

***Man2b1* Cas9-CKO Strategy**

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Reviewer:

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Project Overview

Project Name

Man2b1

Project type

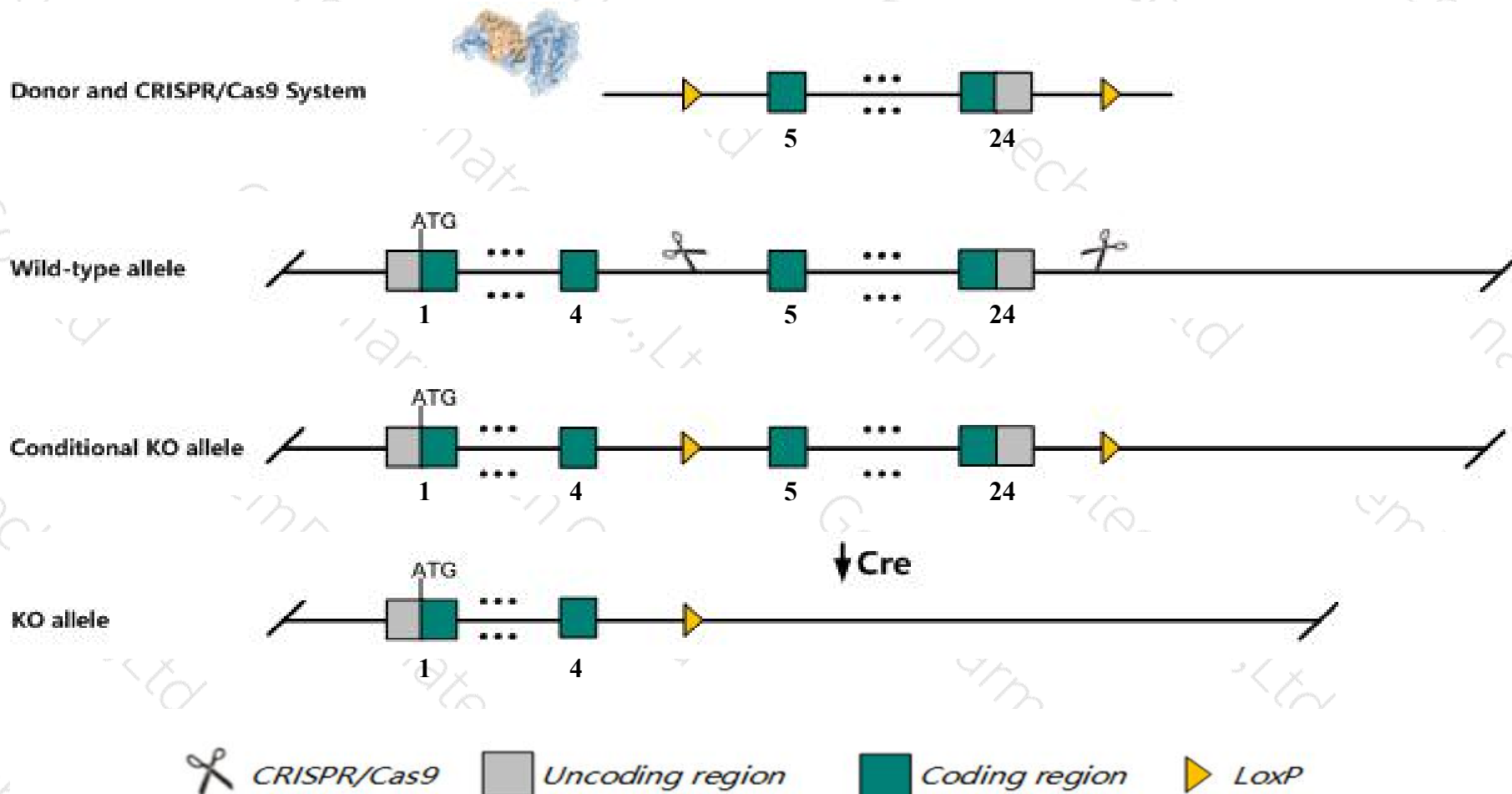
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Man2b1* gene. The schematic diagram is as follows:



- The *Man2b1* gene has 6 transcripts. According to the structure of *Man2b1* gene, exon5-exon24 of *Man2b1-201* (ENSMUST00000034121.10) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Man2b1* 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, Mice homozygous for a knock-out allele show urinary oligosaccharide excretion, storage of neutral sugars, oligosaccharide buildup in spleen, kidney, liver, testis and brain, clear vacuoles and axonal spheroids in CNS, PNS and other cell types, behavioral changes, and enhanced long-term potentiation.
- The *Man2b1* gene is located on the Chr8. 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.

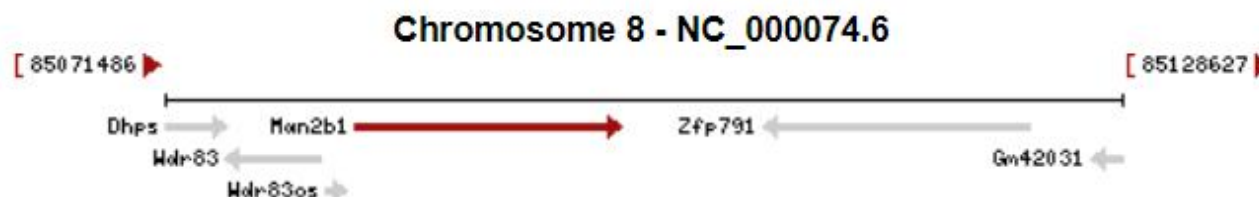
Gene information (NCBI)

Man2b1 mannosidase 2, alpha B1 [*Mus musculus* (house mouse)]

Gene ID: 17159, updated on 12-Aug-2019

Summary

Official Symbol	Man2b1 provided by MGI
Official Full Name	mannosidase 2, alpha B1 provided by MGI
Primary source	MGI:MGI:107286
See related	Ensembl:ENSMUSG000000005142
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	LAMAN; AW107687
Expression	Ubiquitous expression in genital fat pad adult (RPKM 43.7), spleen adult (RPKM 42.9) and 28 other tissues See more
Orthologs	human all

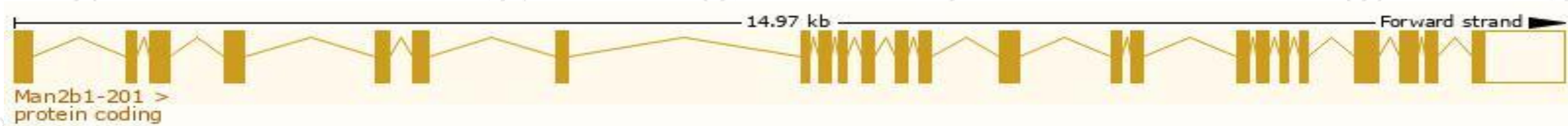


Transcript information (Ensembl)

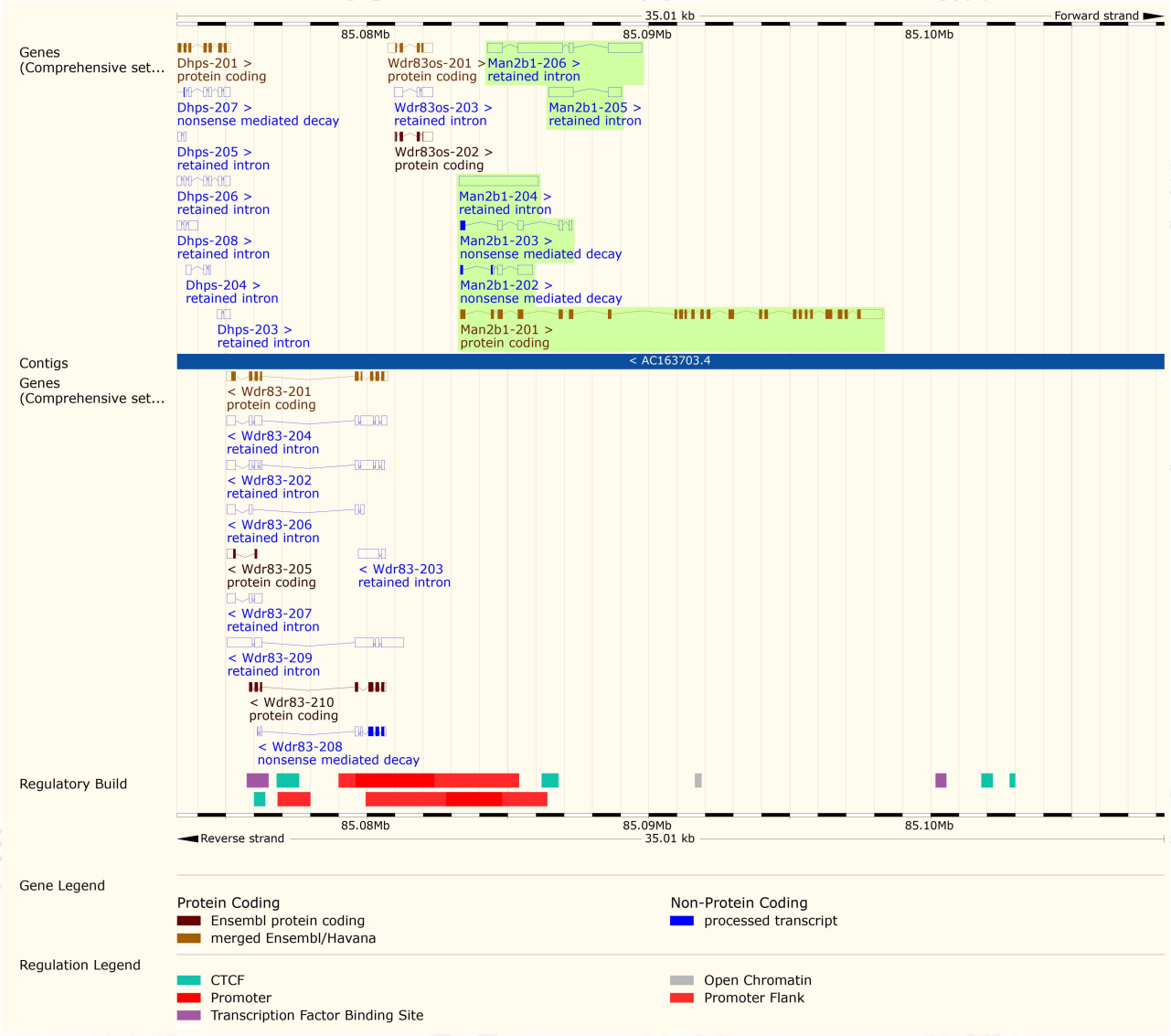
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Man2b1-201	ENSMUST000000034121.10	3822	1013aa	ENSMUSP000000034121.9	Protein coding	CCDS22494	O09159	TSL:1 GENCODE basic APPRIS P1
Man2b1-202	ENSMUST000000209264.1	912	47aa	ENSMUSP000000147441.1	Nonsense mediated decay	-	A0A1B0GRA4	TSL:3
Man2b1-203	ENSMUST000000209361.1	793	54aa	ENSMUSP000000147350.1	Nonsense mediated decay	-	A0A1B0GR27	TSL:3
Man2b1-206	ENSMUST000000211379.1	3459	No protein	-	Retained intron	-	-	TSL:1
Man2b1-204	ENSMUST000000210991.1	2820	No protein	-	Retained intron	-	-	TSL:NA
Man2b1-205	ENSMUST000000211223.1	1341	No protein	-	Retained intron	-	-	TSL:1

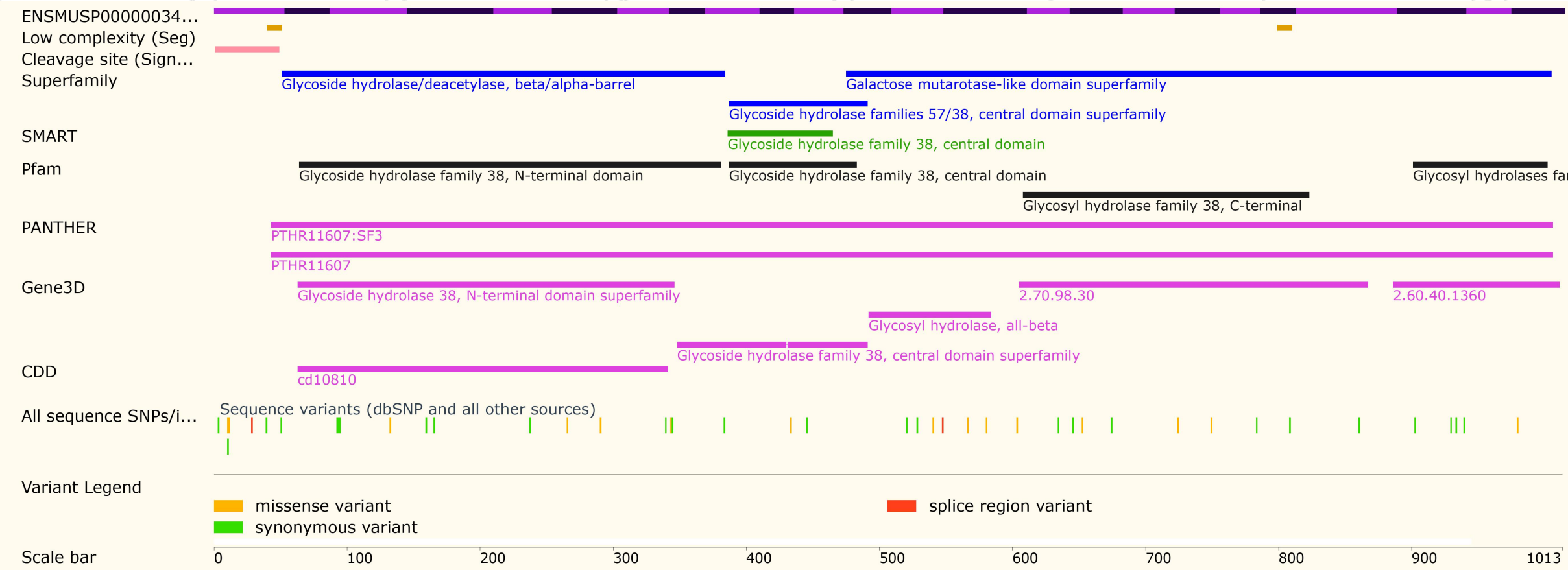
The strategy is based on the design of *Man2b1-201* transcript,The transcription is shown below



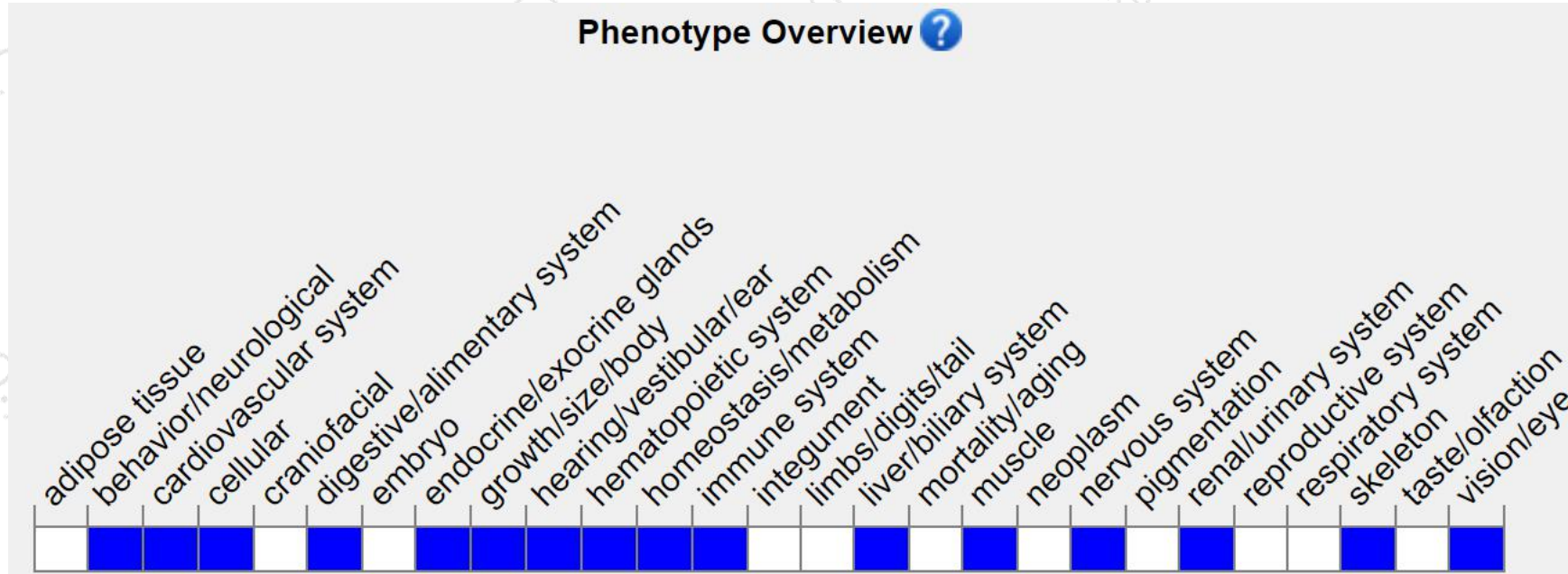
Genomic location distribution



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, Mice homozygous for a knock-out allele show urinary oligosaccharide excretion, storage of neutral sugars, oligosaccharide buildup in spleen, kidney, liver, testis and brain, clear vacuoles and axonal spheroids in CNS, PNS and other cell types, behavioral changes, and enhanced long-term potentiation.

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

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