Gk Cas9-CKO Strategy

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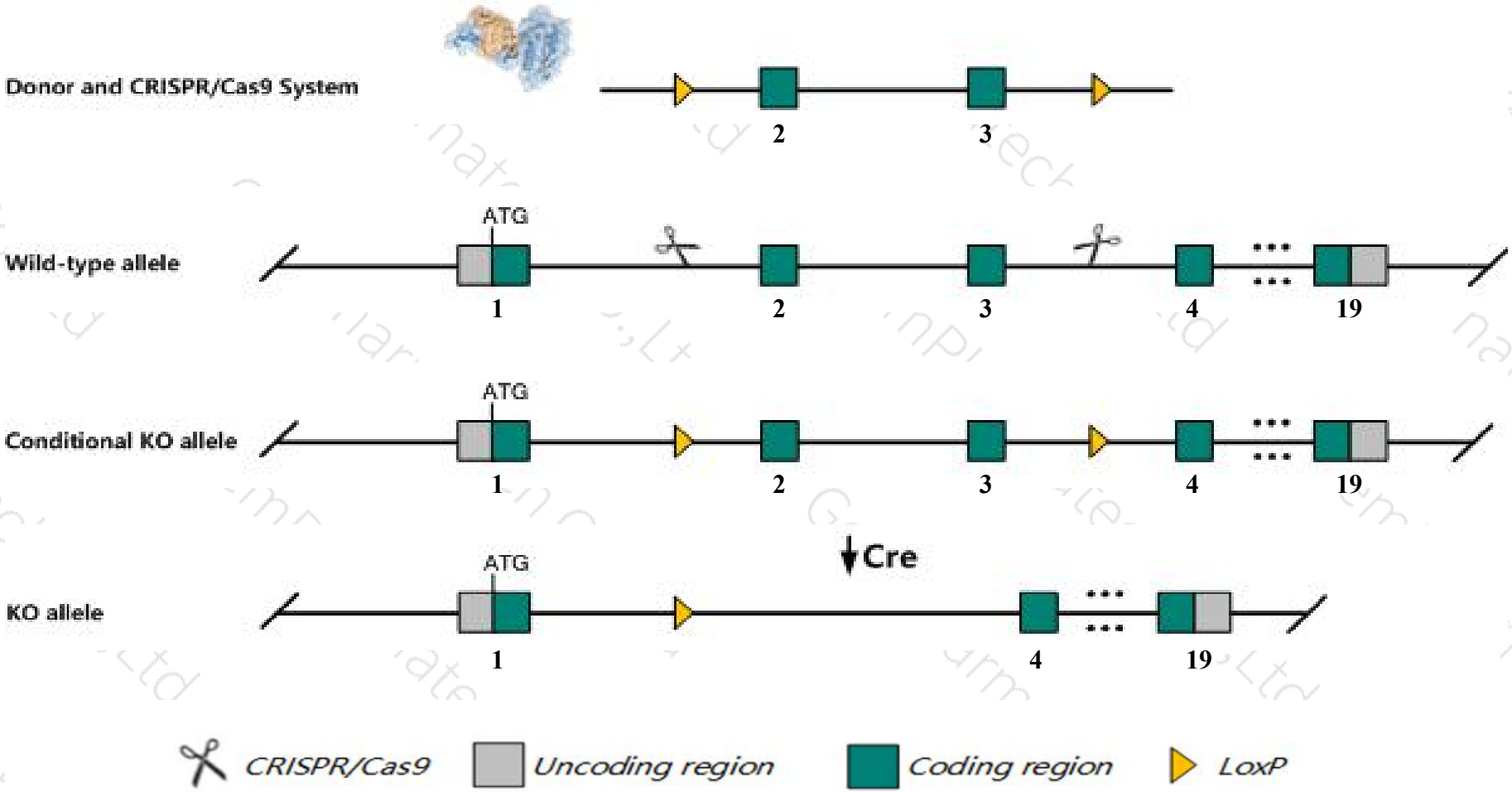
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Gk* gene has 8 transcripts. According to the structure of *Gk* gene, exon2-exon3 of *Gk-201* (ENSMUST00000026039.14) transcript is recommended as the knockout region.The region contains 181bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Gk* 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,Males hemizygous for a targeted null mutation show postnatal growth retardation, altered fat metabolism with severe hyperglycerolemia and increased free fatty acids, autonomous glucocorticoid synthesis and death by 4 days of age. Female heterozygotes show reduced free fatty acid and glucose levels.

- The *Gk* 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 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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Gk glycerol kinase [Mus musculus (house mouse)]

Gene ID: 14933, updated on 31-Jan-2019

Summary

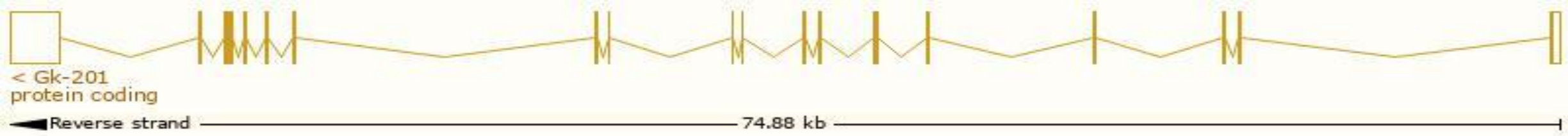
Official Symbol	Gk provided by MGI
Official Full Name	glycerol kinase provided byMGI
Primary source	MGI:MGI:106594
See related	Ensembl:ENSMUSG00000025059
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	D930012N15Rik, Gyk
Expression	Broad expression in kidney adult (RPKM 16.2), liver E18 (RPKM 11.4) and 17 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

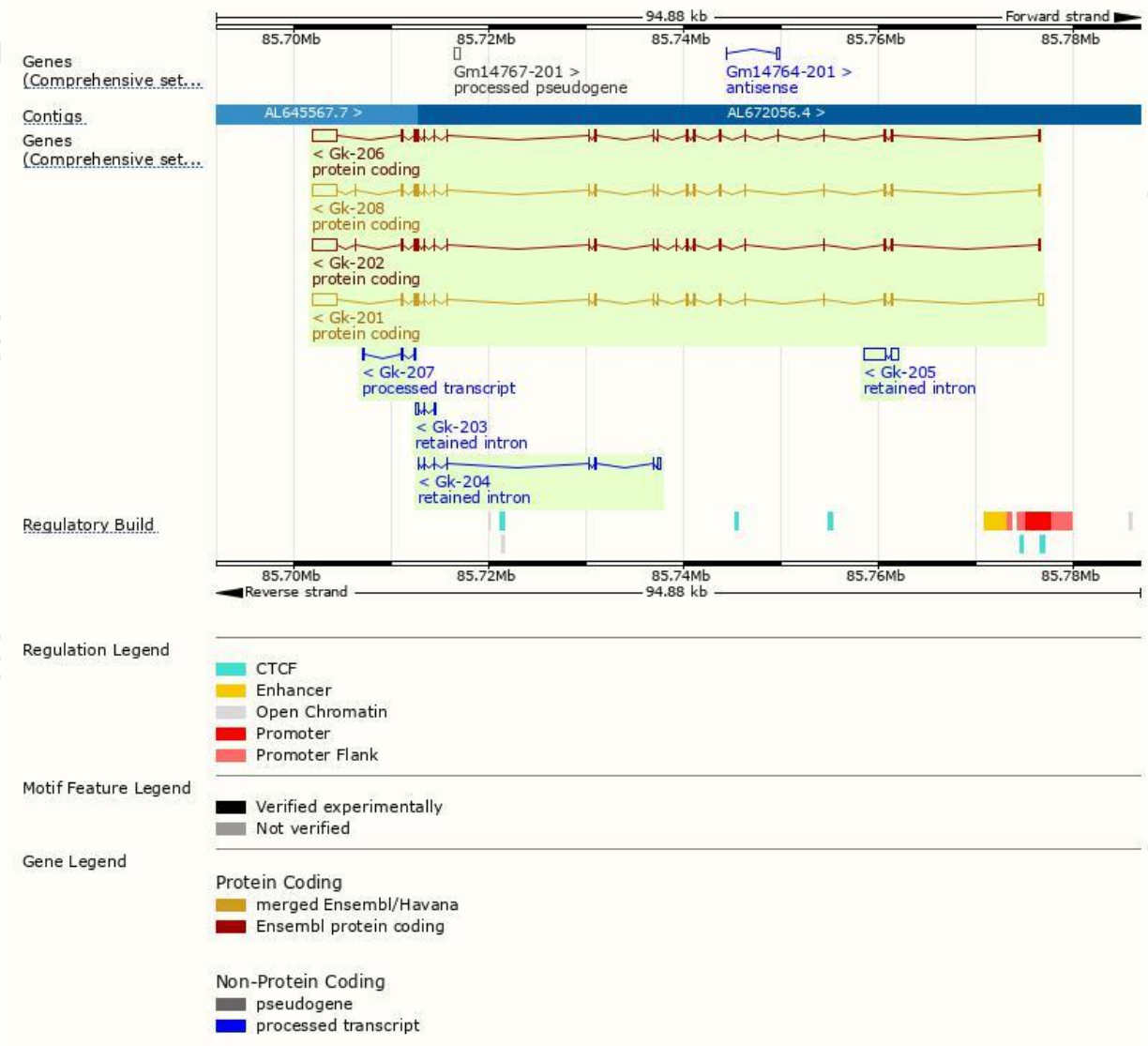
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gk-201	ENSMUST00000026039.14	4340	524aa	Protein coding	CCDS53126	Q64516	TSL:1 GENCODE basic
Gk-202	ENSMUST00000113978.8	4279	559aa	Protein coding	CCDS81149	Q64516	TSL:1 GENCODE basic APPRIS ALT1
Gk-208	ENSMUST00000156390.7	4245	553aa	Protein coding	CCDS41050	Q64516	TSL:1 GENCODE basic APPRIS P3
Gk-206	ENSMUST00000142152.1	4229	552aa	Protein coding	CCDS81150	B1ASZ3	TSL:1 GENCODE basic
Gk-207	ENSMUST00000152221.1	405	No protein	Processed transcript	-	-	TSL:3
Gk-205	ENSMUST00000125081.1	2811	No protein	Retained intron	-	-	TSL:5
Gk-204	ENSMUST00000124386.1	778	No protein	Retained intron	-	-	TSL:5
Gk-203	ENSMUST00000122950.7	527	No protein	Retained intron	-	-	TSL:3

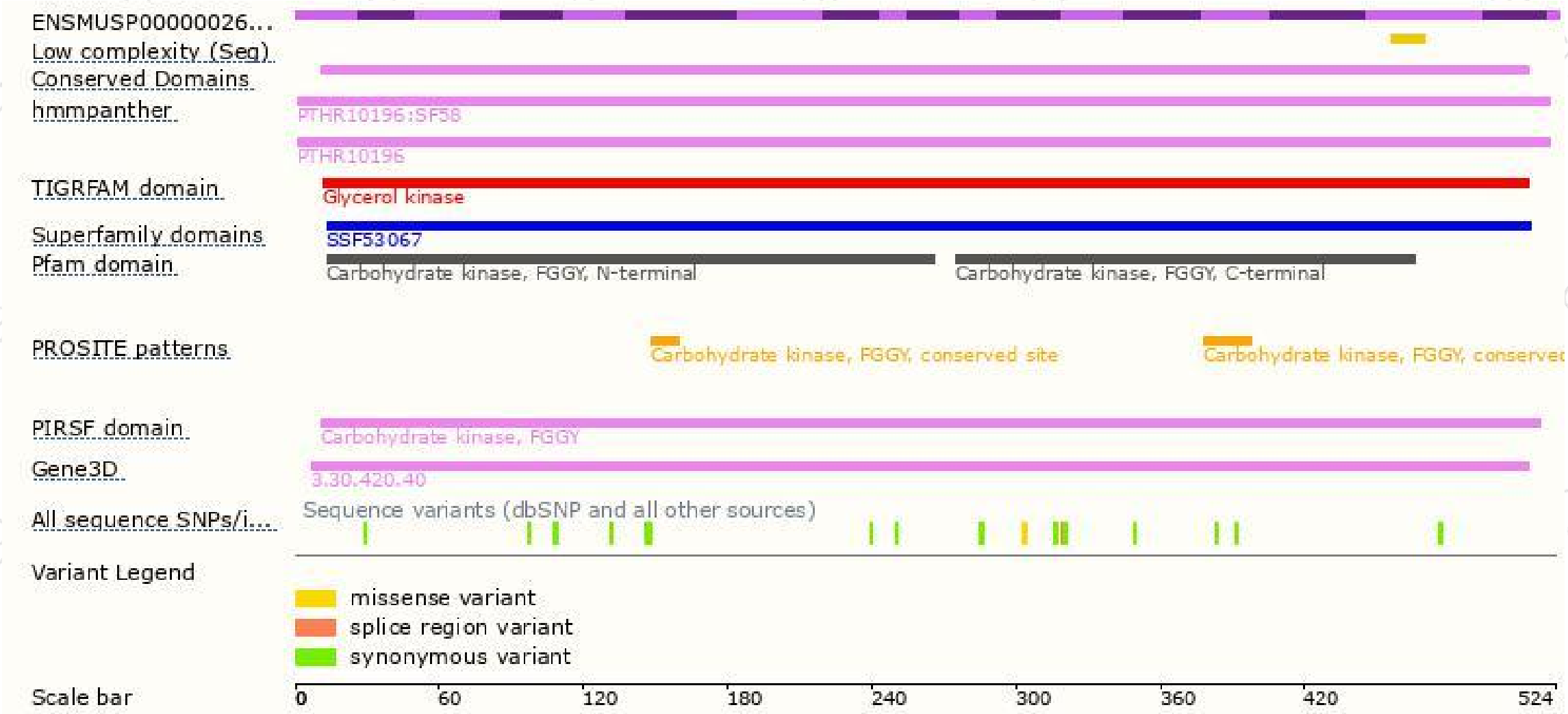
The strategy is based on the design of *Gk-201* transcript,The transcription is shown below



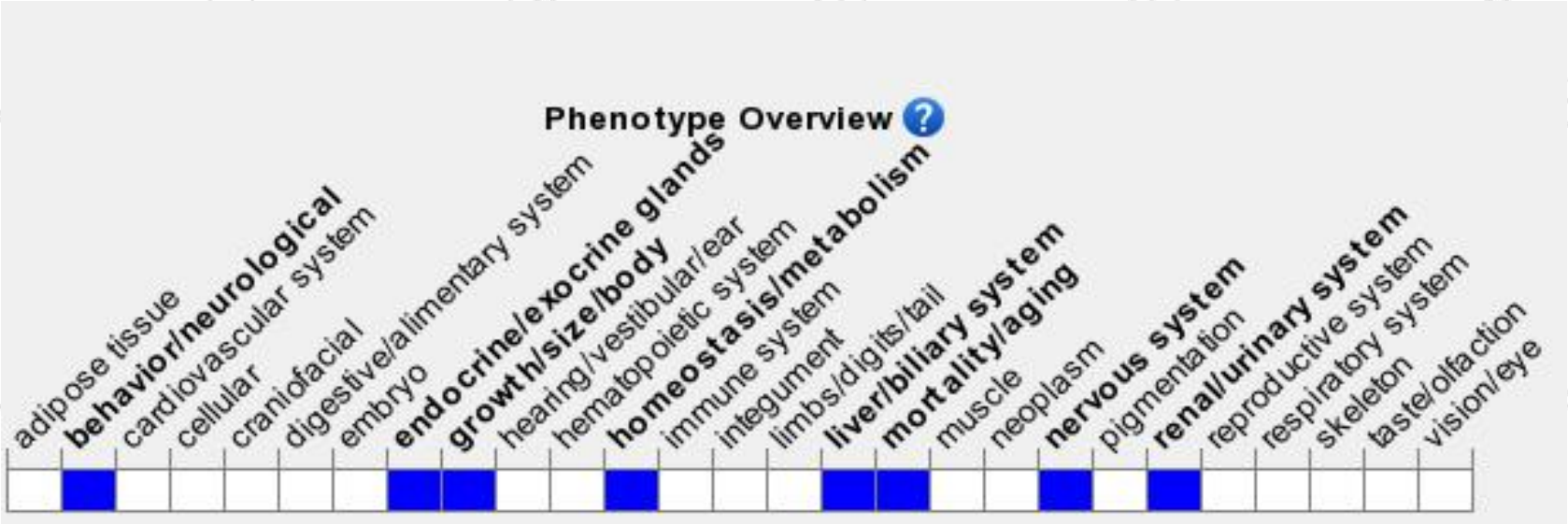
Genomic location distribution



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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