Polg Cas9-CKO Strategy

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Reviewer: Miaomiao Cui

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Project Overview

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

Conditional Knockout strategy

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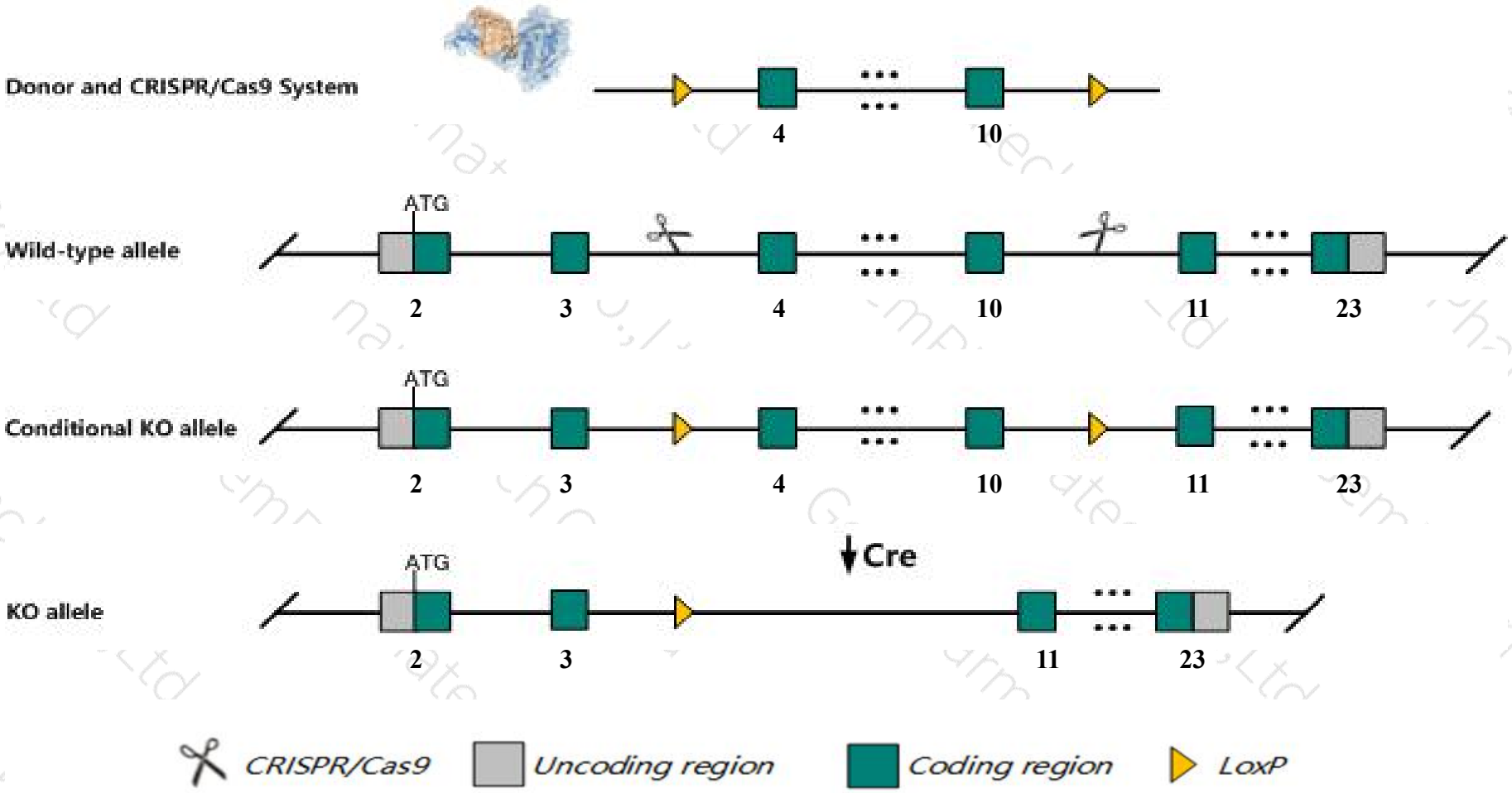
This model will use CRISPR/Cas9 technology to edit the *Polg* gene. The schematic diagram is as follows:



Technical routes

➢ The *Polg* gene has 18 transcripts. According to the structure of *Polg* gene, exon4-exon10 of *Polg-201*(ENSMUST00000073889.13) transcript is recommended as the knockout region.The region contains 1088bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Polg* 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,homozygous proof-reading deficient mutants display reduced life spans and premature aging with weight loss, decreased subcutaneous fat, alopecia, kyphosis, osteoporosis, anemia, reduced fertility, and enlarged hearts. Homozygous null mice display embryonic lethality.

➢Transcript 206, 215,217 and 218 may not be affected.

➢ The *Polg* 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）

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Polg polymerase (DNA directed), gamma [Mus musculus (house mouse)]

Gene ID: 18975, updated on 13-Mar-2020

Summary

Official Symbol	Polg provided by MGI
Official Full Name	polymerase (DNA directed), gamma provided byMGI
Primary source	MGI:MGI:1196389
See related	Ensembl:ENSMUSG00000039176
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	AA409516, PolgA
Expression	Ubiquitous expression in thymus adult (RPKM 26.1), placenta adult (RPKM 20.4) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

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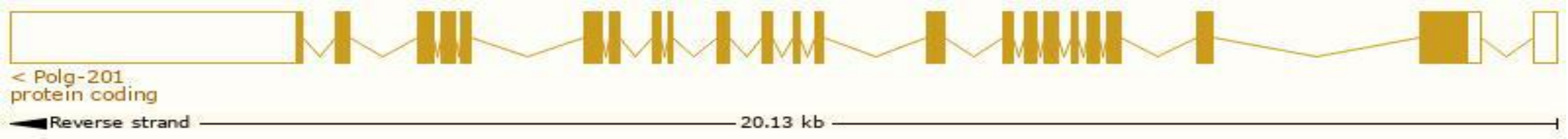
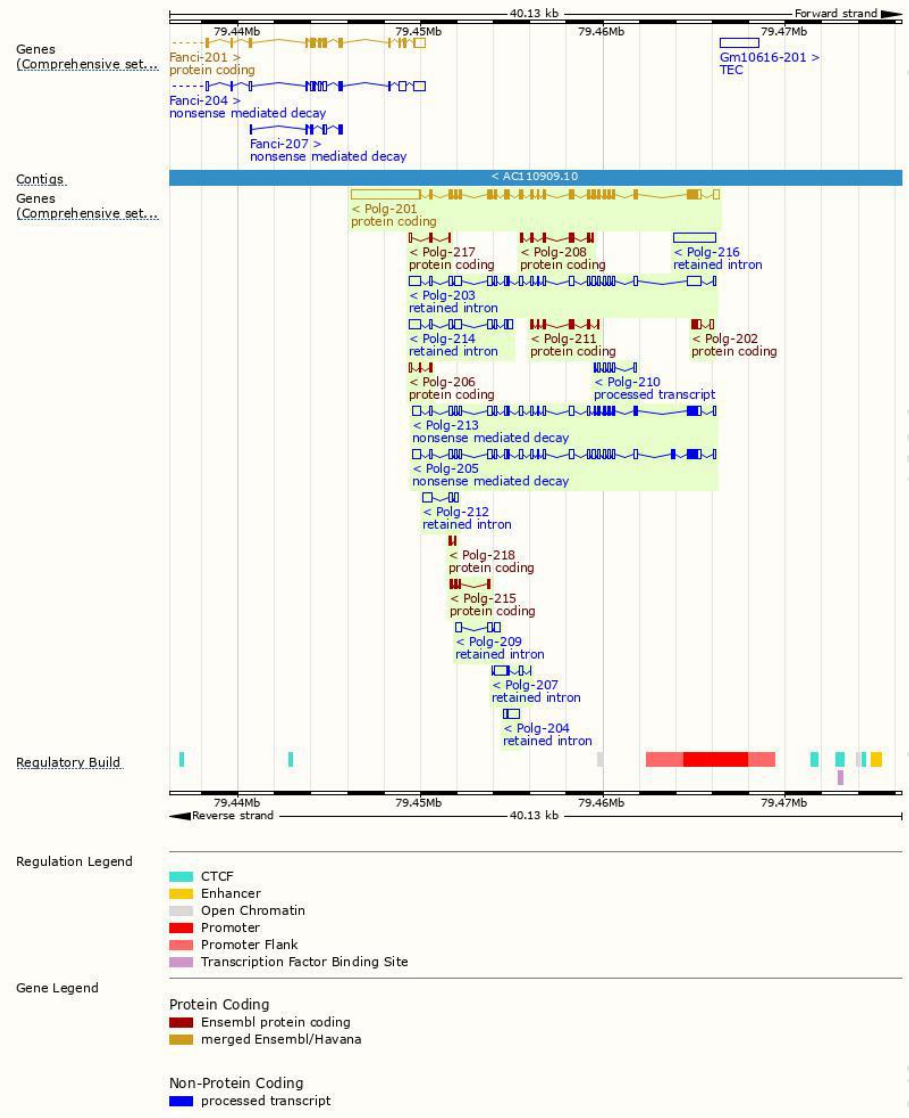
The gene has 18 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Polg-201	ENSMUST00000073889.13	7837	1217aa	Protein coding	CCDS21382	Q75WC0	TSL:1 GENCODE basic APPRIS P1
Polg-211	ENSMUST00000143672.2	728	243aa	Protein coding	-	F7BP45	CDS 5' and 3' incomplete TSL:3
Polg-208	ENSMUST00000139668.7	708	236aa	Protein coding	-	F6YLN2	CDS 5' and 3' incomplete TSL:5
Polg-202	ENSMUST00000125562.1	645	105aa	Protein coding	-	A0A0J9YTR4	CDS 3' incomplete TSL:3
Polg-215	ENSMUST00000201030.1	465	155aa	Protein coding	-	A0A0J9YTZ6	CDS 5' and 3' incomplete TSL:3
Polg-217	ENSMUST00000201662.1	408	94aa	Protein coding	-	A0A0J9YV64	CDS 5' incomplete TSL:3
Polg-206	ENSMUST00000139290.2	375	69aa	Protein coding	-	A0A0J9YU70	CDS 5' incomplete TSL:3
Polg-218	ENSMUST00000201907.1	149	49aa	Protein coding	-	A0A0J9YUA1	CDS 5' and 3' incomplete TSL:1
Polg-205	ENSMUST00000132048.4	4546	261aa	Nonsense mediated decay	-	A0A0J9YU03	TSL:5
Polg-213	ENSMUST00000149444.7	4247	498aa	Nonsense mediated decay	-	D6RIP1	TSL:1
Polg-210	ENSMUST00000142915.2	724	No protein	Processed transcript	-	-	TSL:3
Polg-203	ENSMUST00000127082.7	4589	No protein	Retained intron	-	-	TSL:1
Polg-216	ENSMUST00000201557.1	2279	No protein	Retained intron	-	-	TSL:NA
Polg-214	ENSMUST00000154403.7	2167	No protein	Retained intron	-	-	TSL:1
Polg-207	ENSMUST00000139597.5	919	No protein	Retained intron	-	-	TSL:5
Polg-212	ENSMUST00000145154.2	910	No protein	Retained intron	-	-	TSL:2
Polg-209	ENSMUST00000139795.1	849	No protein	Retained intron	-	-	TSL:2
Polg-204	ENSMUST00000127734.2	765	No protein	Retained intron	-	-	TSL:2

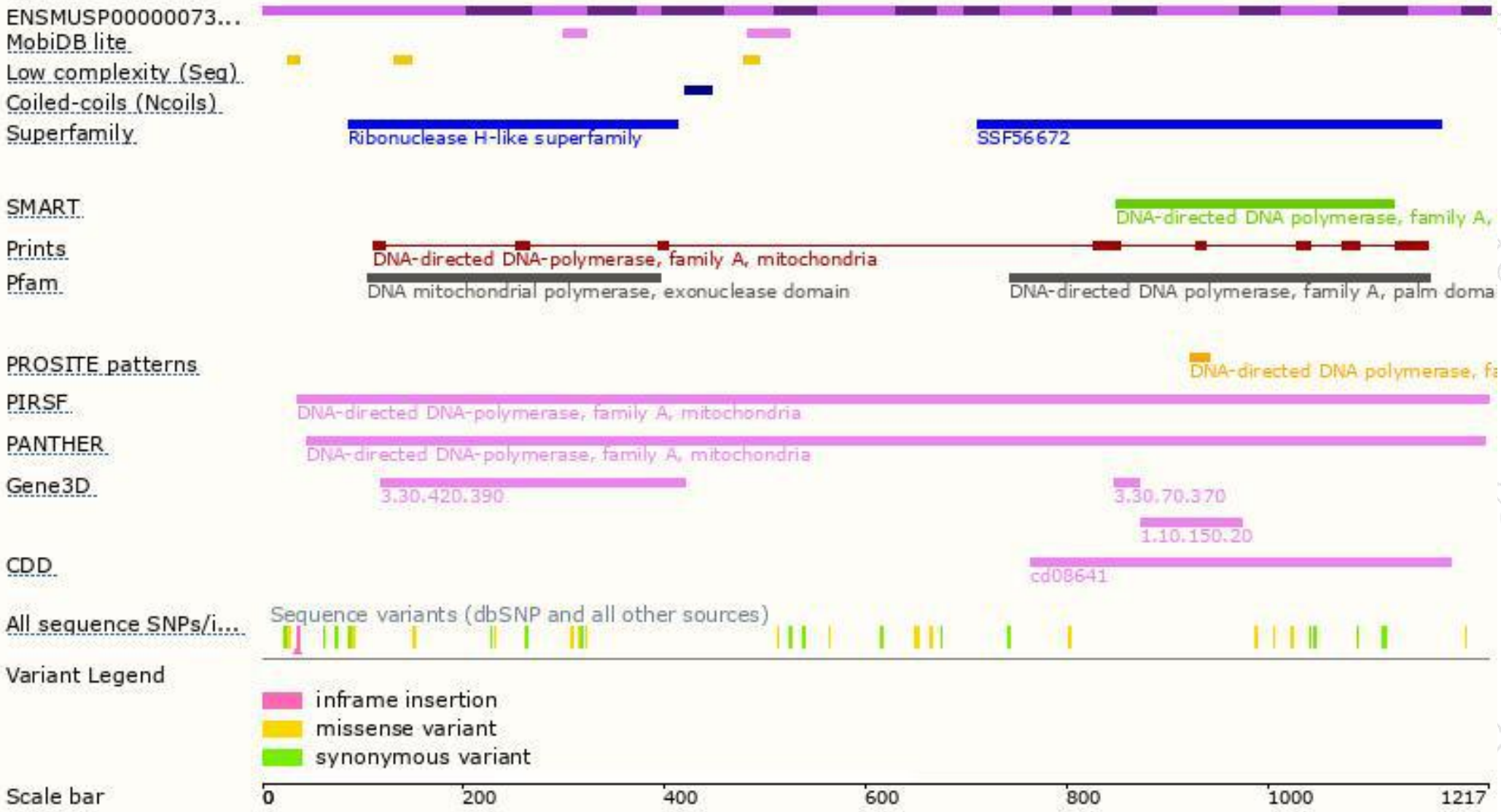
The strategy is based on the design of *Polg-201* transcript,the transcription is shown below:



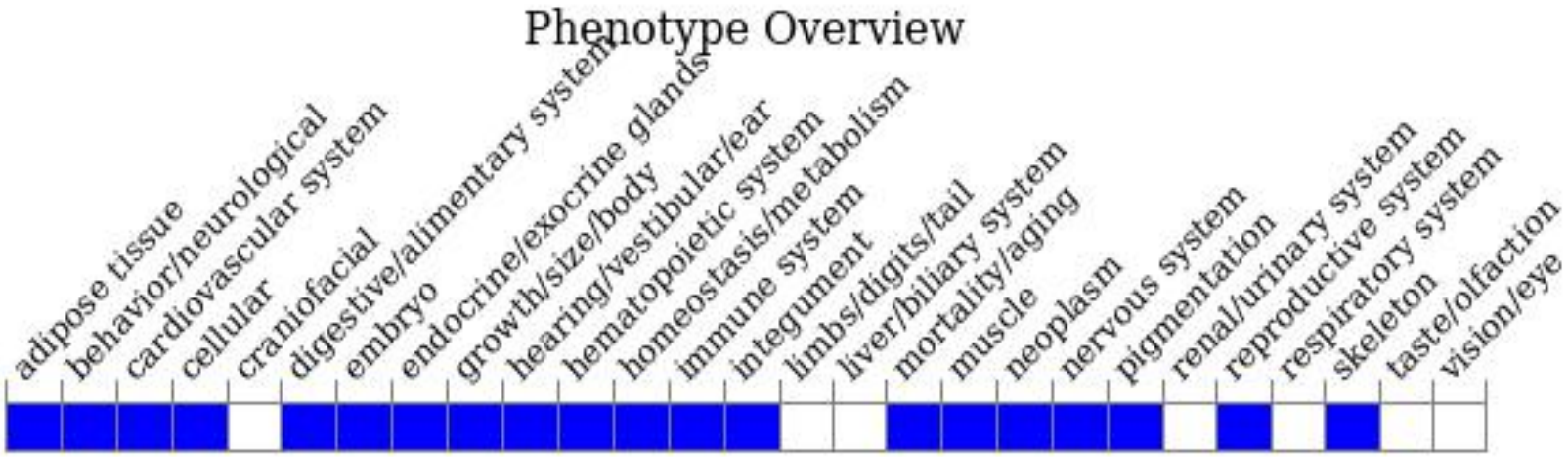
Genomic location distribution



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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