Klhl42 Cas9-CKO Strategy

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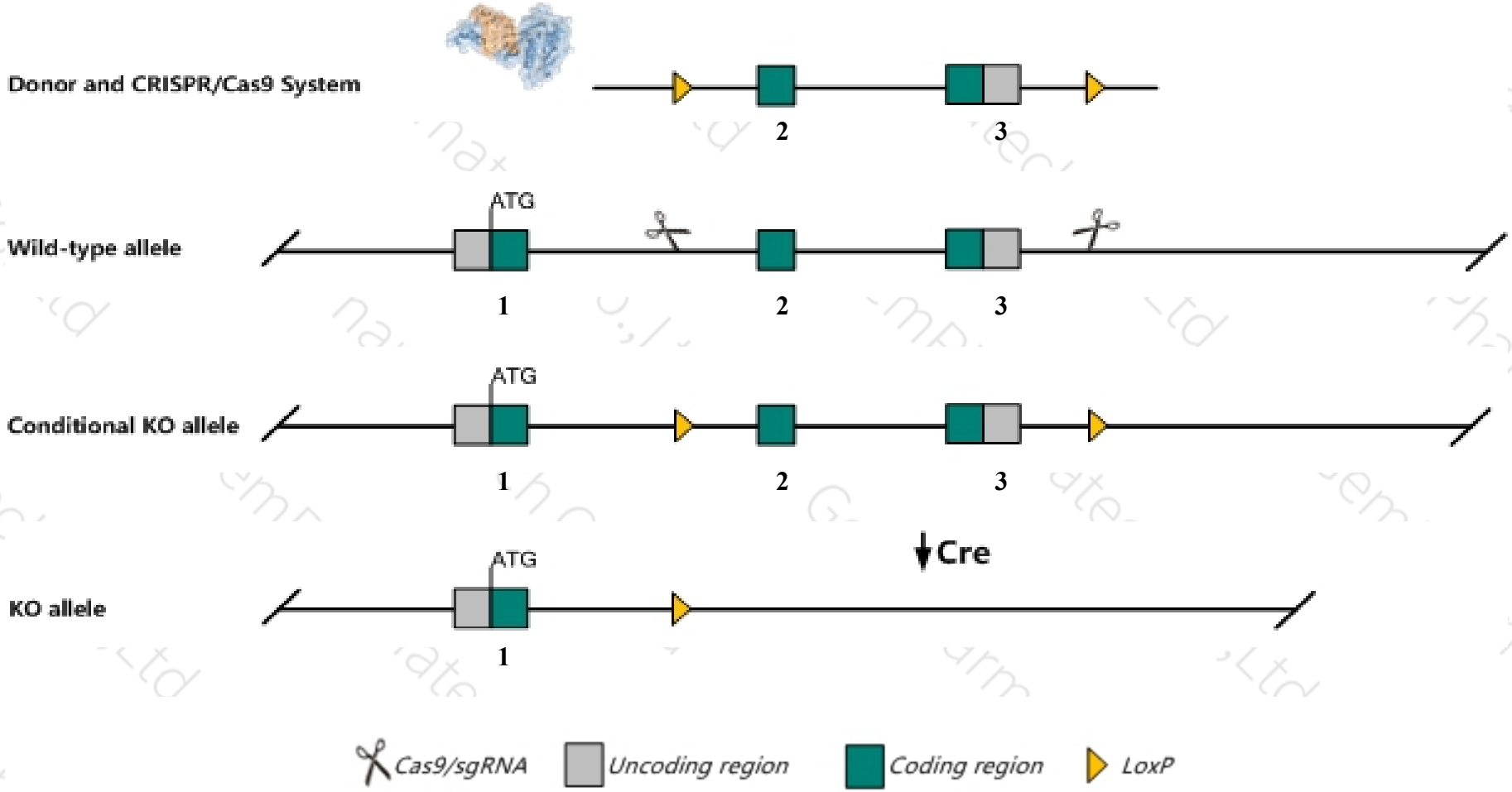
Project Overview

Project Name	***Klhl42***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Klhl42* gene. The schematic diagram is as follows:



Technical routes

➢ The *Klhl42* gene has 3 transcripts. According to the structure of *Klhl42* gene, exon2-exon3 of *Klhl42-201*(ENSMUST00000036003.7) transcript is recommended as the knockout region.The region contains 646bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Klhl42* 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

➢ The *Klhl42* 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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Klhl42 kelch-like 42 [Mus musculus (house mouse)]

Gene ID: 232539, updated on 13-Mar-2020

Summary

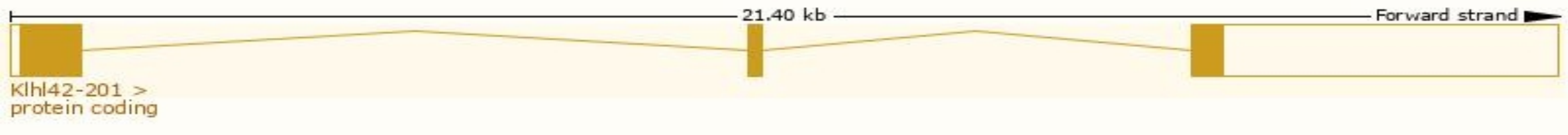
Official Symbol	Klhl42 provided by MGI
Official Full Name	kelch-like 42 provided byMGI
Primary source	MGI:MGI:2444786
See related	Ensembl:ENSMUSG00000040102
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	A330079N23Rik, AI450355, C230080I20Rik, Ctb9, Klhdc5, mKIAA1340
Expression	Ubiquitous expression in ovary adult (RPKM 10.6), adrenal adult (RPKM 8.4) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

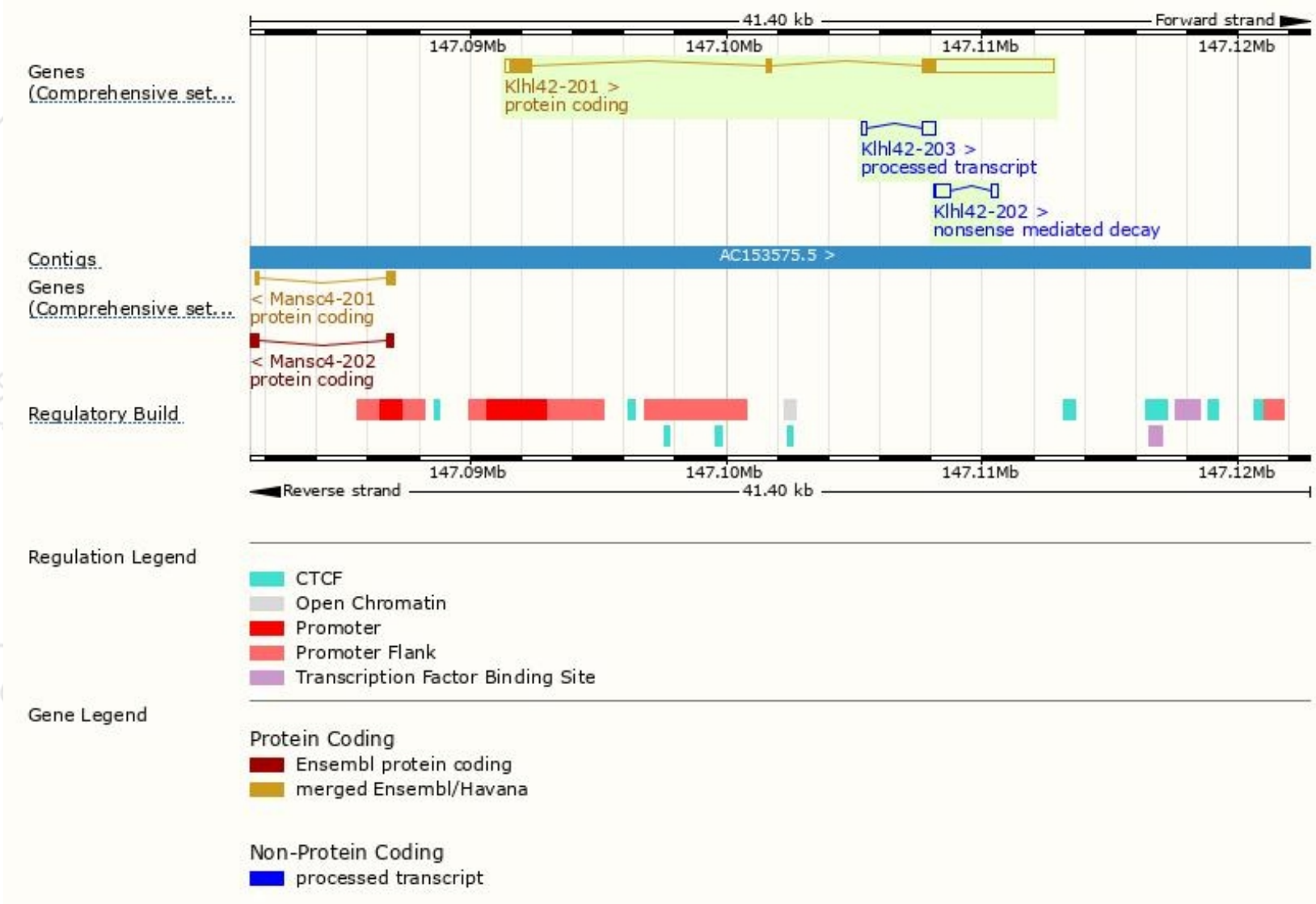
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Klhl42-201	ENSMUST00000036003.7	6267	493aa	Protein coding	CCDS39716	B2KFS7 Q8BFQ9	TSL:1 GENCODE basic APPRIS P1
Klhl42-202	ENSMUST00000203441.1	881	17aa	Nonsense mediated decay	-	A0A0N4SWA9	CDS 5' incomplete TSL:3
Klhl42-203	ENSMUST00000203866.1	660	No protein	Processed transcript	-	-	TSL:5

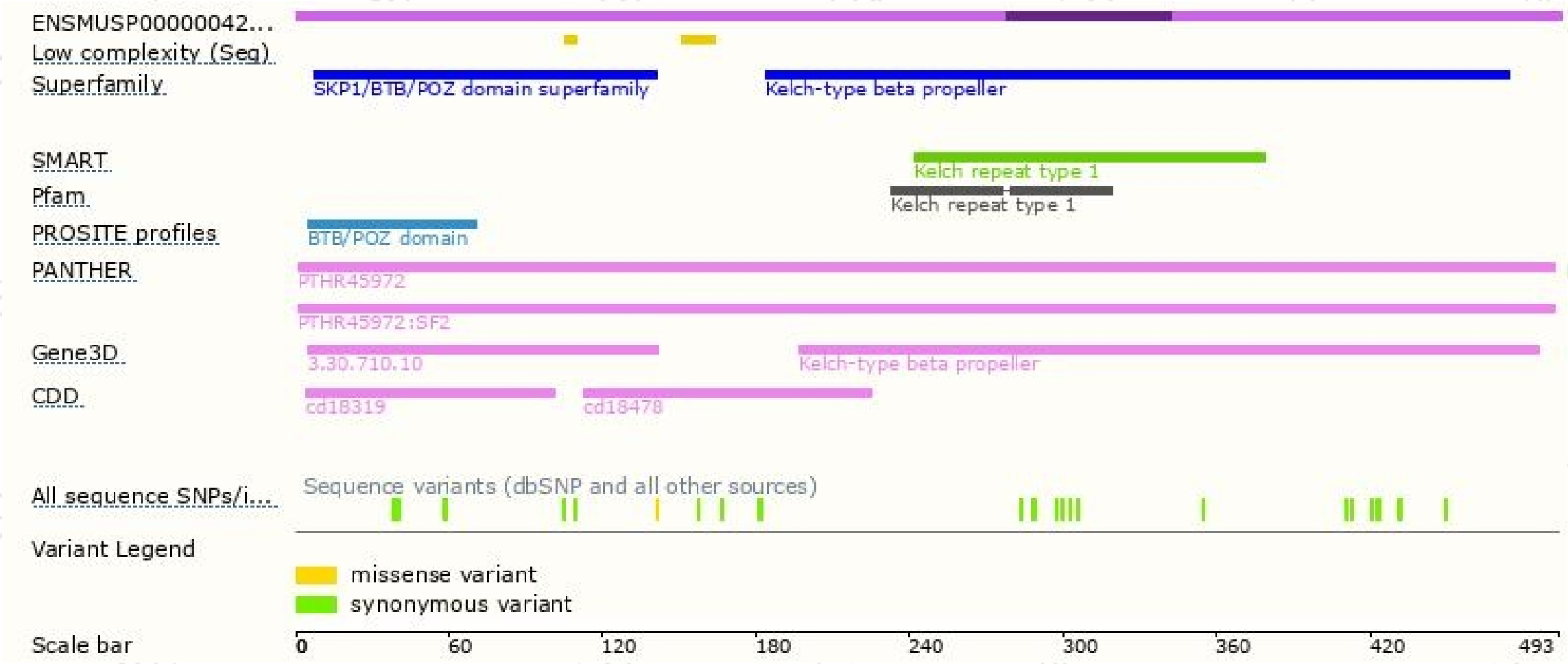
The strategy is based on the design of *Klhl42-201* transcript,the transcription is shown below:



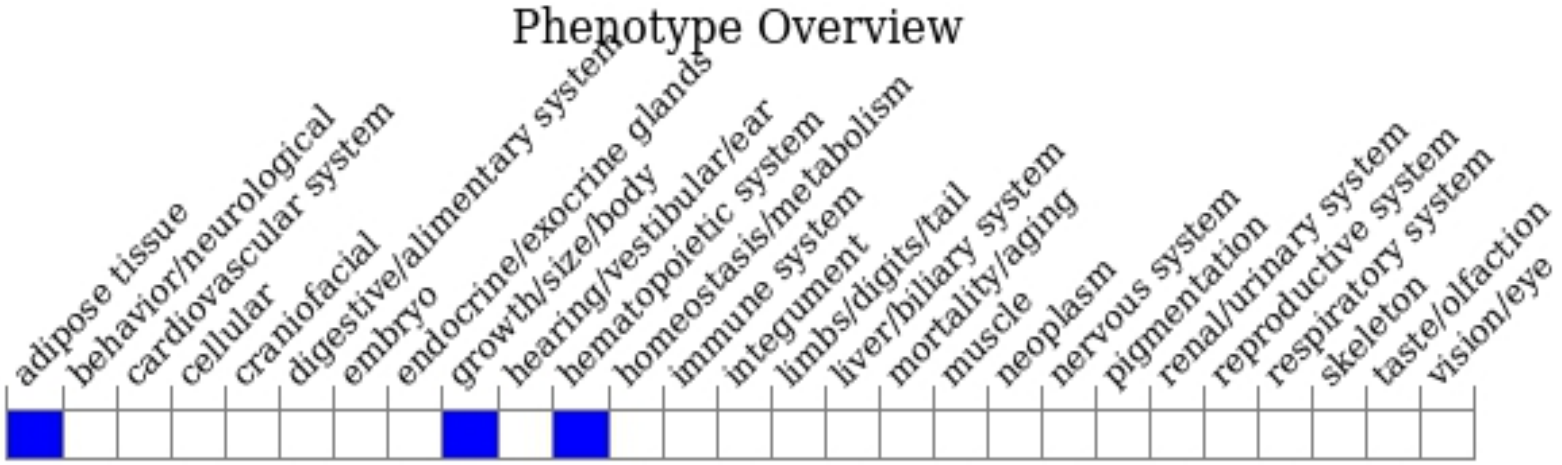
Genomic location distribution



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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