Cldn2 Cas9-CKO *Strategy*

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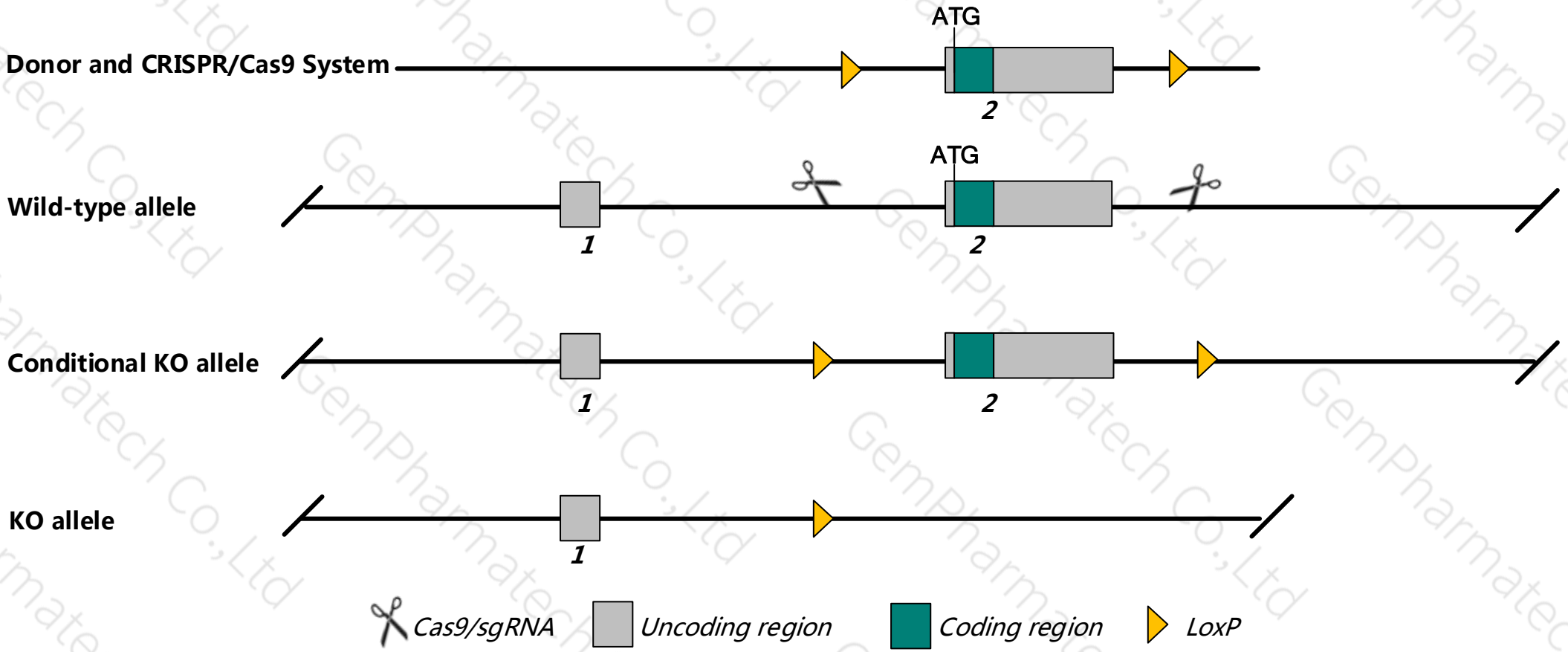
Project Overview

Project Name	***Cldn2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cldn2* gene. The schematic diagram is as follows:



Technical routes

- The Cldn2 gene has 3 transcripts.According to the structure of Cldn2 gene, exon2 of Cldn2-201 (ENSMUST00000054889.3)transcript is recommended as the knockout region.The region contains all the coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Cldn2* 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 or cell types.

Notice

- According to the existing MGI data，nullizygous females show altered Na+ and water reabsorption in the kidney proximal tubules. Males hemizygous for a null allele show increased transcellular Na+ reabsorption in the thick ascending limb, higher renal oxygen consumption, medullary hypoxia, and susceptibility to ischemic renal injury.
- The *Cldn2* 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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Cldn2 claudin 2 [*Mus musculus* (house mouse)]

Gene ID: 12738, updated on 12-Nov-2019

Summary

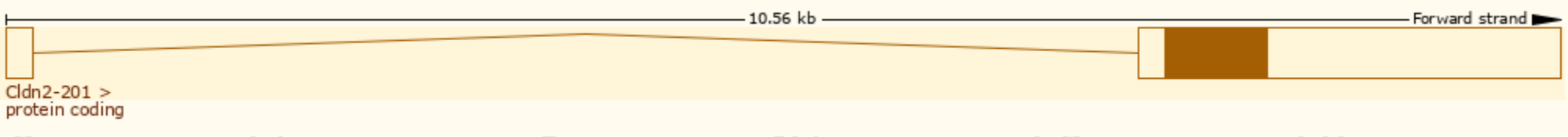
Official Symbol	Cldn2 provided by MGI
Official Full Name	claudin 2 provided by MGI
Primary source	MGI:MGI:1276110
See related	Ensembl:ENSMUSG00000047230
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AL022813
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. The knockout mice lacking this gene display normal appearance, activity, growth and behavior, but are defective in the leaky and cation-selective paracellular permeability properties of renal proximal tubules. The proteins encoded by this gene and another family member Cldn12 are also critical for vitamin D-dependent Ca2+ absorption between enterocytes. [provided by RefSeq, Aug 2010]
Expression	Biased expression in kidney adult (RPKM 78.3), genital fat pad adult (RPKM 58.9) and 6 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

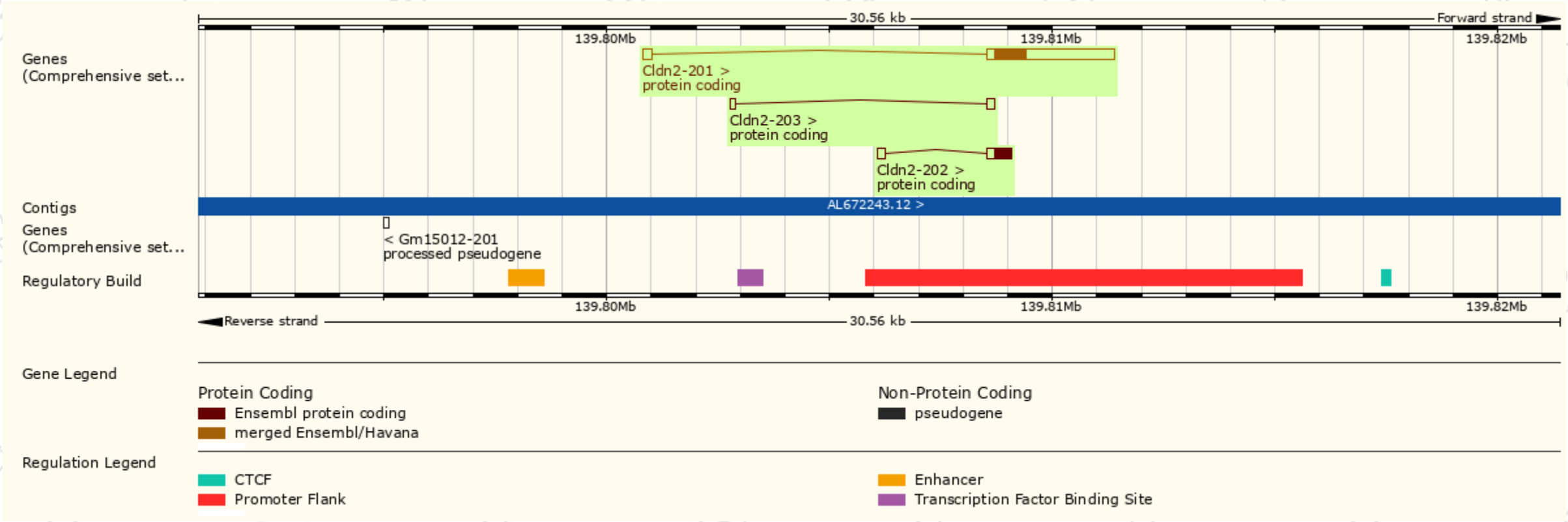
The gene has 3 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cldn2-201	ENSMUST00000054889.3	3044	230aa	Protein coding	CCDS30437	O88552	TSL:1 GENCODE basic APPRIS P1
Cldn2-202	ENSMUST00000135224.1	755	131aa	Protein coding	-	A3KGB5	CDS 3' incomplete TSL:3
Cldn2-203	ENSMUST00000172779.1	290	2aa	Protein coding	-	-	CDS 3' incomplete TSL:3

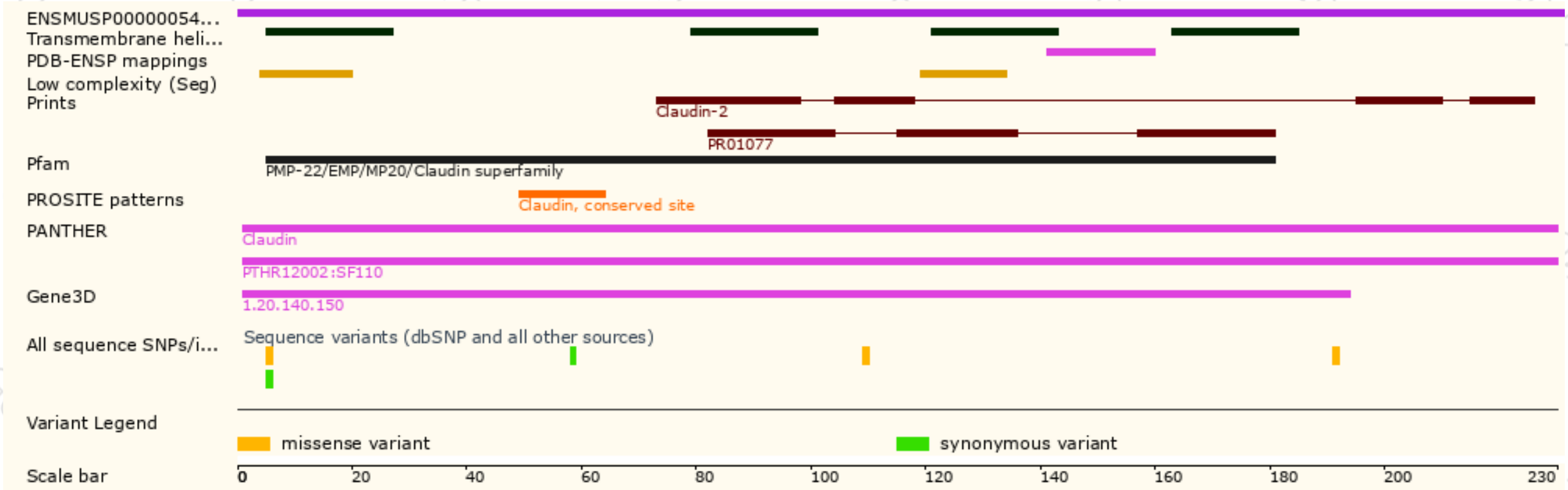
The strategy is based on the design of *Cldn2*-201 transcript,the transcription is shown below:



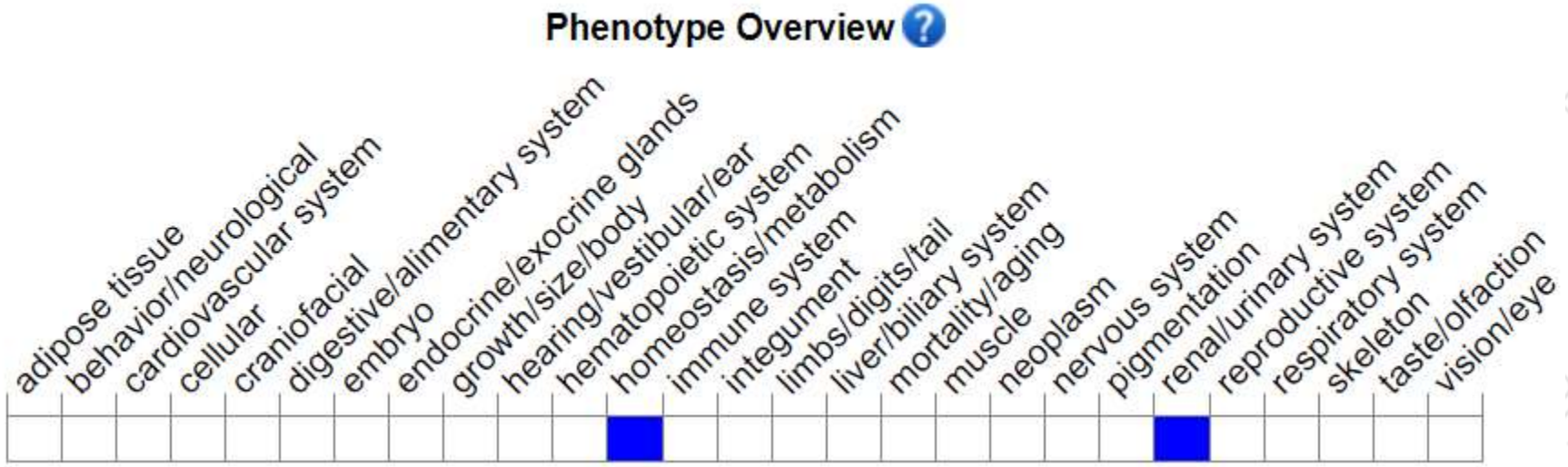
Genomic location distribution



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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