Kbtbd2 Cas9-CKO Strategy

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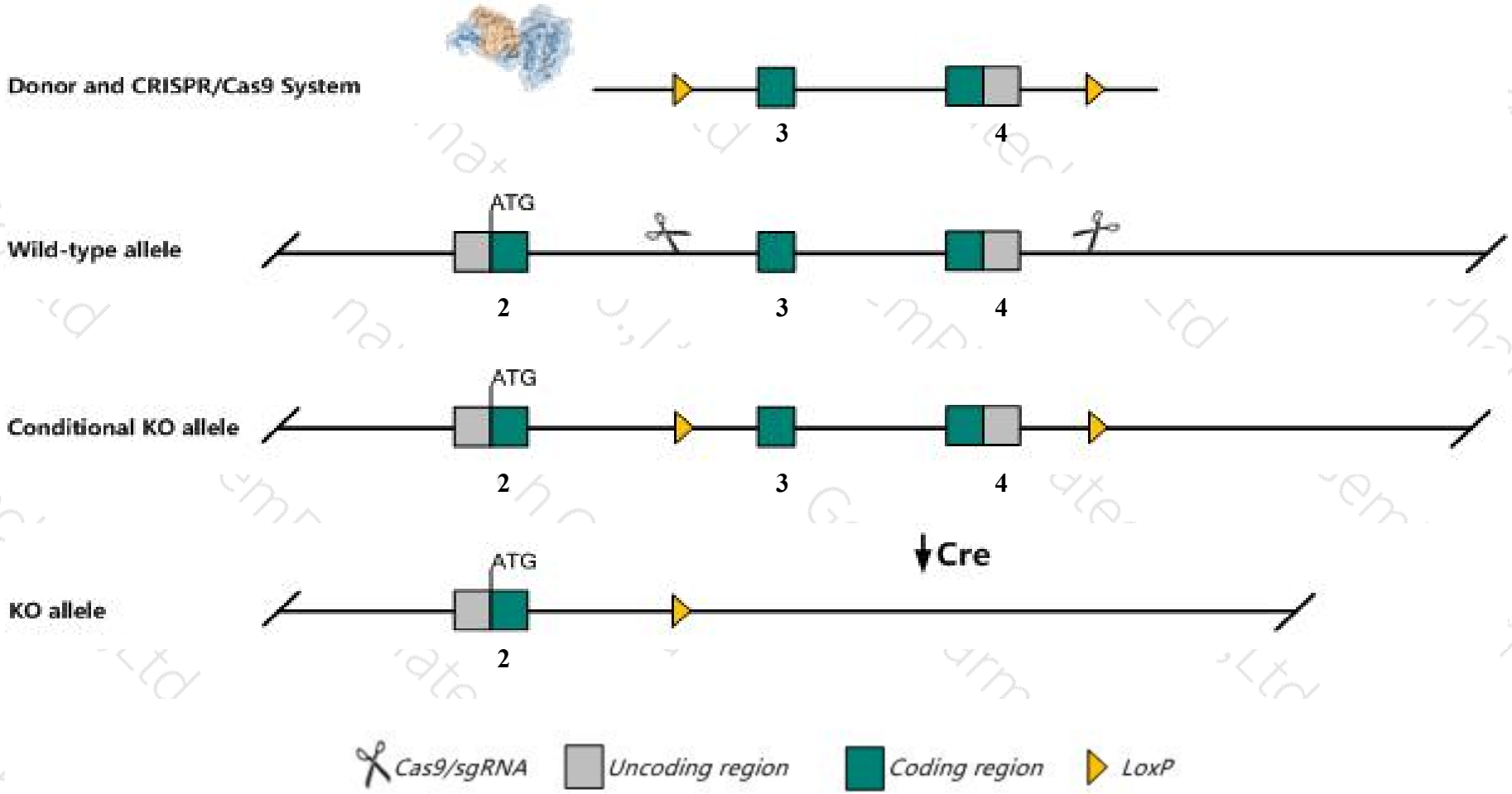
Project Overview

Project Name	***Kbtbd2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Kbtbd2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Kbtbd2* gene has 4 transcripts. According to the structure of *Kbtbd2* gene, exon3-exon4 of *Kbtbd2-201*(ENSMUST00000114321.1) transcript is recommended as the knockout region.The region contains 1702bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Kbtbd2* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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,mice homozygous for a knock-out allele or mutation exhibit diabetes, lipodystrophy, and hepatic steatosis.

➢ The *Kbtbd2* gene is located on the Chr6. 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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Kbtbd2 kelch repeat and BTB (POZ) domain containing 2 [Mus musculus (house mouse)]

Gene ID: 210973, updated on 13-Mar-2020

Summary

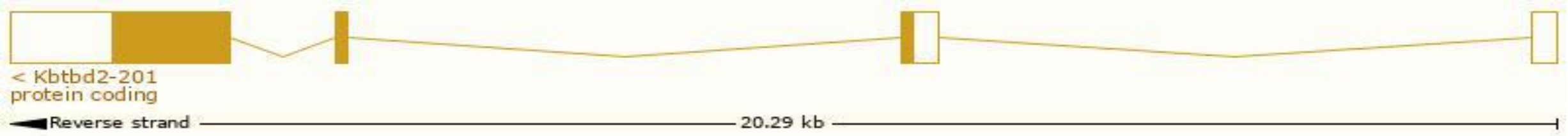
Official Symbol	Kbtbd2 provided by MGI
Official Full Name	kelch repeat and BTB (POZ) domain containing 2 provided byMGI
Primary source	MGI:MGI:2384811
See related	Ensembl:ENSMUSG00000059486
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	BC022962, Bklhd1, mKIAA1489
Expression	Ubiquitous expression in CNS E11.5 (RPKM 8.6), placenta adult (RPKM 8.5) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

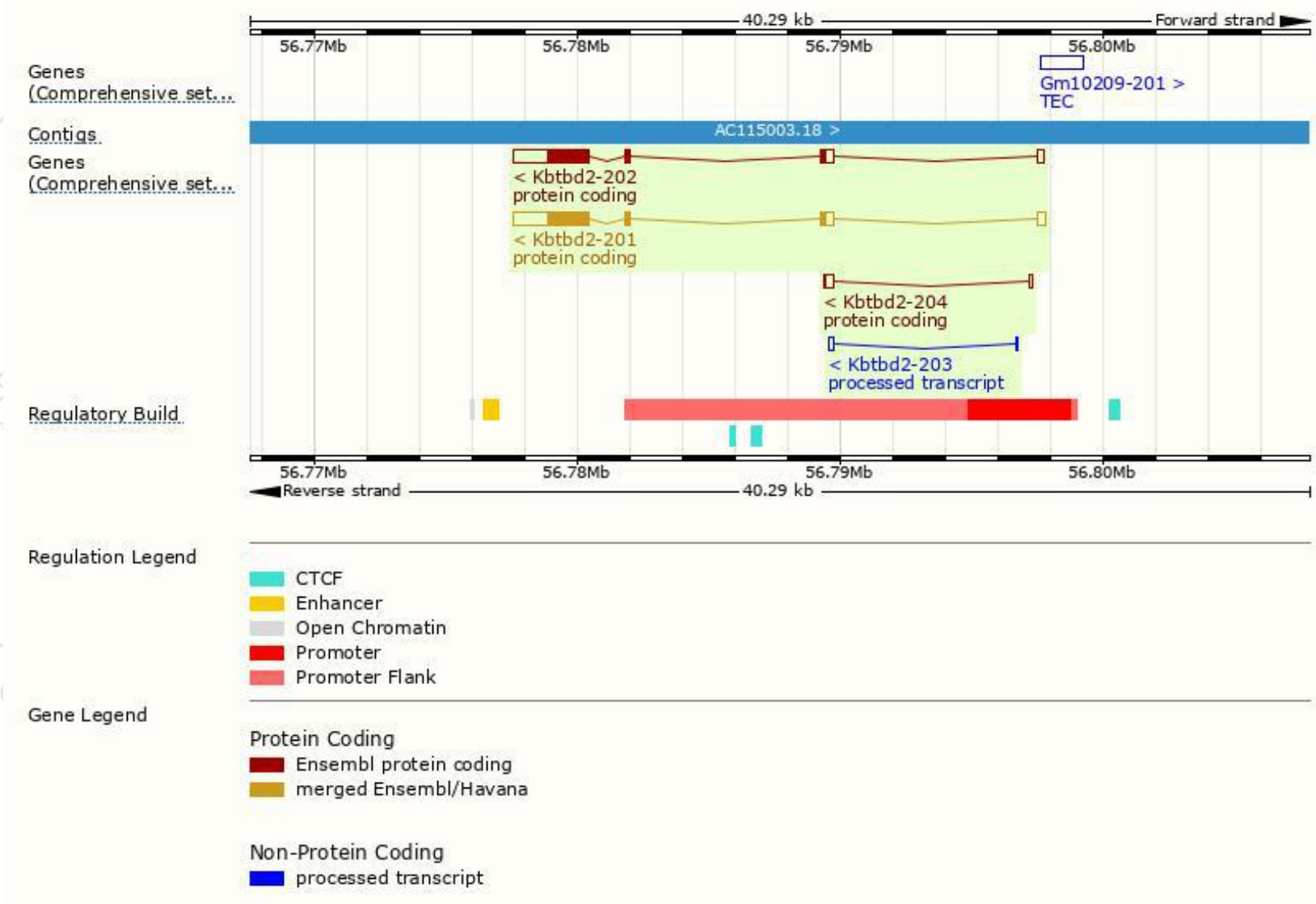
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Kbtbd2-201	ENSMUST00000114321.1	3868	623aa	Protein coding	CCDS51790	G3X9X1	TSL:1 GENCODE basic APPRIS P1
Kbtbd2-202	ENSMUST00000114323.7	3816	623aa	Protein coding	CCDS51790	G3X9X1	TSL:1 GENCODE basic APPRIS P1
Kbtbd2-204	ENSMUST00000151308.1	440	3aa	Protein coding	-	-	CDS 3' incomplete TSL:3
Kbtbd2-203	ENSMUST00000124994.1	251	No protein	Processed transcript	-	-	TSL:3

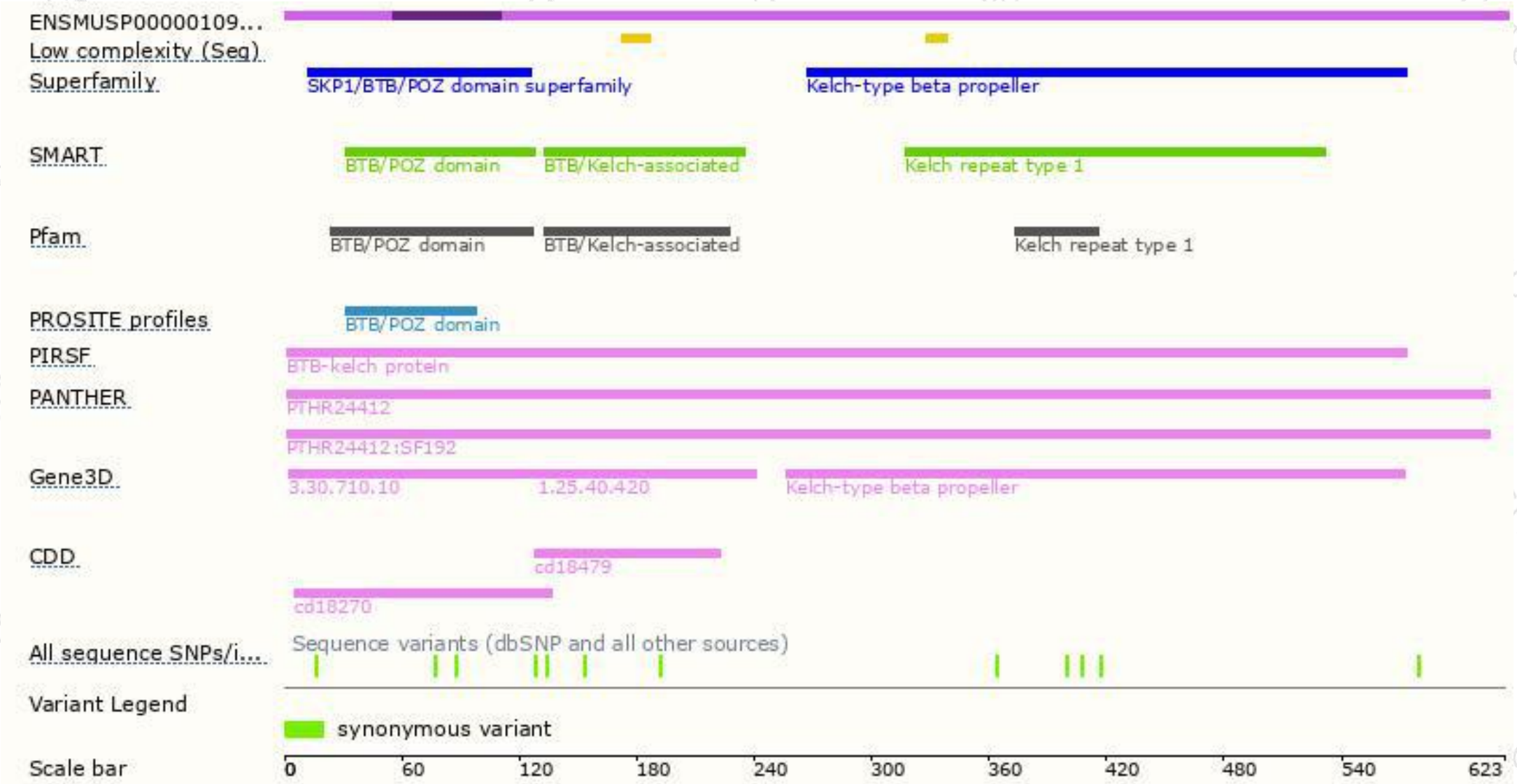
The strategy is based on the design of *Kbtbd2-201* transcript,the transcription is shown below:



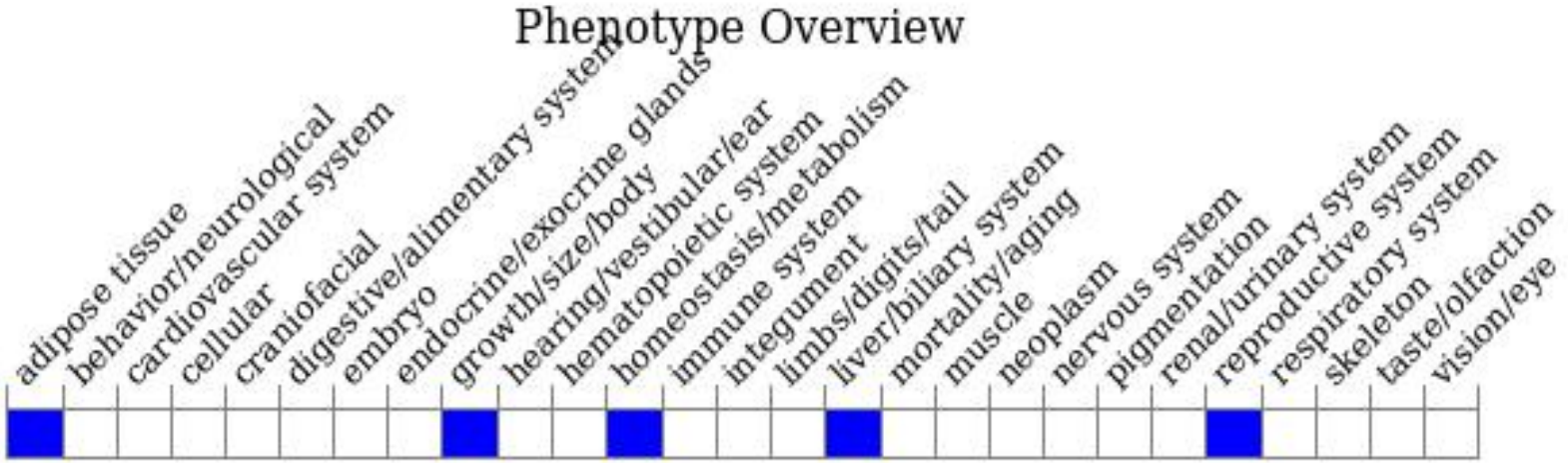
Genomic location distribution



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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