Slc12a3 Cas9-CKO Strategy

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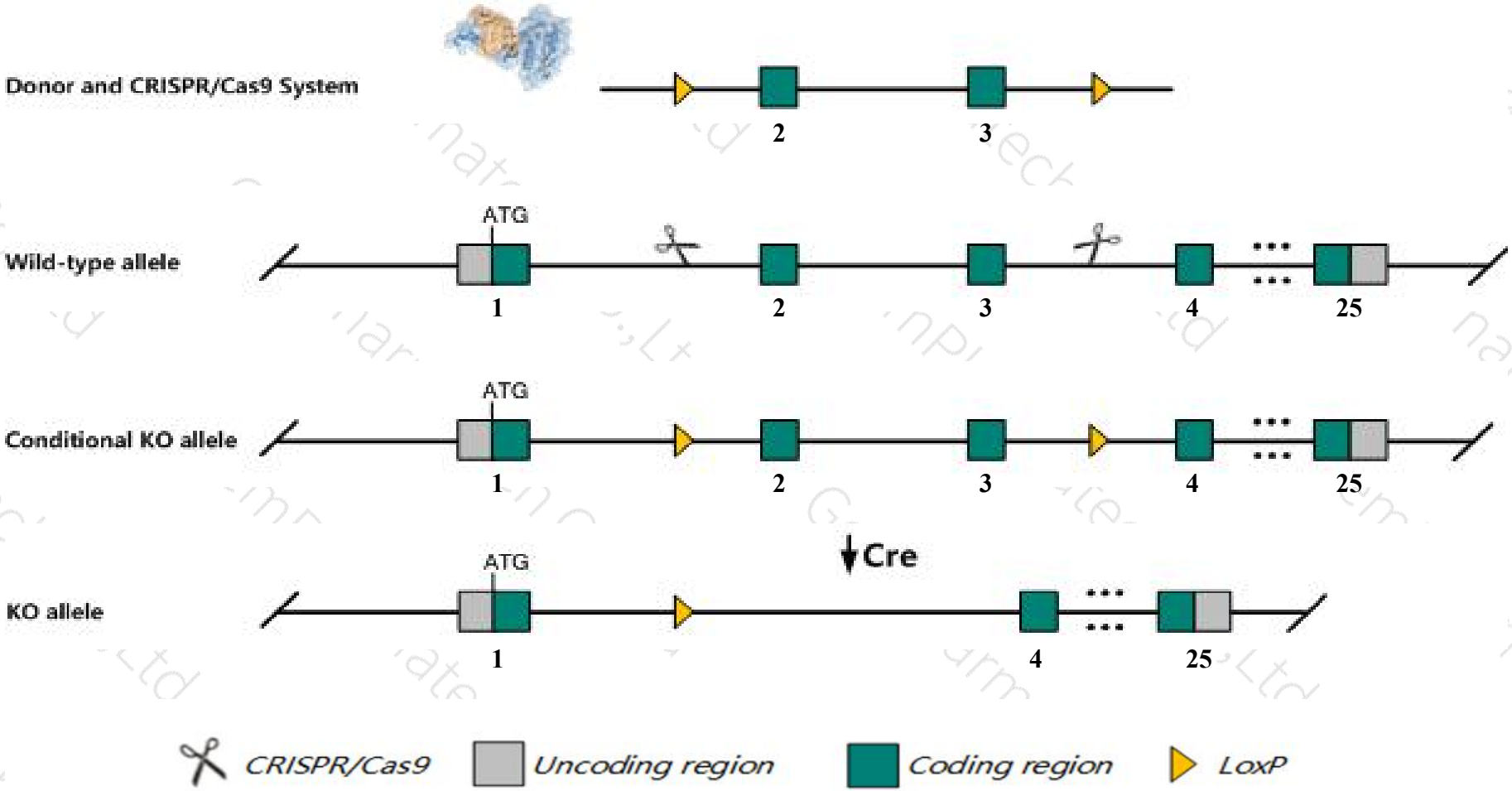
Project Overview

Project Name	***Slc12a3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Slc12a3* gene. The schematic diagram is as follows:



Technical routes

- The *Slc12a3* gene has 7 transcripts. According to the structure of *Slc12a3* gene, exon2-exon3 of *Slc12a3-201* (ENSMUST00000034218.4) transcript is recommended as the knockout region.The region contains 223bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Slc12a3* 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 hypomagnesemia, hypocalciurua and abnormal renal distal convoluted tubule morphology, and show significantly reduced arterial blood pressure on a sodium-depleted diet. Mutant kidney cortical collecting ductsdisplay thiazide-sensitive NaCl absorption.
- Transcript *Slc12a3-202* may not be affected.
- The *Slc12a3* gene is located on the Chr8. 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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Slc12a3 solute carrier family 12, member 3 [Mus musculus (house mouse)]

Gene ID: 20497, updated on 19-Mar-2019

Summary

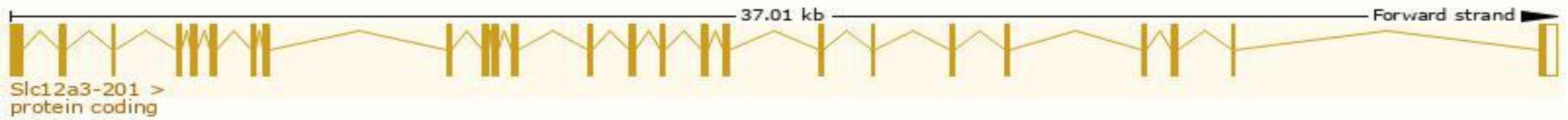
Official Symbol	Slc12a3 provided by MGI
Official Full Name	solute carrier family 12, member 3 provided byMGI
Primary source	MGI:MGI:108114
See related	Ensembl:ENSMUSG00000031766
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI035291, NCC, NCCT, TSC
Expression	Restricted expression toward kidney adult (RPKM 195.7)See more
Orthologs	human all

Transcript information（Ensembl）

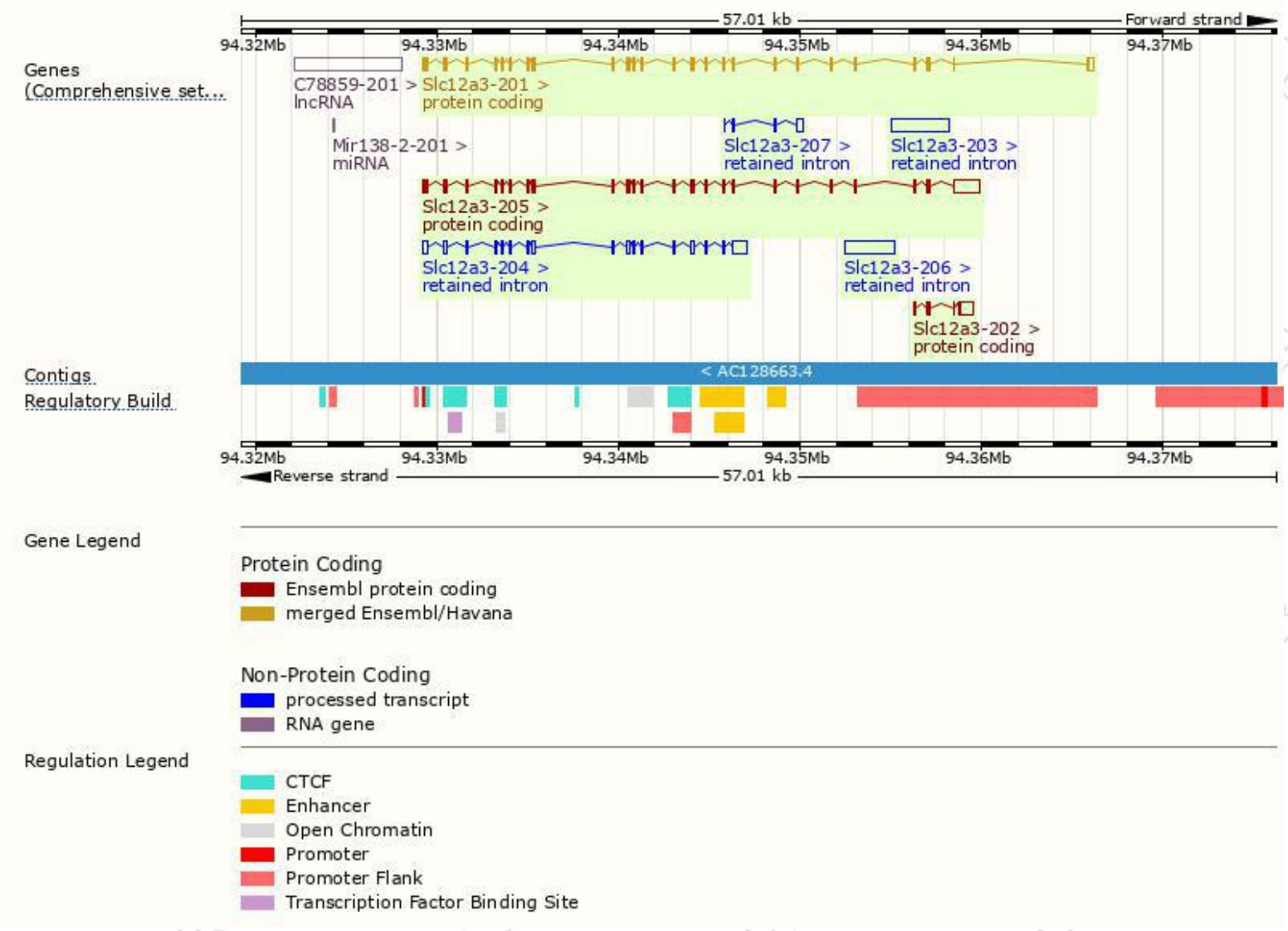
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Slc12a3-201	ENSMUST00000034218.4	3299	1002aa	Protein coding	CCDS57632	P59158 Q543E4	TSL:1 GENCODE basic APPRIS P2
Slc12a3-205	ENSMUST00000212134.1	4310	956aa	Protein coding	-	A0A1D5RLP7	TSL:1 GENCODE basic APPRIS ALT2
Slc12a3-202	ENSMUST00000211905.1	1147	130aa	Protein coding	-	A0A1D5RLX1	CDS 5' incomplete TSL:1
Slc12a3-203	ENSMUST00000211942.1	3161	No protein	Retained intron	-	-	TSL:NA
Slc12a3-204	ENSMUST00000212041.1	2910	No protein	Retained intron	-	-	TSL:1
Slc12a3-206	ENSMUST00000212632.1	2763	No protein	Retained intron	-	-	TSL:NA
Slc12a3-207	ENSMUST00000212915.1	684	No protein	Retained intron	-	-	TSL:3

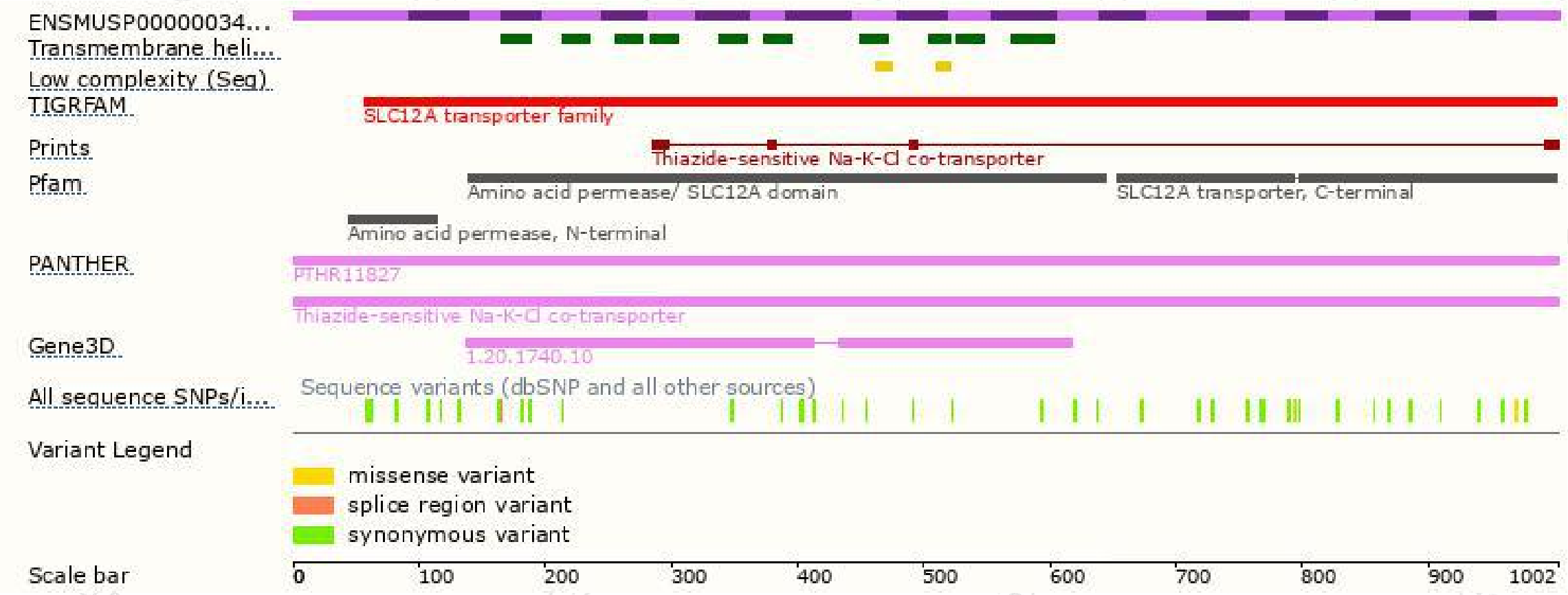
The strategy is based on the design of *Slc12a3-201* transcript,The transcription is shown below



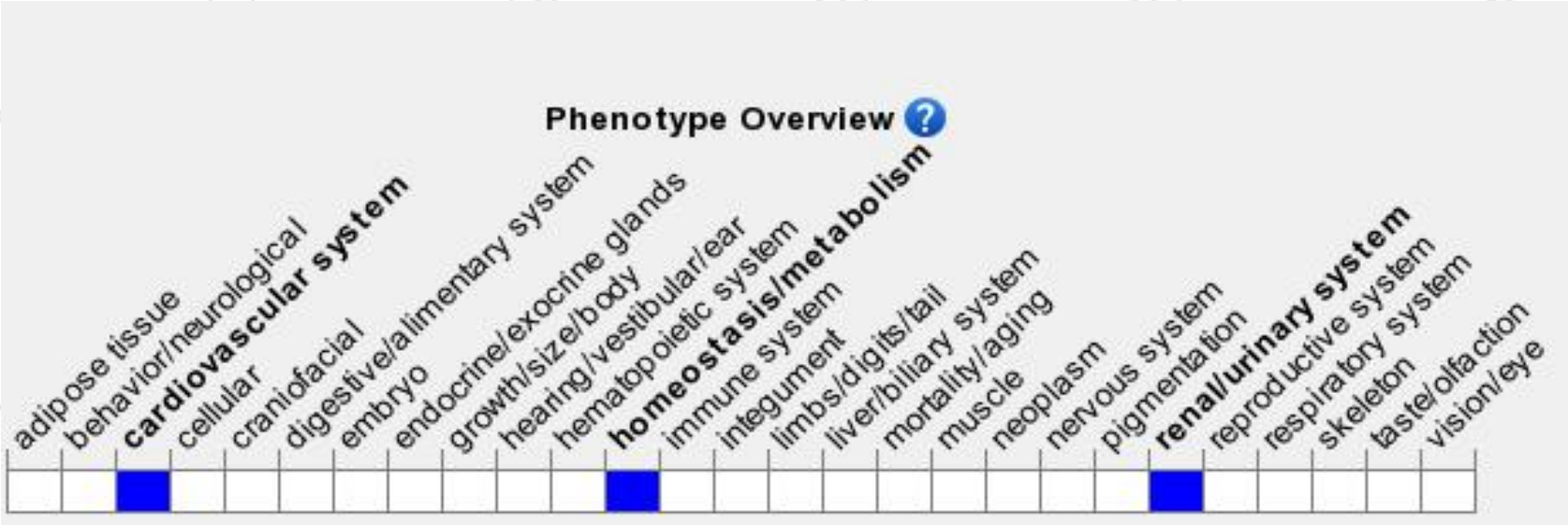
Genomic location distribution



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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