

***Man2a2* Cas9-CKO Strategy**

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Reviewer: JiaYu

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Project Overview

Project Name

Man2a2

Project type

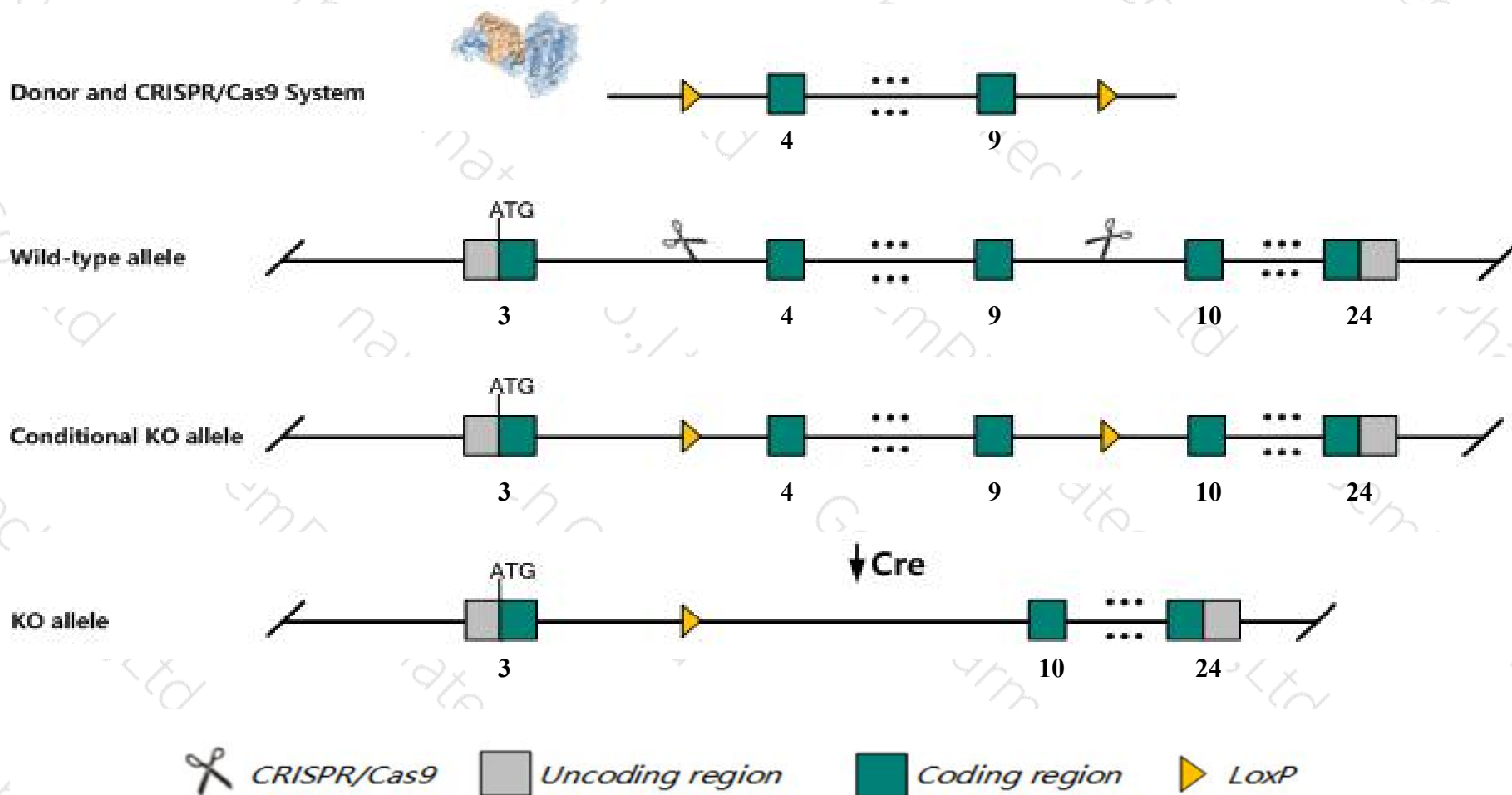
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Man2a2* gene. The schematic diagram is as follows:



Technical routes

- The *Man2a2* gene has 11 transcripts. According to the structure of *Man2a2* gene, exon4-exon9 of *Man2a2-201*(ENSMUST00000098346.4) transcript is recommended as the knockout region. The region contains 1064bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Man2a2* 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.

- According to the existing MGI data, homozygous null males are infertile due to a defect during spermatogenesis involving the premature release of germ cells from the seminiferous tubules into the epididymis.
- The *Man2a2* gene is located on the Chr7. 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)

Man2a2 mannosidase 2, alpha 2 [Mus musculus (house mouse)]

Gene ID: 140481, updated on 13-Mar-2020

Summary



Official Symbol Man2a2 provided by [MGI](#)

Official Full Name mannosidase 2, alpha 2 provided by [MGI](#)

Primary source [MGI:MGI:2150656](#)

See related [Ensembl:ENSMUSG00000038886](#)

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 1700052022Rik, 4931438M07Rik, AI480988, MX, Man IIx

Expression Ubiquitous expression in adrenal adult (RPKM 32.8), mammary gland adult (RPKM 28.0) and 26 other tissues [See more](#)

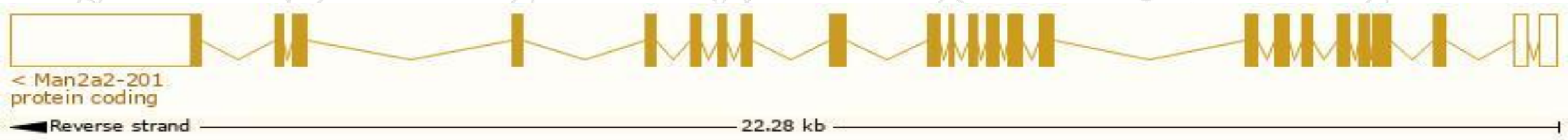
Orthologs [human all](#)

Transcript information (Ensembl)

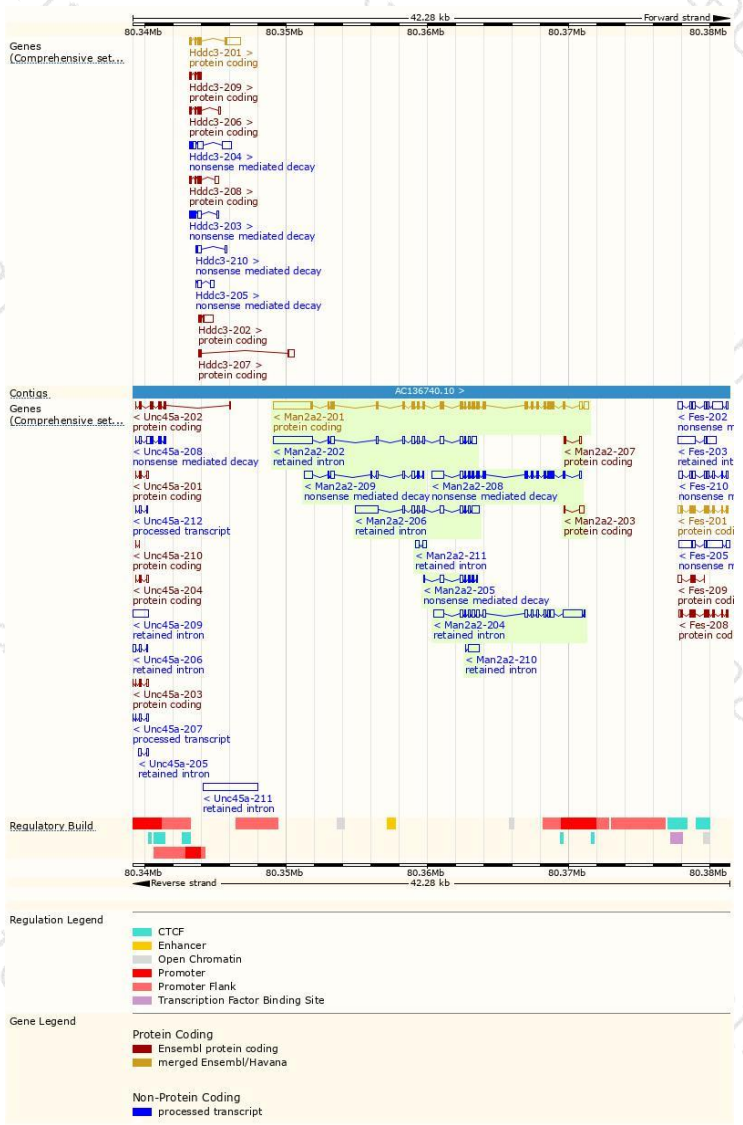
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Man2a2-201	ENSMUST00000098346.4	6554	1152aa	Protein coding	CCDS57556	Q197W7 Q8BRK9	TSL:1 GENCODE basic APPRIS P1
Man2a2-203	ENSMUST00000205436.1	431	19aa	Protein coding	-	A0A0U1RNS8	CDS 3' incomplete TSL:2
Man2a2-207	ENSMUST00000206212.1	262	29aa	Protein coding	-	A0A0U1RP42	CDS 3' incomplete TSL:5
Man2a2-208	ENSMUST00000206301.1	3007	583aa	Nonsense mediated decay	-	Q3TSU5	TSL:1
Man2a2-209	ENSMUST00000206807.1	1607	67aa	Nonsense mediated decay	-	A0A0U1RQ19	CDS 5' incomplete TSL:5
Man2a2-205	ENSMUST00000205853.1	747	69aa	Nonsense mediated decay	-	A0A0U1RPB8	CDS 5' incomplete TSL:3
Man2a2-202	ENSMUST00000205318.1	4610	No protein	Retained intron	-	-	TSL:1
Man2a2-204	ENSMUST00000205535.1	4020	No protein	Retained intron	-	-	TSL:1
Man2a2-206	ENSMUST00000206066.1	3190	No protein	Retained intron	-	-	TSL:1
Man2a2-210	ENSMUST00000206917.1	783	No protein	Retained intron	-	-	TSL:5
Man2a2-211	ENSMUST00000206973.1	537	No protein	Retained intron	-	-	TSL:2

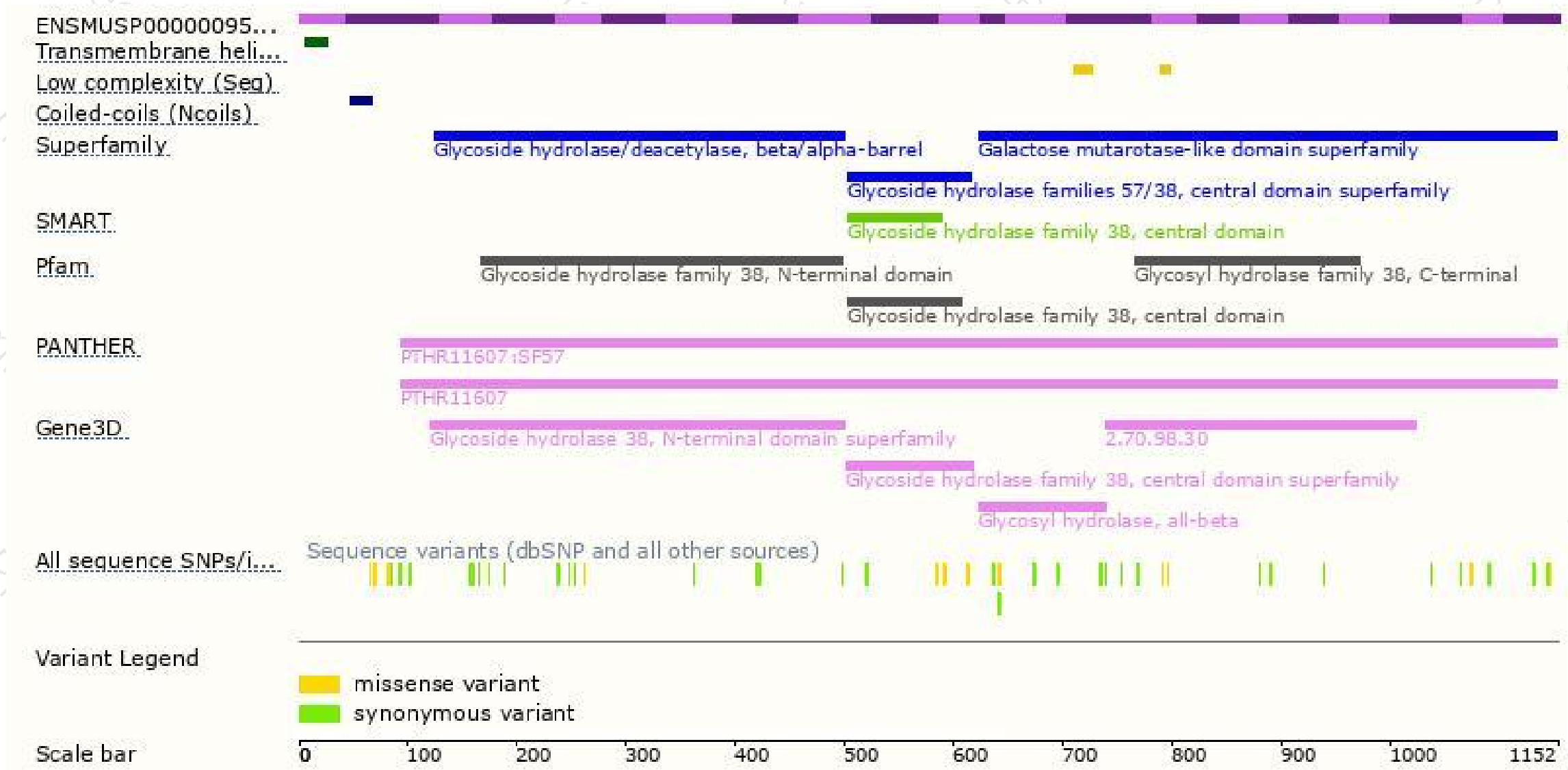
The strategy is based on the design of *Man2a2-201* transcript,the transcription is shown below:



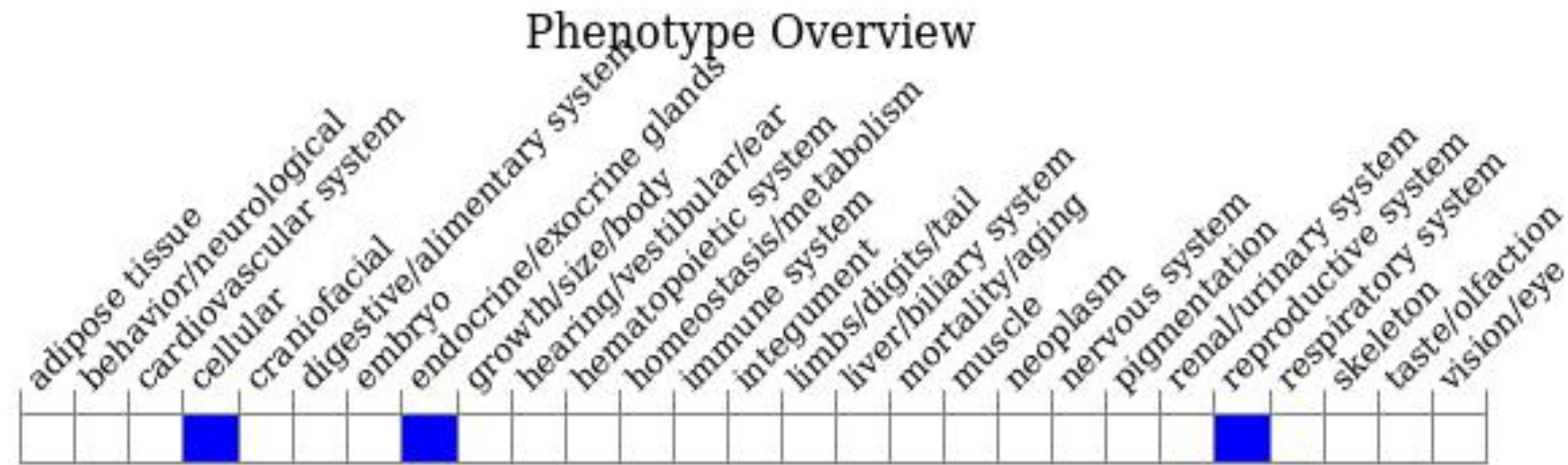
Genomic location distribution



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, homozygous null males are infertile due to a defect during spermatogenesis involving the premature release of germ cells from the seminiferous tubules into the epididymis.

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

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