Cldn18 Cas9-CKO Strategy

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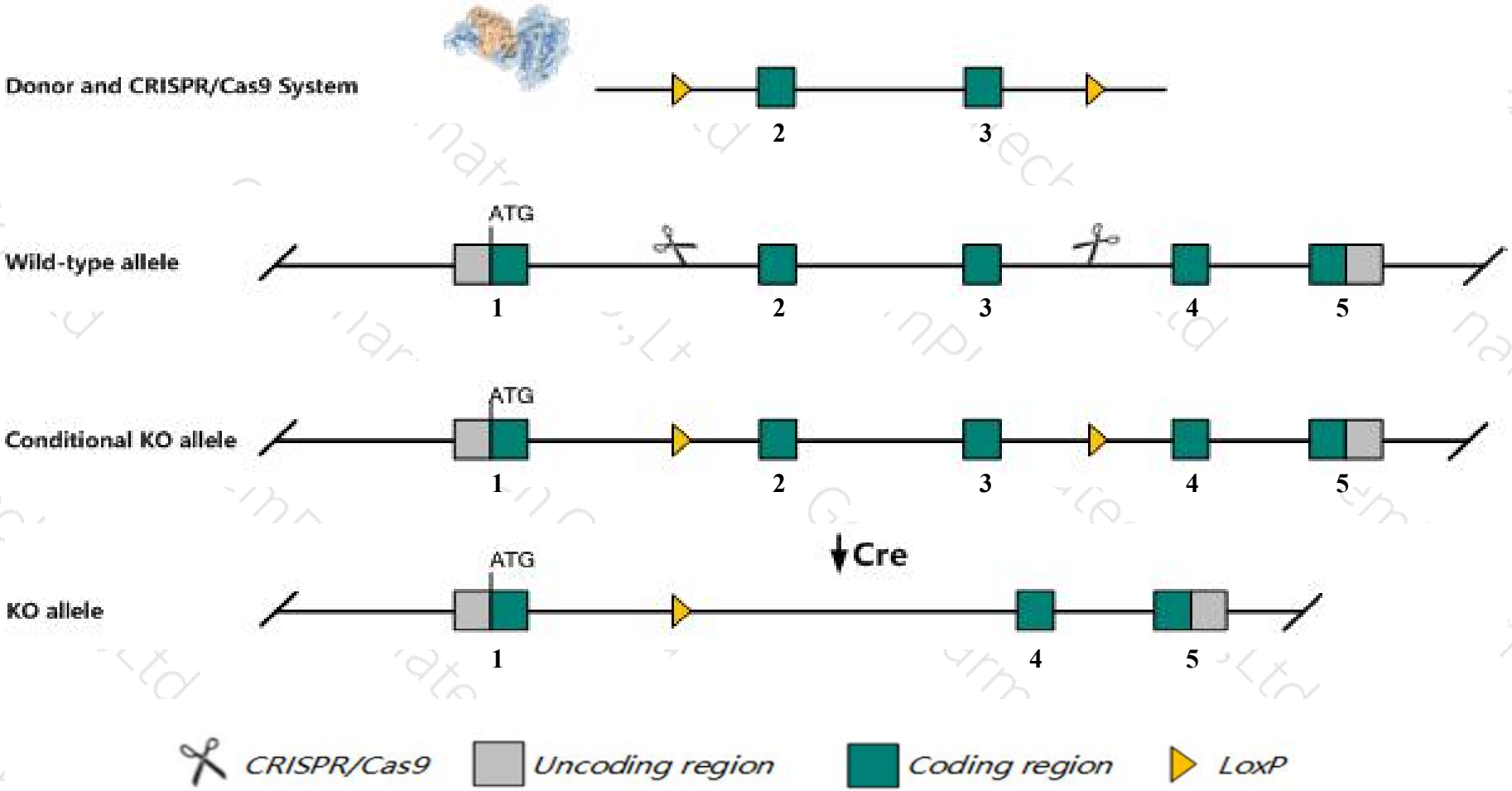
Project Overview

Project Name	***Cldn18***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cldn18* gene. The schematic diagram is as follows:



Technical routes

- The *Cldn18* gene has 4 transcripts. According to the structure of *Cldn18* gene, exon2-exon3 of *Cldn18-201* (ENSMUST00000035048.11) transcript is recommended as the knockout region.The region contains 292bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Cldn18* 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,Mice homozygous for a knock-out allele exhibit increased bone resorption and osteoclast differentiation. Homozygotes for another knock-out allele have impaired alveolarization and alveolar epithelial barrier function.

➢ The *Cldn18* gene is located on the Chr9. 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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Cldn18 claudin 18 [Mus musculus (house mouse)]

Gene ID: 56492, updated on 31-Jan-2019

Summary

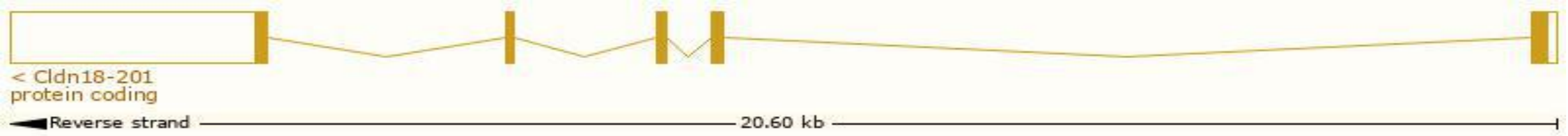
Official Symbol	Cldn18 provided by MGI
Official Full Name	claudin 18 provided by MGI
Primary source	MGI:MGI:1929209
See related	Ensembl:ENSMUSG00000032473
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Summary	This gene encodes a member of the claudin family. Claudins are integral membrane proteins and components of tight junction strands. Tight junction strands serve as a physical barrier to prevent solutes and water from passing freely through the paracellular space between epithelial or endothelial cell sheets, and also play critical roles in maintaining cell polarity and signal transductions. This gene is a downstream target gene regulated by the T/EBP/NKX2.1 homeodomain transcription factor. Four alternatively spliced transcript variants resulted from alternative promoters and alternative splicing have been identified, which encode two lung-specific isoforms and two stomach-specific isoforms respectively. This gene is also expressed in colons, inner ear and skin, and its expression is increased in both experimental colitis and ulcerative colitis. [provided by RefSeq, Aug 2010]
Expression	Biased expression in lung adult (RPKM 177.1), stomach adult (RPKM 118.5) and 1 other tissue See more
Orthologs	human all

Transcript information（Ensembl）

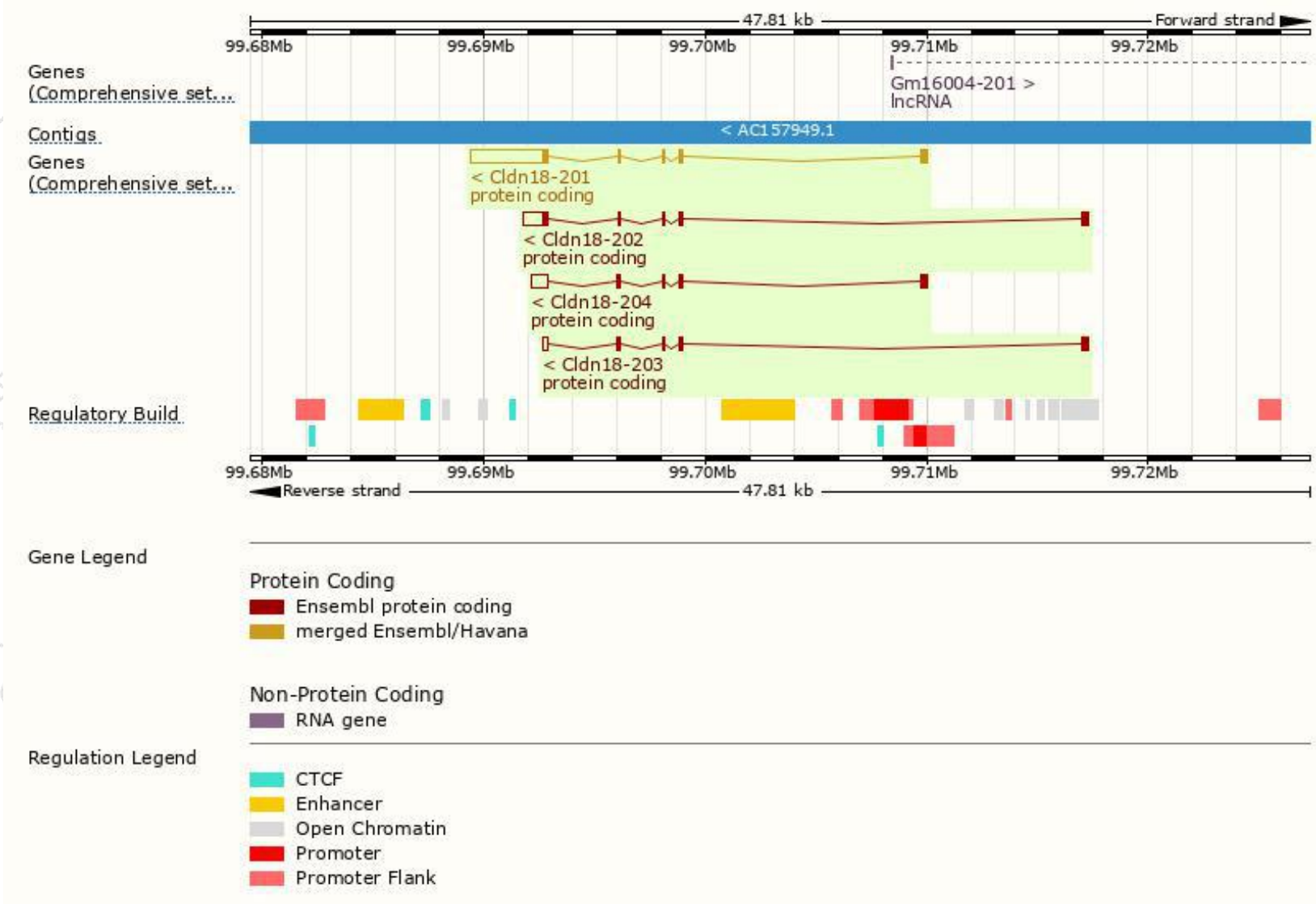
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cldn18-201	ENSMUST00000035048.11	4172	264aa	Protein coding	CCDS23437	P56857	TSL:1 GENCODE basic APPRIS P3
Cldn18-202	ENSMUST00000112882.8	1750	264aa	Protein coding	CCDS57694	P56857	TSL:1 GENCODE basic APPRIS ALT1
Cldn18-204	ENSMUST00000136429.7	1409	208aa	Protein coding	CCDS57692	P56857	TSL:1 GENCODE basic
Cldn18-203	ENSMUST00000131922.1	860	208aa	Protein coding	CCDS57693	P56857	TSL:1 GENCODE basic

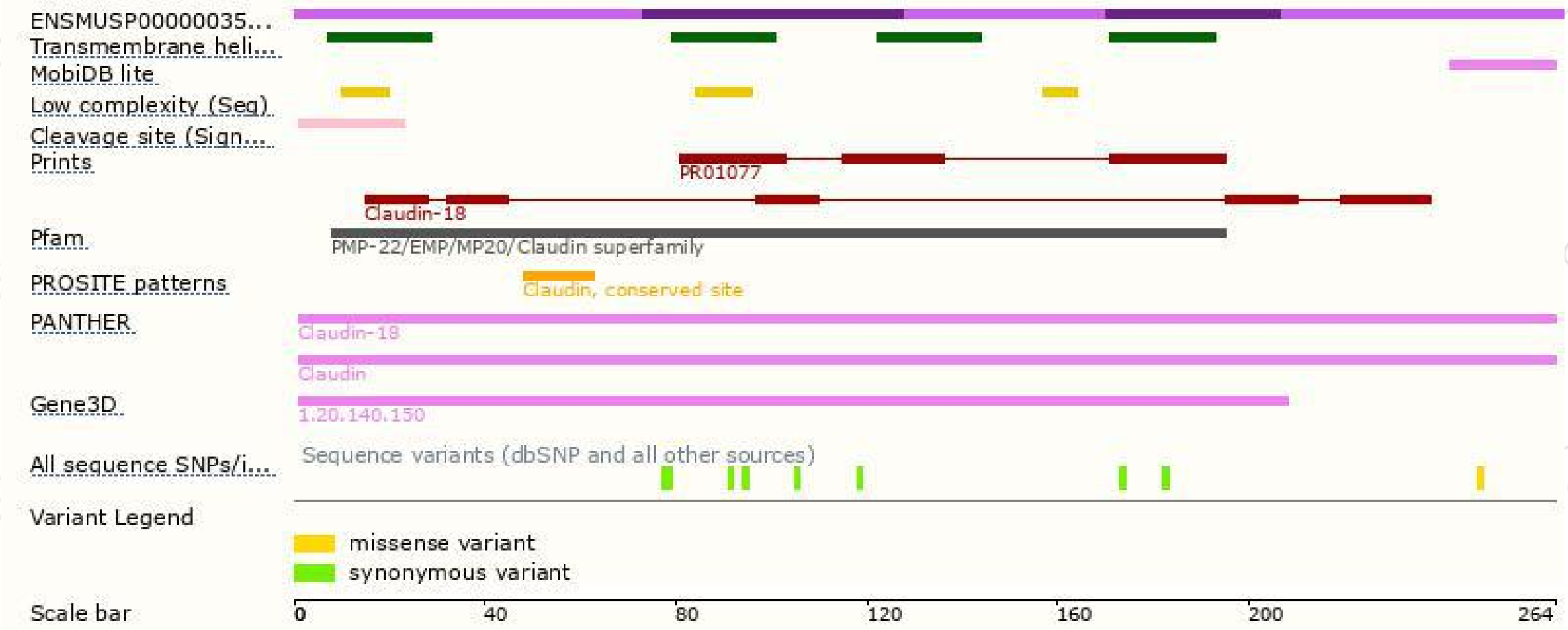
The strategy is based on the design of *Cldn18-201* transcript,The transcription is shown below



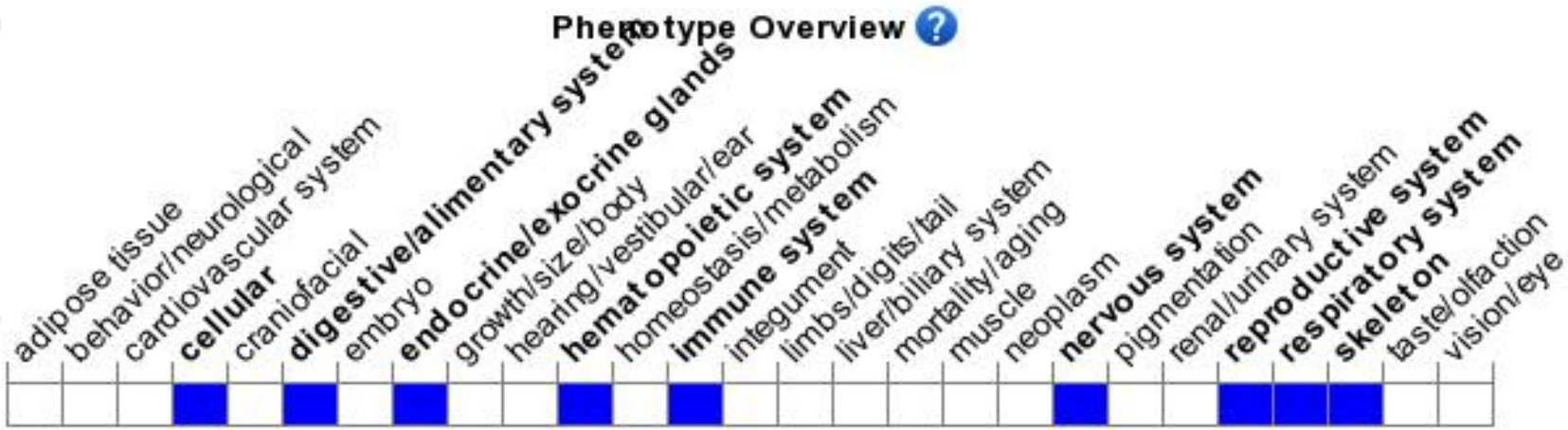
Genomic location distribution



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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