

Cul4a Cas9-CKO Strategy

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Project Overview

Project Name

Cul4a

Project type

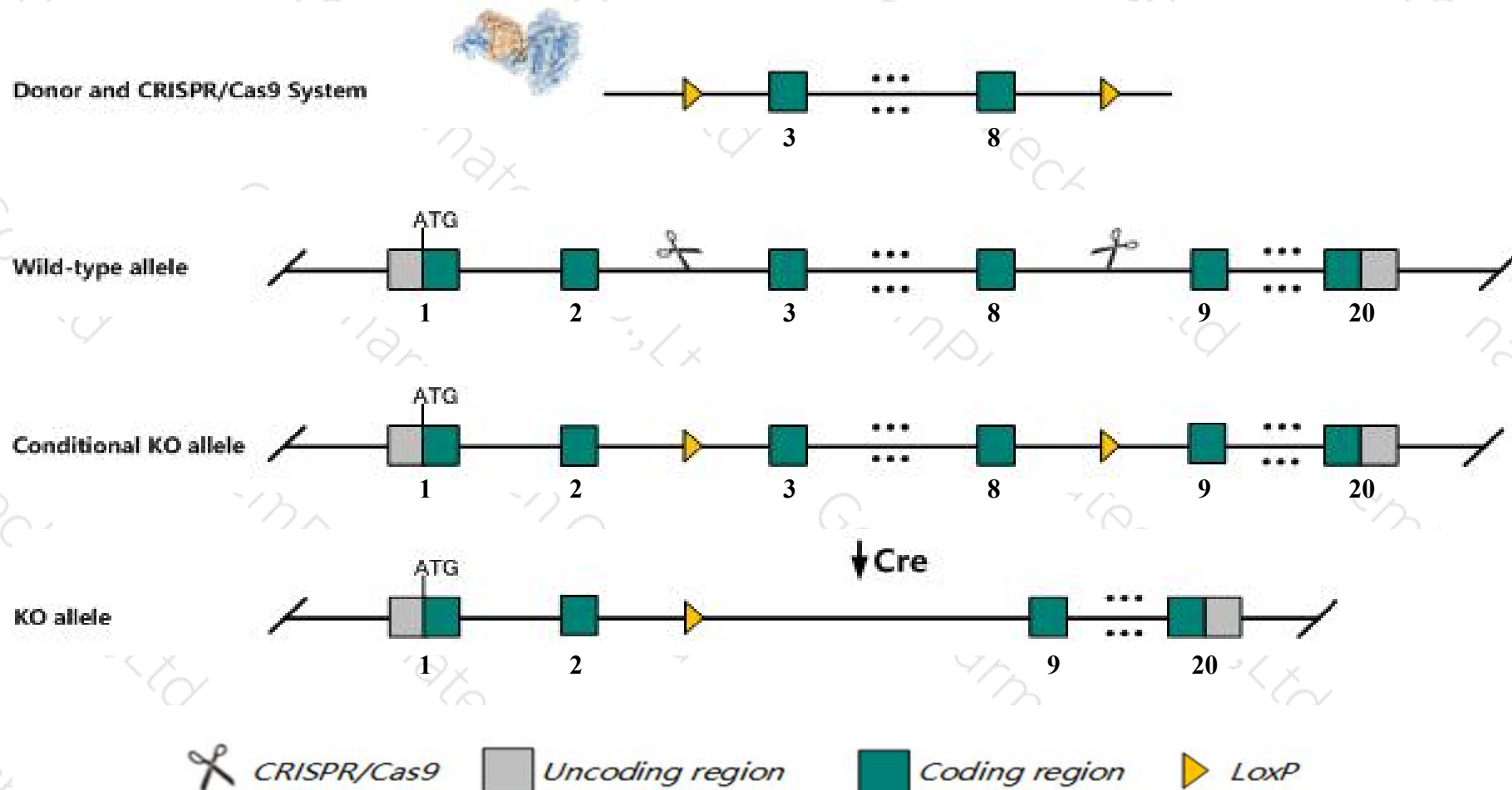
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cul4a* gene. The schematic diagram is as follows:



- The *Cul4a* gene has 8 transcripts. According to the structure of *Cul4a* gene, exon3-exon8 of *Cul4a-201* (ENSMUST00000016680.13) transcript is recommended as the knockout region. The region contains 584bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cul4a* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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, Mice homozygous for one knock-out allele exhibit reduced female fertility, male infertility, impaired spermatogenesis, and impaired DNA repair.
- Transcript *Cul4a*-204&206&208 may not be affected.
- The floxed region is near to the N-terminal of *Pcid2* gene, this strategy may influence the regulatory function of the N-terminal of *Pcid2* gene.
- The *Cul4a* 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)

Cul4a cullin 4A [*Mus musculus* (house mouse)]

Gene ID: 99375, updated on 12-Aug-2019

Summary

Official Symbol Cul4a provided by [MGI](#)
Official Full Name cullin 4A provided by [MGI](#)
Primary source [MGI:MGI:1914487](#)
See related [Ensembl:ENSMUSG00000031446](#)
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 AW495282; 2810470J21Rik
Expression Ubiquitous expression in liver E14 (RPKM 25.1), adrenal adult (RPKM 24.4) and 28 other tissues [See more](#)
Orthologs [human](#) [all](#)

Genomic context

Location: 8; 8 A1.1

See Cul4a in [Genome Data Viewer](#)

Exon count: 20

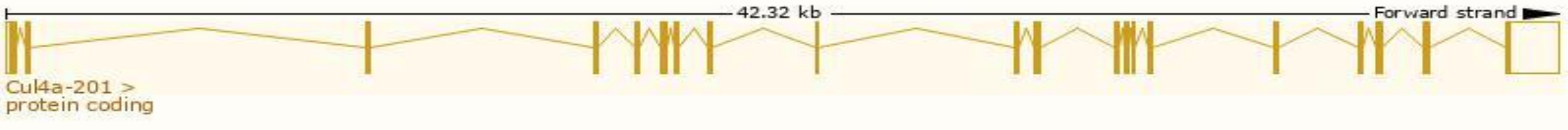
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	8	NC_000074.6 (13105623..13147940)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	8	NC_000074.5 (13105721..13147940)

Transcript information (Ensembl)

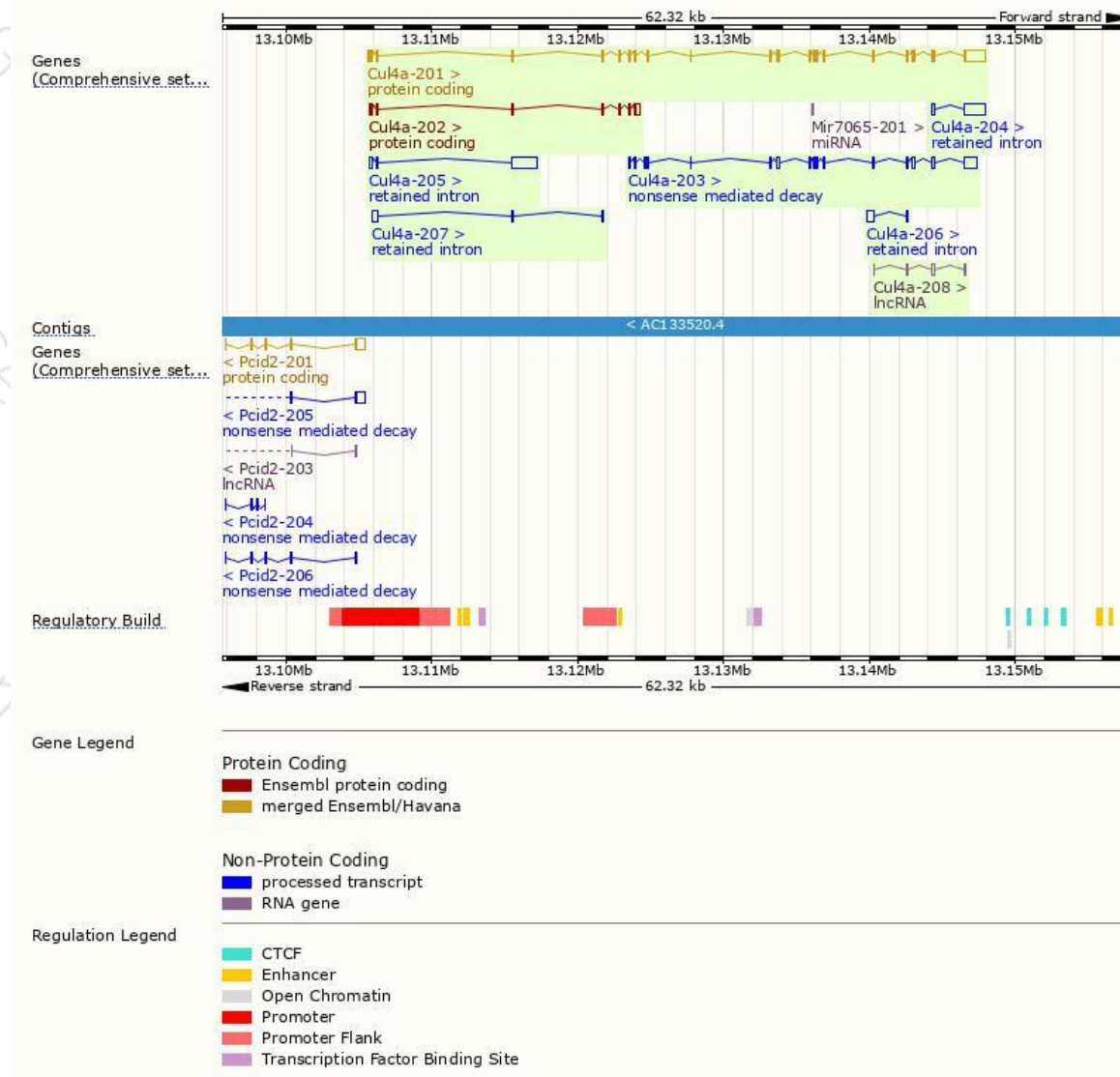
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cul4a-201	ENSMUST00000016680.13	3728	759aa	Protein coding	CCDS52485	Q3TCH7	TSL:1 GENCODE basic APPRIS P1
Cul4a-202	ENSMUST00000121426.1	1044	265aa	Protein coding	-	E9PXT5	TSL:1 GENCODE basic
Cul4a-203	ENSMUST00000125514.7	2570	87aa	Nonsense mediated decay	-	F6UV36	CDS 5' incomplete TSL:1
Cul4a-205	ENSMUST00000135371.1	2006	No protein	Retained intron	-	-	TSL:1
Cul4a-204	ENSMUST00000125646.1	1614	No protein	Retained intron	-	-	TSL:1
Cul4a-206	ENSMUST00000139316.1	574	No protein	Retained intron	-	-	TSL:3
Cul4a-207	ENSMUST00000141315.1	548	No protein	Retained intron	-	-	TSL:3
Cul4a-208	ENSMUST00000142700.1	357	No protein	lncRNA	-	-	TSL:1

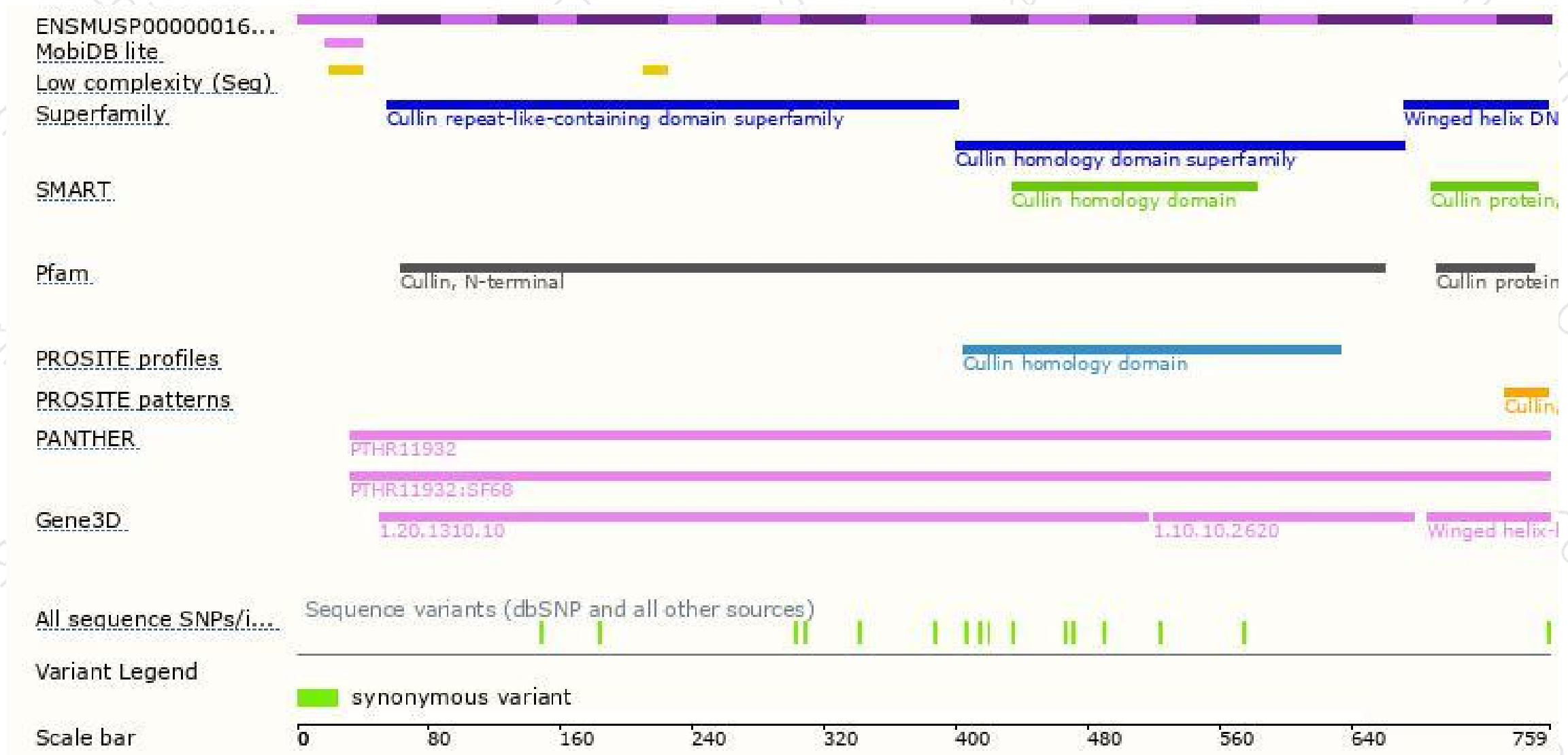
The strategy is based on the design of *Cul4a-201* transcript,The transcription is shown below



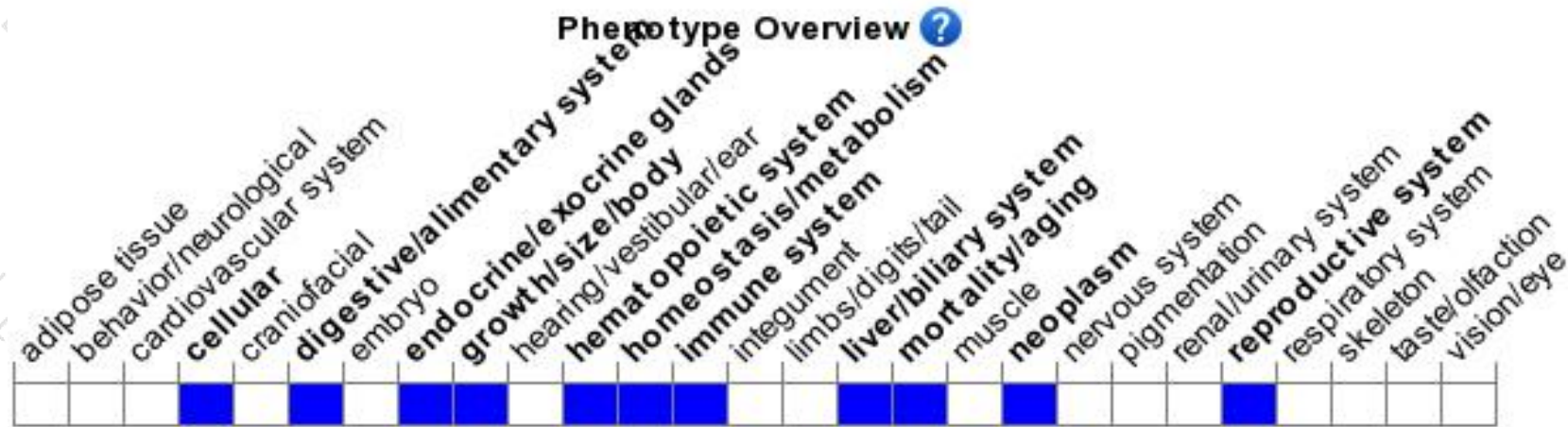
Genomic location distribution



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 one knock-out allele exhibit reduced female fertility, male infertility, impaired spermatogenesis, and impaired DNA repair.

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

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