

Cyld Cas9-CKO Strategy

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Project Overview

Project Name

Cyld

Project type

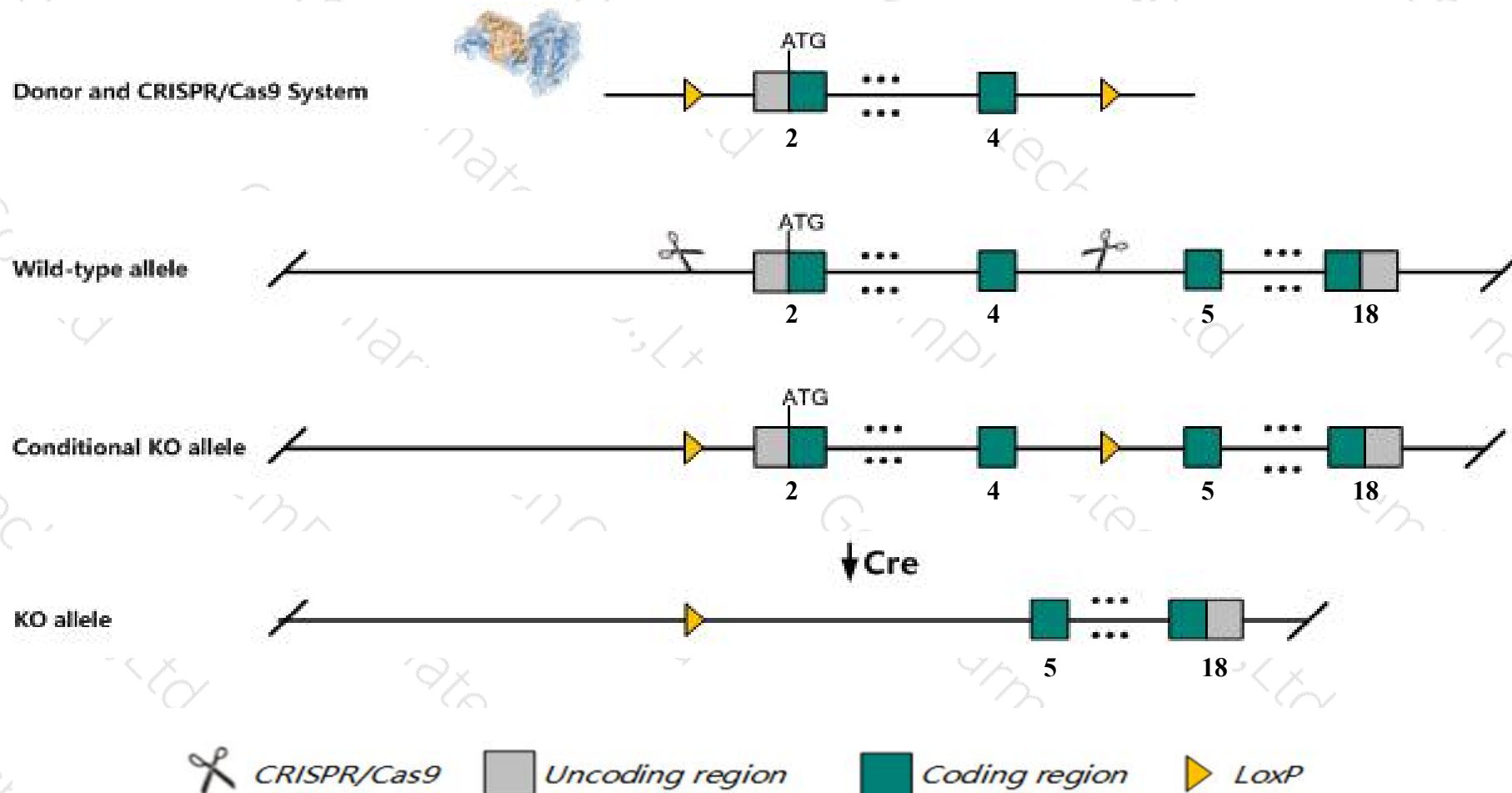
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



Technical routes

- The *Cyld* gene has 11 transcripts. According to the structure of *Cyld* gene, exon2-exon4 of *Cyld*-205 (ENSMUST00000209532.1) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cyld* 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, Various knockout models with different exon deletions have been created. Observed phenotypes include altered T cell and B cell development, susceptibility to induced skin tumors, resistance to lethal lung infection, high colon tumor incidence, kinky tails, and neonatal death due to lung dysfunction.
- Transcript *Cyld*-207&209&211 may not be affected.
- The *Cyld* 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)

Cyld CYLD lysine 63 deubiquitinase [Mus musculus (house mouse)]

Gene ID: 74256, updated on 31-Jan-2019

Summary

Official Symbol	Cyld provided by MGI
Official Full Name	CYLD lysine 63 deubiquitinase provided by MGI
Primary source	MGI:MGI:1921506
See related	Ensembl:ENSMUSG00000036712
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	2010013M14Rik, 2900009M21Rik, C130039D01Rik, CDMT, CYLD1, EAC, mKIAA0849
Summary	<p>This gene encodes a protein that is a member of the ubiquitin C-terminal hydrolase subfamily of the deubiquitinating enzyme family. Members of this family catalyze the removal of ubiquitin from a substrate or another ubiquitin molecule and thereby play important roles in regulating signaling pathways, recycling ubiquitin and regulating protein stability. This protein removes ubiquitin from K-63-linked ubiquitin chains from proteins involved in NF-kappaB signaling and thus acts as a negative regulator of this pathway. In humans mutations in this gene have been associated with cylindromatosis, an autosomal dominant predisposition to tumors of skin appendages. In mouse deficiency of this gene impairs thymocyte development and increases susceptibility to skin and colon tumors. A pseudogene of this gene has been identified on chromosome 1. Alternative splicing results in multiple transcript variants that encode different protein isoforms. [provided by RefSeq, Jan 2013]</p>
Expression	Ubiquitous expression in frontal lobe adult (RPKM 5.8), cortex adult (RPKM 5.5) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

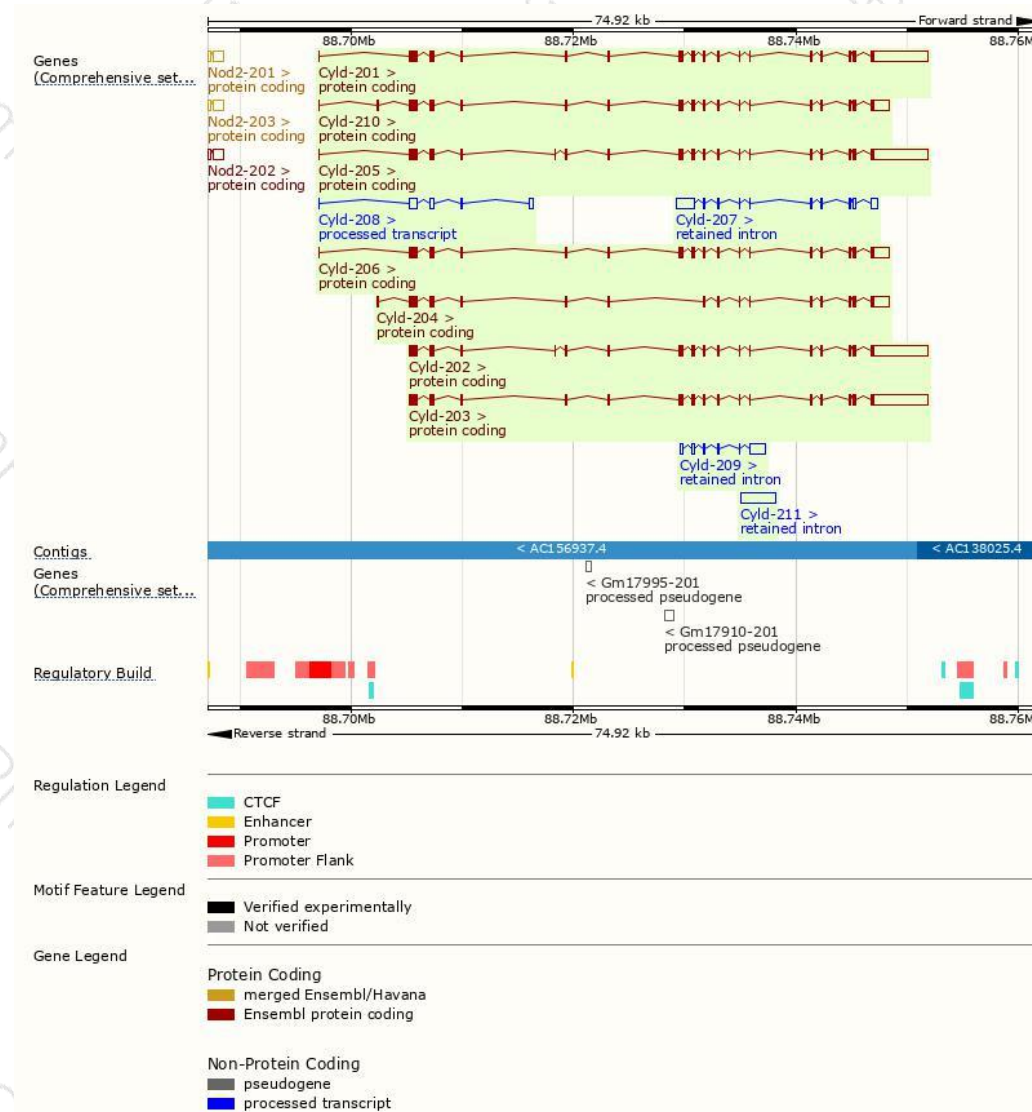
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cyld-205	ENSMUST00000209532.1	7990	955aa	Protein coding	CCDS52633	Q80TQ2	TSL:5 GENCODE basic APPRIS ALT 1
Cyld-202	ENSMUST0000098519.10	7954	955aa	Protein coding	CCDS52633	Q80TQ2	TSL:5 GENCODE basic APPRIS ALT 1
Cyld-203	ENSMUST00000109626.3	7945	952aa	Protein coding	CCDS22513	Q80TQ2	TSL:5 GENCODE basic APPRIS P3
Cyld-210	ENSMUST00000211554.1	4638	952aa	Protein coding	CCDS22513	Q80TQ2	TSL:1 GENCODE basic APPRIS P3
Cyld-206	ENSMUST00000209559.1	4476	952aa	Protein coding	CCDS22513	Q80TQ2	TSL:1 GENCODE basic APPRIS P3
Cyld-204	ENSMUST00000209206.1	4032	770aa	Protein coding	CCDS85579	A0A1B0GSE5	TSL:5 GENCODE basic
Cyld-201	ENSMUST0000043526.14	8022	955aa	Protein coding	-	Z4YJJ5	TSL:1 GENCODE basic APPRIS ALT 1
Cyld-208	ENSMUST00000209742.1	1477	No protein	Processed transcript	-	-	TSL:1
Cyld-207	ENSMUST00000209722.1	3119	No protein	Retained intron	-	-	TSL:1
Cyld-211	ENSMUST00000211671.1	3096	No protein	Retained intron	-	-	TSL:NA
Cyld-209	ENSMUST00000210302.1	2214	No protein	Retained intron	-	-	TSL:2

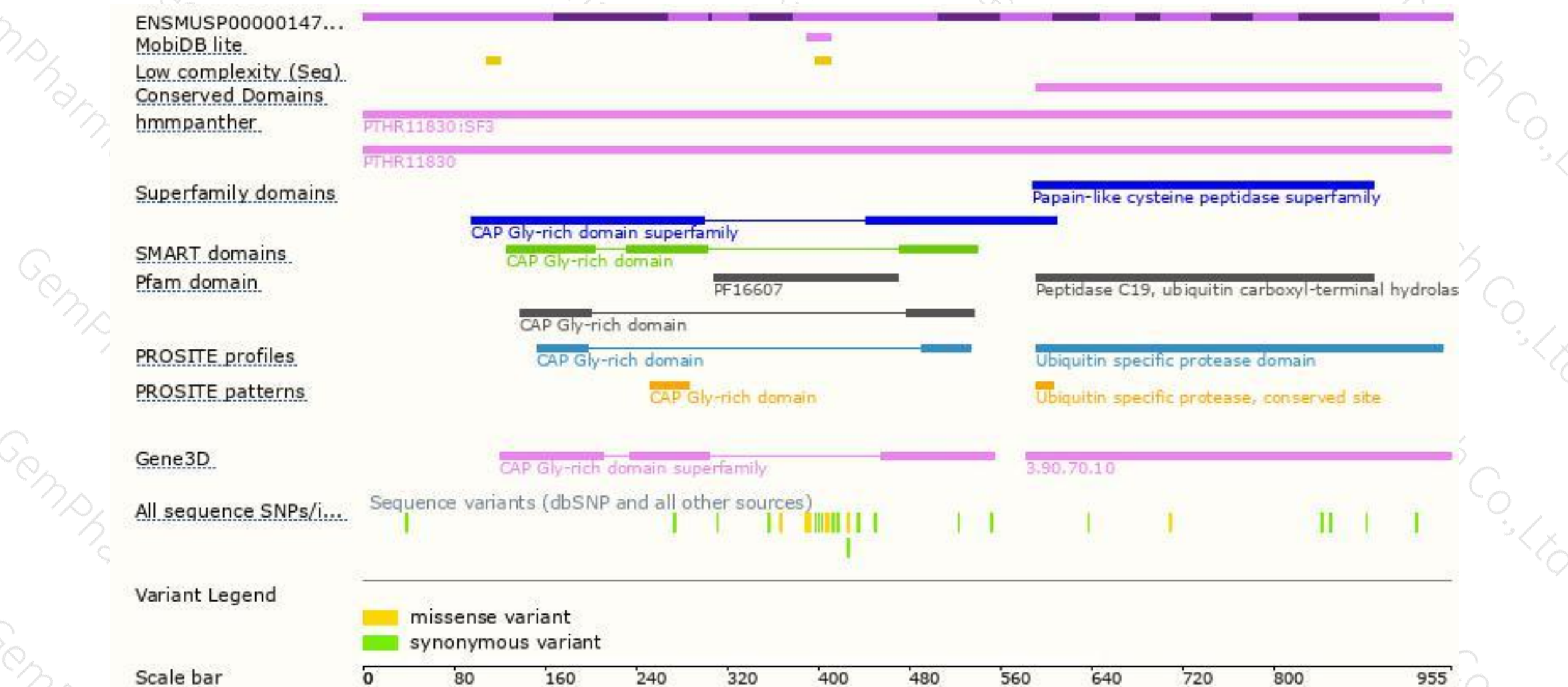
The strategy is based on the design of *Cyld-205* transcript,The transcription is shown below



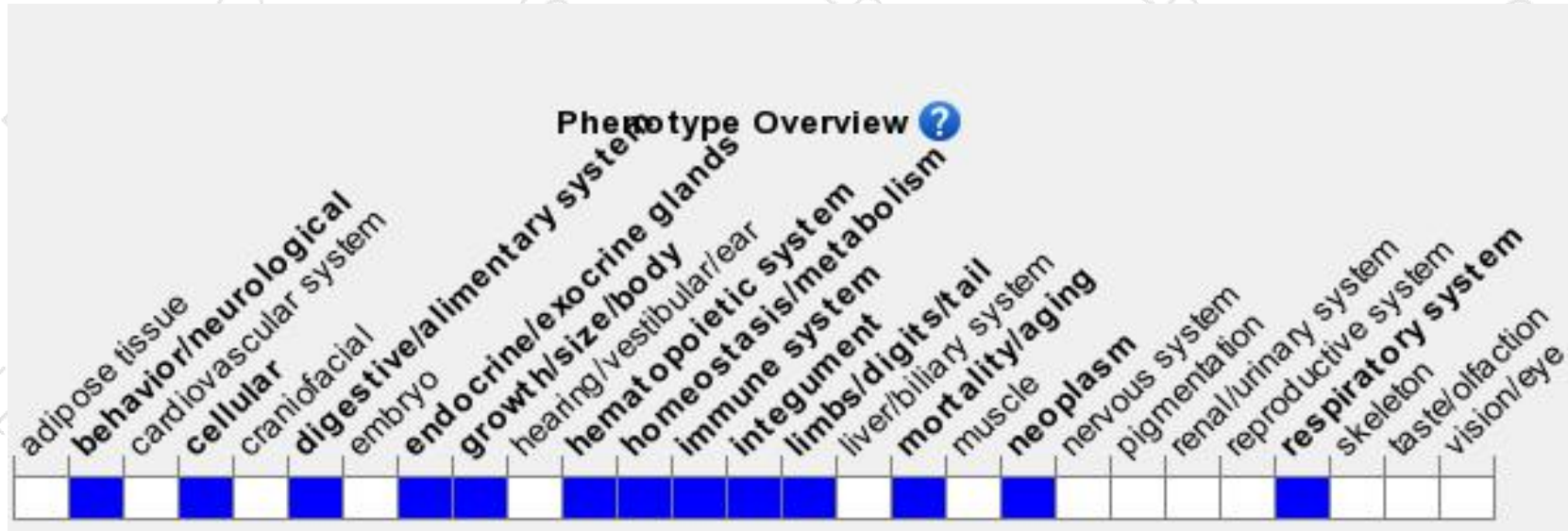
Genomic location distribution



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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