

Piga Cas9-CKO Strategy

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Project Overview

Project Name

Piga

Project type

Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

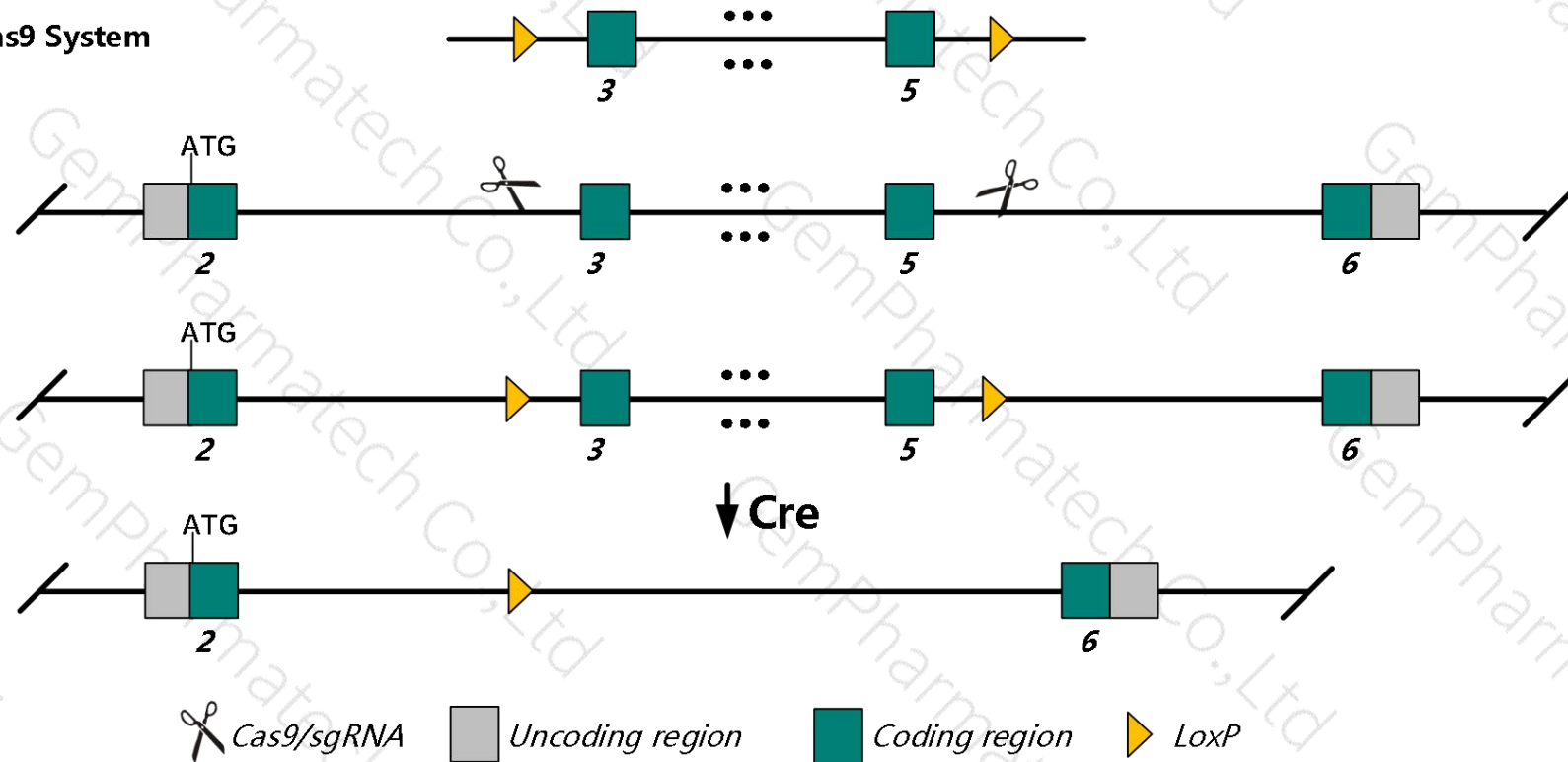
This model will use CRISPR/Cas9 technology to edit the *Piga* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Wild-type allele

Conditional KO allele

KO allele



Technical routes

- The *Piga* gene has 9 transcripts. According to the structure of *Piga* gene, exon3-exon5 of *Piga-201* (ENSMUST00000033754.14) transcript is recommended as the knockout region. The region contains 473bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Piga* 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.

Notice

- According to the existing MGI data, Mutations produce lethal neural tube defects. Specific allele combinations are viable but oocytes are defective.
- The *Piga* gene is located on the ChrX. 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

Piga phosphatidylinositol glycan anchor biosynthesis, class A [Mus musculus (house mouse)]

Gene ID: 18700, updated on 31-Jan-2019

Summary



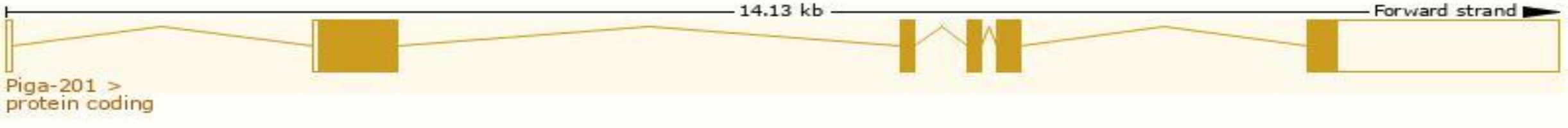
Official Symbol	Piga provided by MGI
Official Full Name	phosphatidylinositol glycan anchor biosynthesis, class A provided by MGI
Primary source	MGI:MGI:99461
See related	Ensembl:ENSMUSG000000031381
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI194334, Pig-a
Expression	Ubiquitous expression in liver E14.5 (RPKM 4.8), liver E14 (RPKM 4.5) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

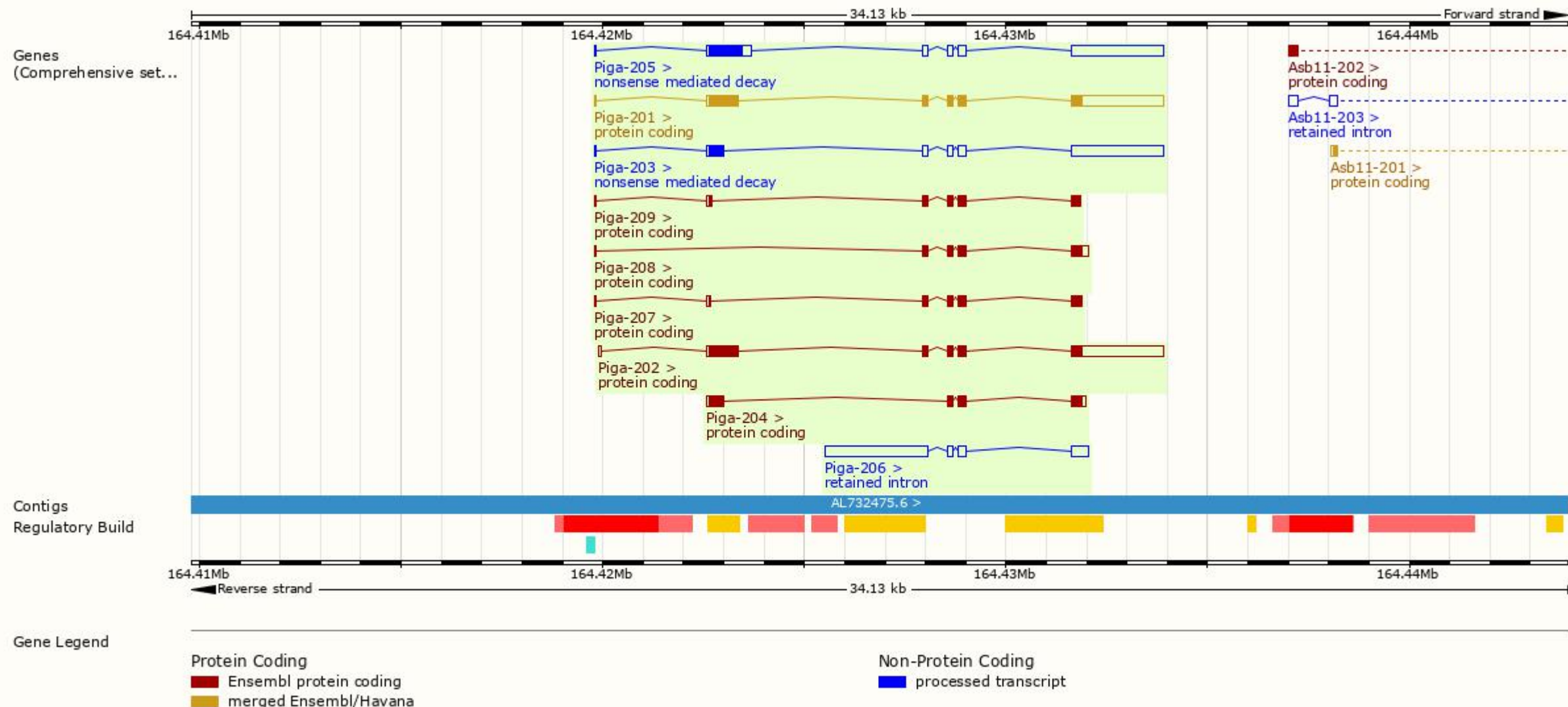
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Piga-202	ENSMUST00000112255.7	3597	485aa	Protein coding	CCDS30522	A2AIH5 Q64323	TSL:1 GENCODE basic APPRIS P1
Piga-201	ENSMUST00000033754.14	3594	485aa	Protein coding	CCDS30522	A2AIH5 Q64323	TSL:1 GENCODE basic APPRIS P1
Piga-204	ENSMUST00000133813.2	1086	315aa	Protein coding	-	A2AIH4	TSL:5 GENCODE basic
Piga-208	ENSMUST00000208697.1	947	250aa	Protein coding	-	A0A140LI97	TSL:5 GENCODE basic
Piga-209	ENSMUST00000208741.1	854	246aa	Protein coding	-	A0A140LJB2	CDS 3' incomplete TSL:5
Piga-207	ENSMUST00000208261.1	831	245aa	Protein coding	-	A0A140LIG1	CDS 3' incomplete TSL:5
Piga-205	ENSMUST00000151911.8	3903	267aa	Nonsense mediated decay	-	M0QWY7	TSL:5
Piga-203	ENSMUST00000112257.9	3212	115aa	Nonsense mediated decay	-	M0QWH5	TSL:1
Piga-206	ENSMUST00000155849.1	3321	No protein	Retained intron	-	-	TSL:1

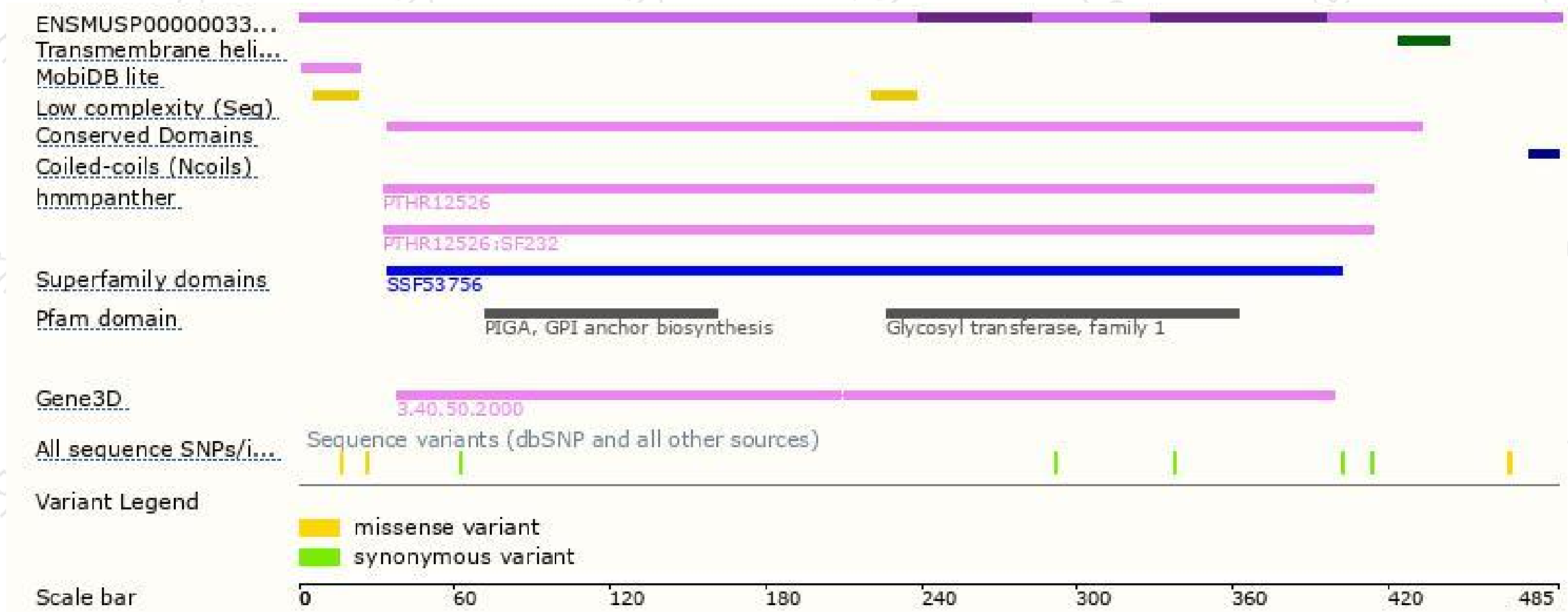
The strategy is based on the design of *Piga-201* transcript,The transcription is shown below



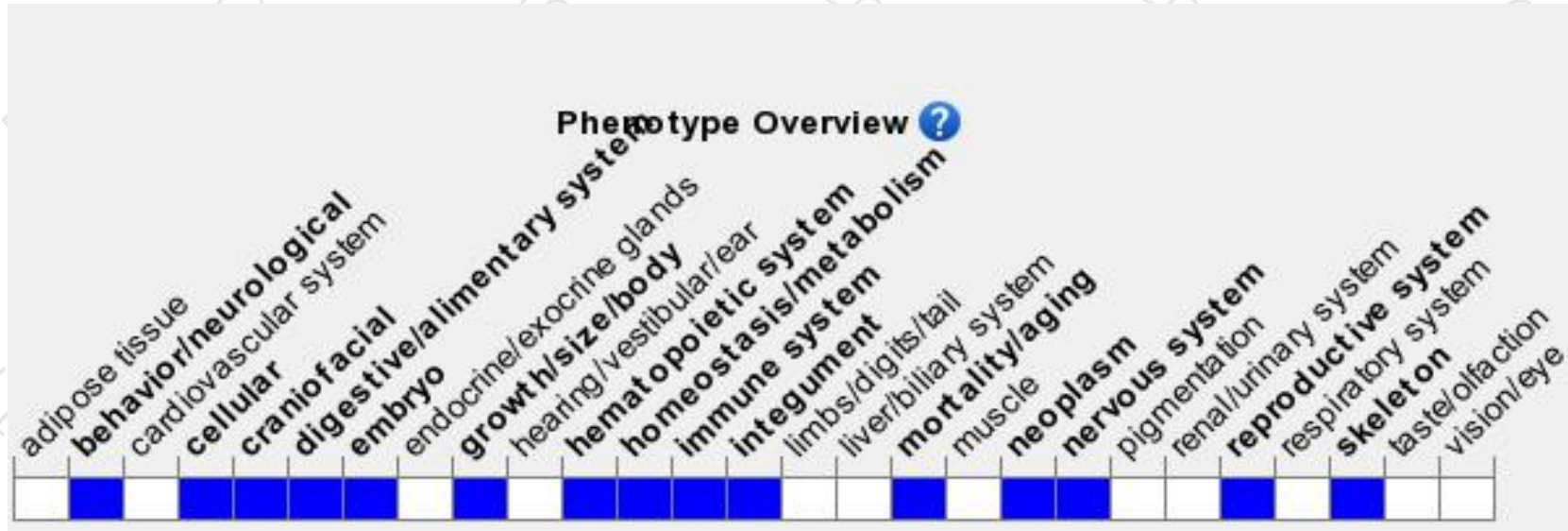
Genomic location distribution



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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