Gpx4 Cas9-CKO Strategy

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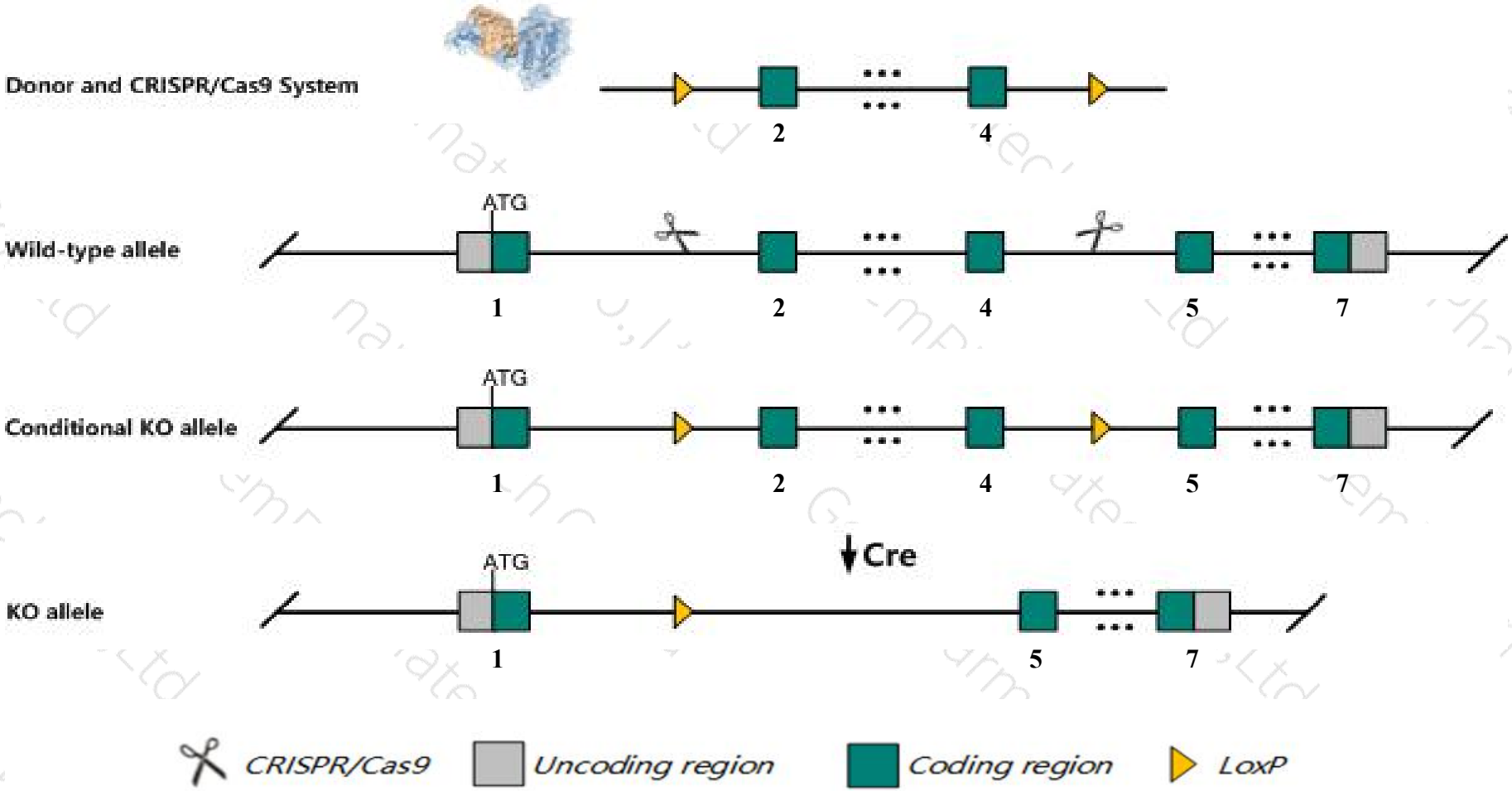
Project Overview

Project Name	***Gpx4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Gpx4* gene. The schematic diagram is as follows:



Technical routes

➢ The *Gpx4* gene has 7 transcripts. According to the structure of *Gpx4* gene, exon2-exon4 of *Gpx4-201*(ENSMUST00000097227.9) transcript is recommended as the knockout region.The region contains 392bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Gpx4* 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,gastrulation is impaired and homozygous mutant embryos consequently die during early embryonic development.

➢ The Intron1 and Intron4 are only 213bp and 803bp,loxp insertion may affect mRNA splicing.

➢ The *Gpx4* gene is located on the Chr10. 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）

集萃药康 GemPharmatech

Gpx4 glutathione peroxidase 4 [Mus musculus (house mouse)]

Gene ID: 625249, updated on 19-Mar-2019

Summary

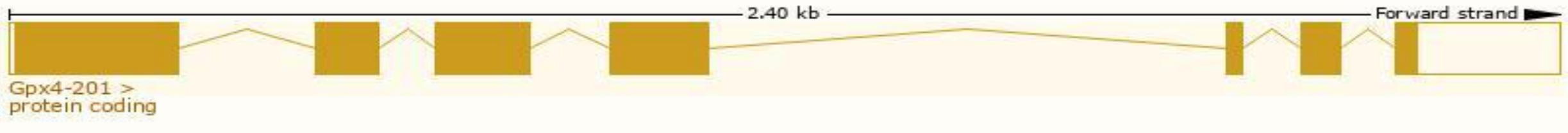
Official Symbol	Gpx4 provided by MGI
Official Full Name	glutathione peroxidase 4 provided by MGI
Primary source	MGI:MGI:104767
See related	Ensembl:ENSMUSG00000075706
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	GPx-4, GSHPx-4, PHGPx, mtPHGPx, snGPx
Summary	The protein encoded by this gene belongs to the glutathione peroxidase family, members of which catalyze the reduction of hydrogen peroxide, organic hydroperoxides and lipid hydroperoxides, and thereby protect cells against oxidative damage. Several isozymes of this gene family exist in vertebrates, which vary in cellular location and substrate specificity. This isozyme has a high preference for lipid hydroperoxides and protects cells against membrane lipid peroxidation and cell death. It is also required for normal sperm development; thus, it has been identified as a 'moonlighting' protein because of its ability to serve dual functions as a peroxidase, as well as a structural protein in mature spermatozoa. Disruption of this gene in mouse spermatocytes is associated with male infertility. This isozyme is also a selenoprotein, containing the rare amino acid selenocysteine (Sec) at its active site. Sec is encoded by the UGA codon, which normally signals translation termination. The 3' UTRs of selenoprotein mRNAs contain a conserved stem-loop structure, designated the Sec insertion sequence (SECIS) element, that is necessary for the recognition of UGA as a Sec codon, rather than as a stop signal. Transcript variants resulting from alternative splicing or use of alternate promoters have been described to encode isoforms with different subcellular localization. Pseudogenes of this locus have been identified on chromosomes 10 and 17. [provided by RefSeq, Jan 2019]
Expression	Ubiquitous expression in testis adult (RPKM 772.1), kidney adult (RPKM 308.6) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

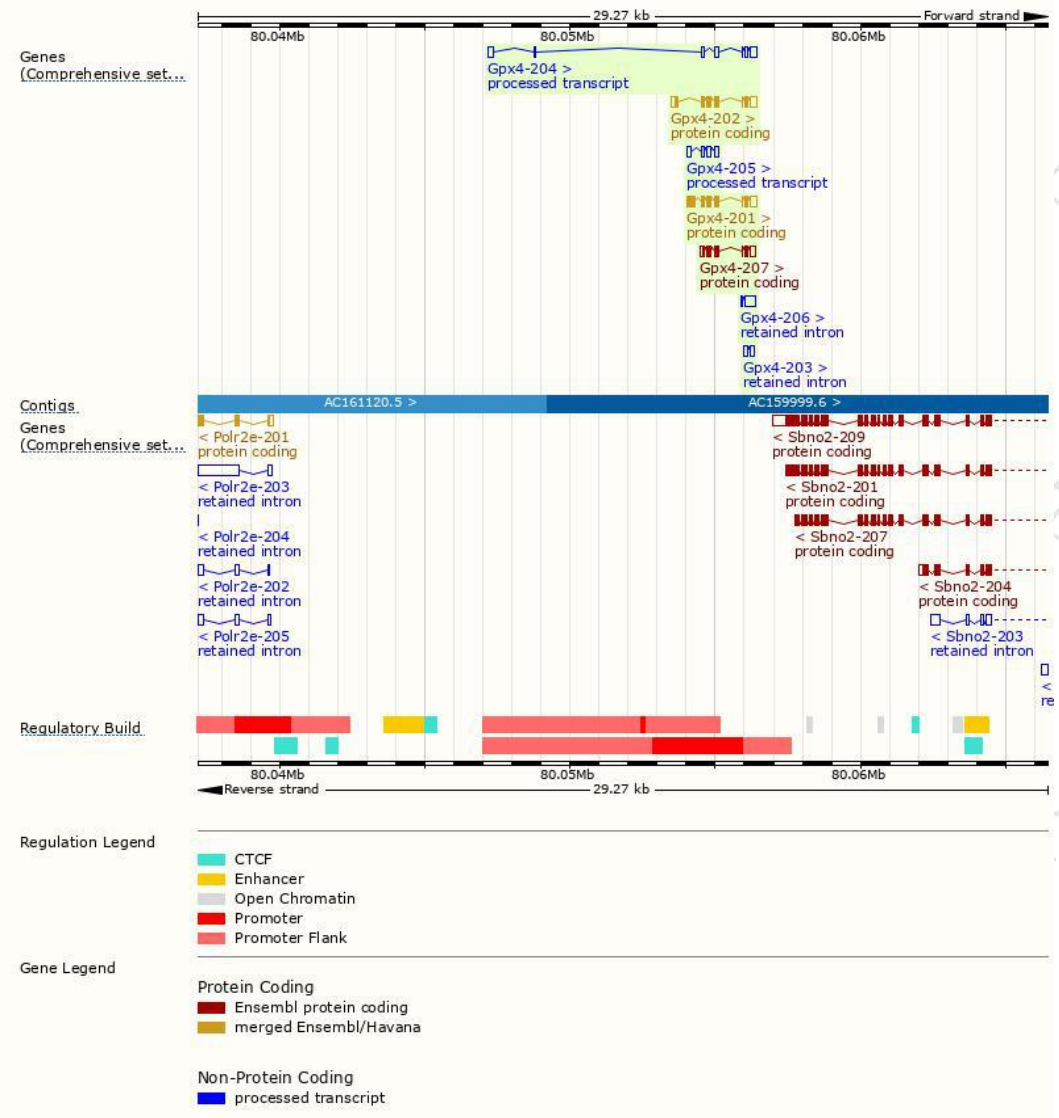
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gpx4-201	ENSMUST00000097227.9	995	253aa	Protein coding	CCDS24007	O70325 Q76LV0	TSL:1 GENCODE basic
Gpx4-202	ENSMUST00000105372.7	959	197aa	Protein coding	CCDS35973	O70325	TSL:1 GENCODE basic APPRIS P1
Gpx4-207	ENSMUST00000183037.1	735	157aa	Protein coding	-	S4R1E5	TSL:2 GENCODE basic
Gpx4-206	ENSMUST00000154095.1	380	No protein	Retained intron	-	-	TSL:2
Gpx4-203	ENSMUST00000136081.1	280	No protein	Retained intron	-	-	TSL:2
Gpx4-204	ENSMUST00000144698.7	879	No protein	lncRNA	-	-	TSL:1
Gpx4-205	ENSMUST00000145703.1	516	No protein	lncRNA	-	-	TSL:3

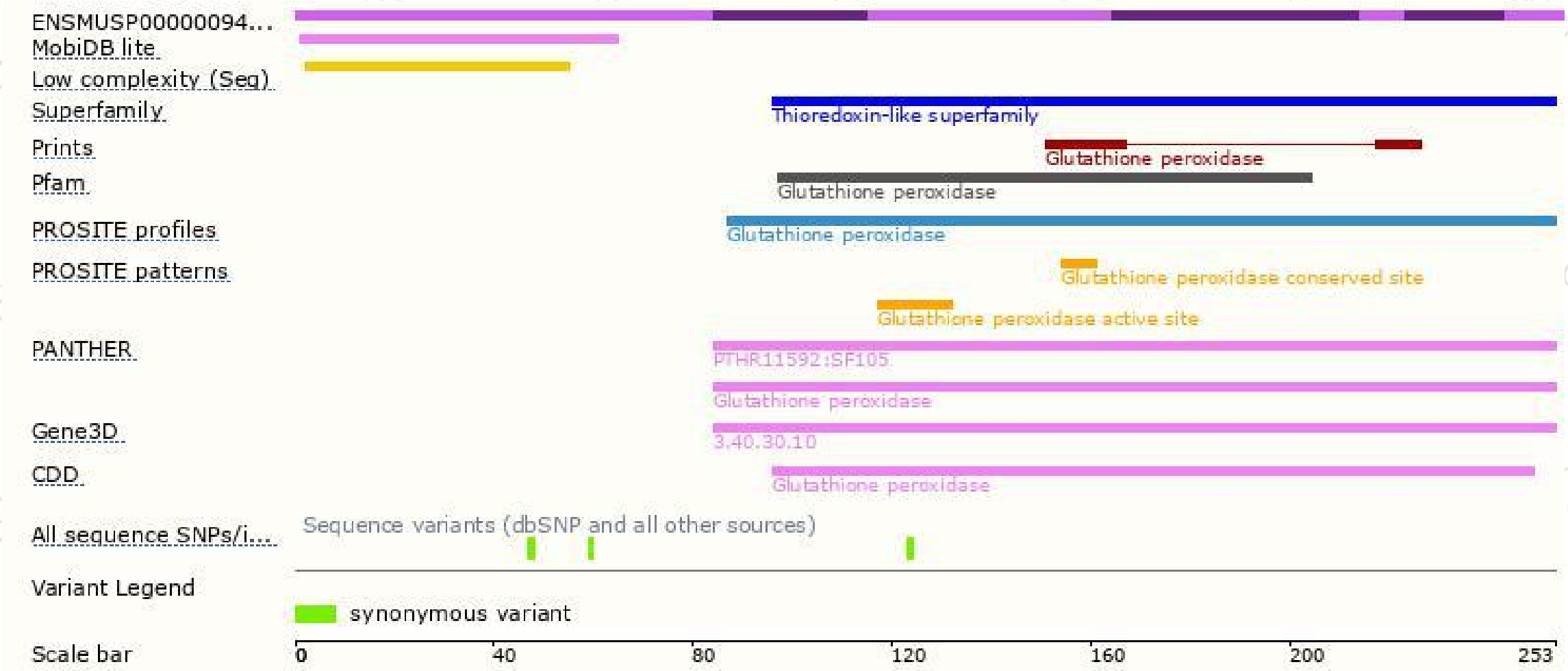
The strategy is based on the design of *Gpx4-201* transcript,the transcription is shown below:



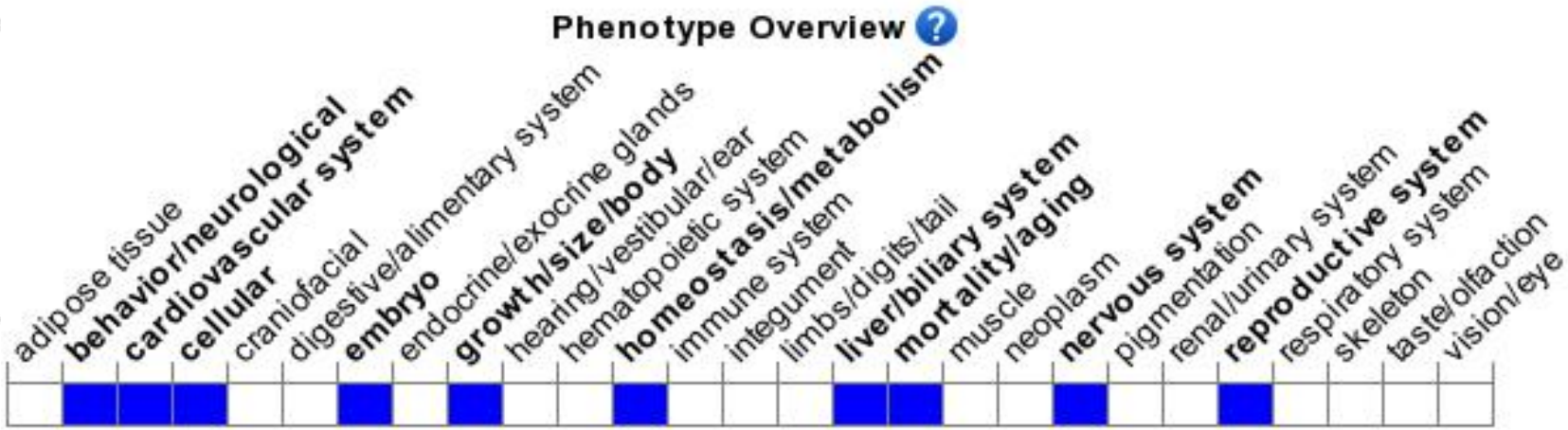
Genomic location distribution



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,gastrulation is impaired and homozygous mutant embryos consequently die during early embryonic development.

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

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