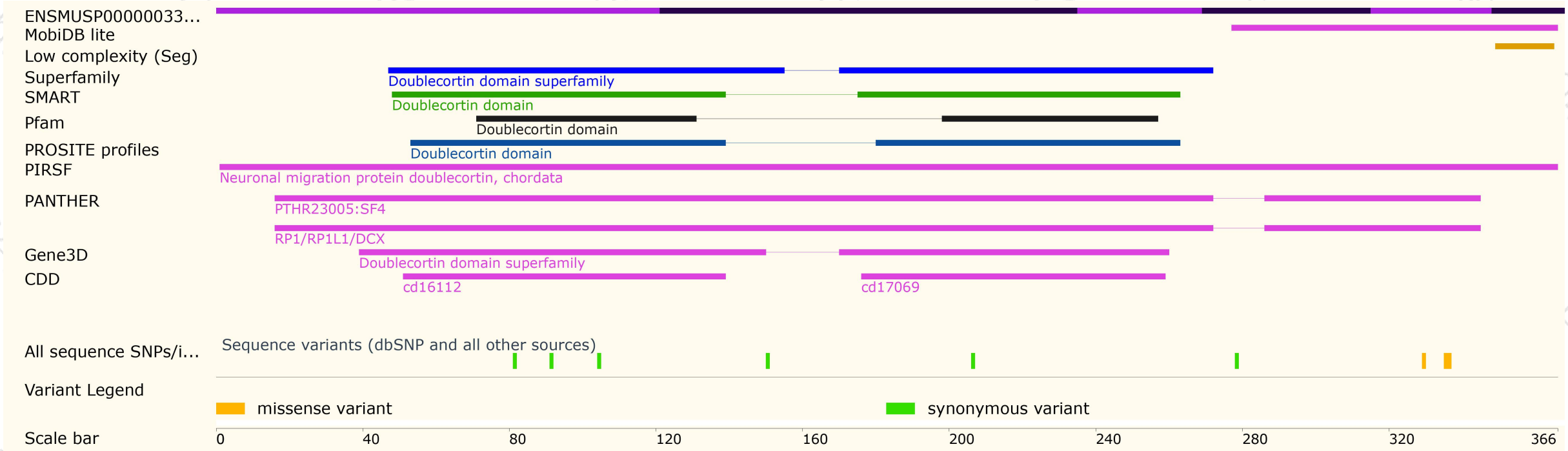
The strategy is based on the design of *Dcx-201* transcript,The transcription is shown below



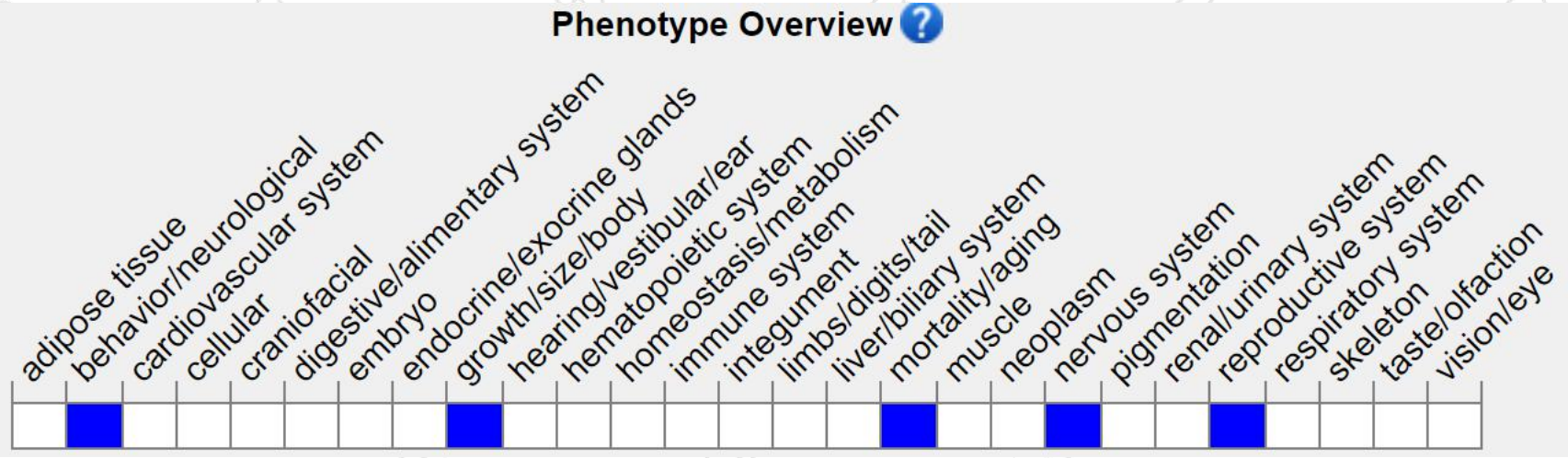
Genomic location distribution



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/).

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

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