Vps33a Cas9-CKO *Strategy*

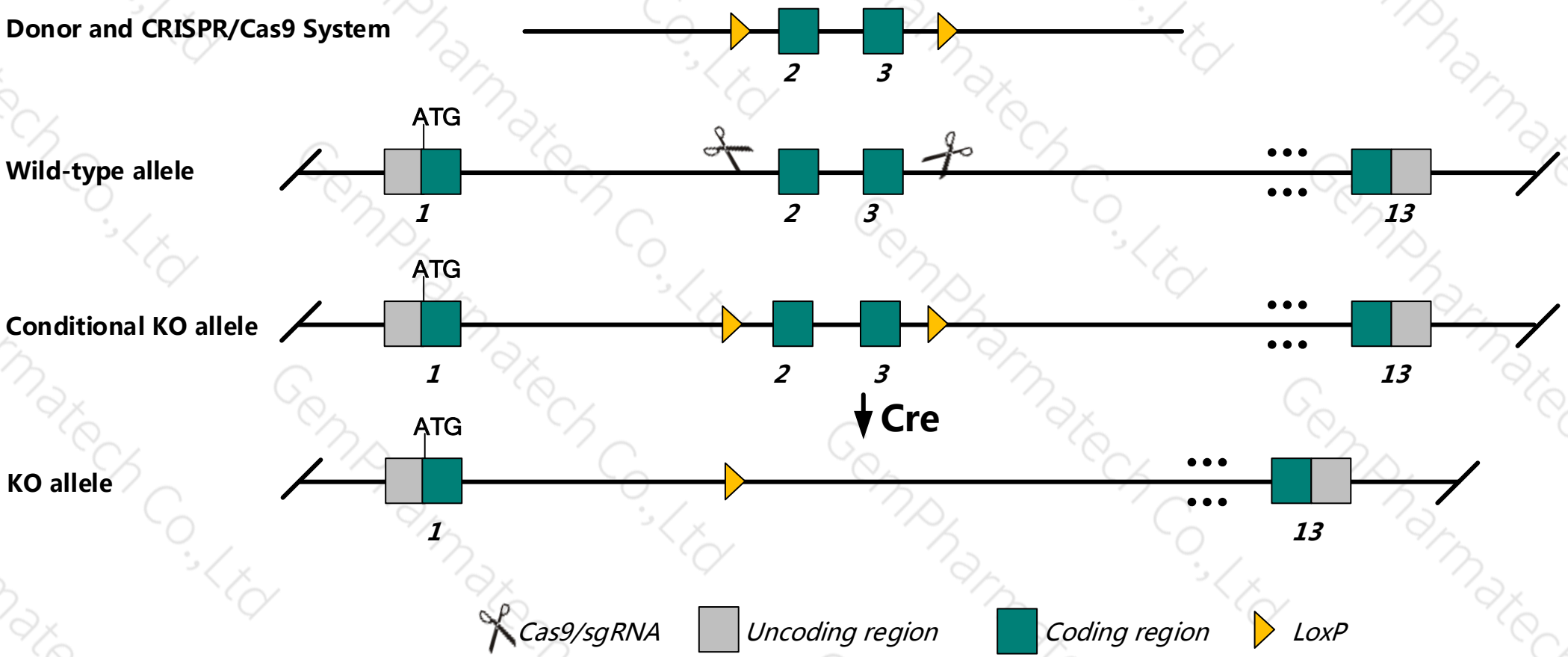
Designer: **Daohua Xu**

Project Overview

Project Name	***Vps33a***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Vps33a* gene. The schematic diagram is as follows:



Technical routes

- The *Vps33a* gene has 4 transcripts.According to the structure of *Vps33a* gene, exon2-exon3 of *Vps33a*-201 (NSMUST00000031388.12) transcript is recommended as the knockout region.The region contains 194bp coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Vps33a* 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，Mutations in this gene produce hypopigmentation, an extended bleeeding time and abnormal kidney function.
- The *Vps33a* gene is located on the Chr5. 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）

Vps33a VPS33A CORVET/HOPS core subunit [*Mus musculus* (house mouse)]

Gene ID: 77573, updated on 2-Oct-2018

Summary

Official Symbol	Vps33a provided by MGI
Official Full Name	VPS33A CORVET/HOPS core subunit provided by MGI
Primary source	MGI:MGI:1924823
See related	Ensembl:ENSMUSG00000029434 Vega:OTTMUSG00000054672
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	bf; AI503300; AW048546; AW554476; 3830421M04Rik
Expression	Ubiquitous expression in cortex adult (RPKM 12.1), frontal lobe adult (RPKM 11.8) and 28 other tissues See more
Orthologs	human all

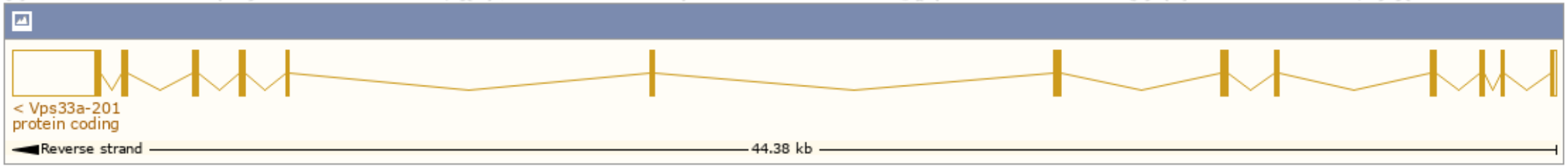
Transcript information（ Ensembl ）

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

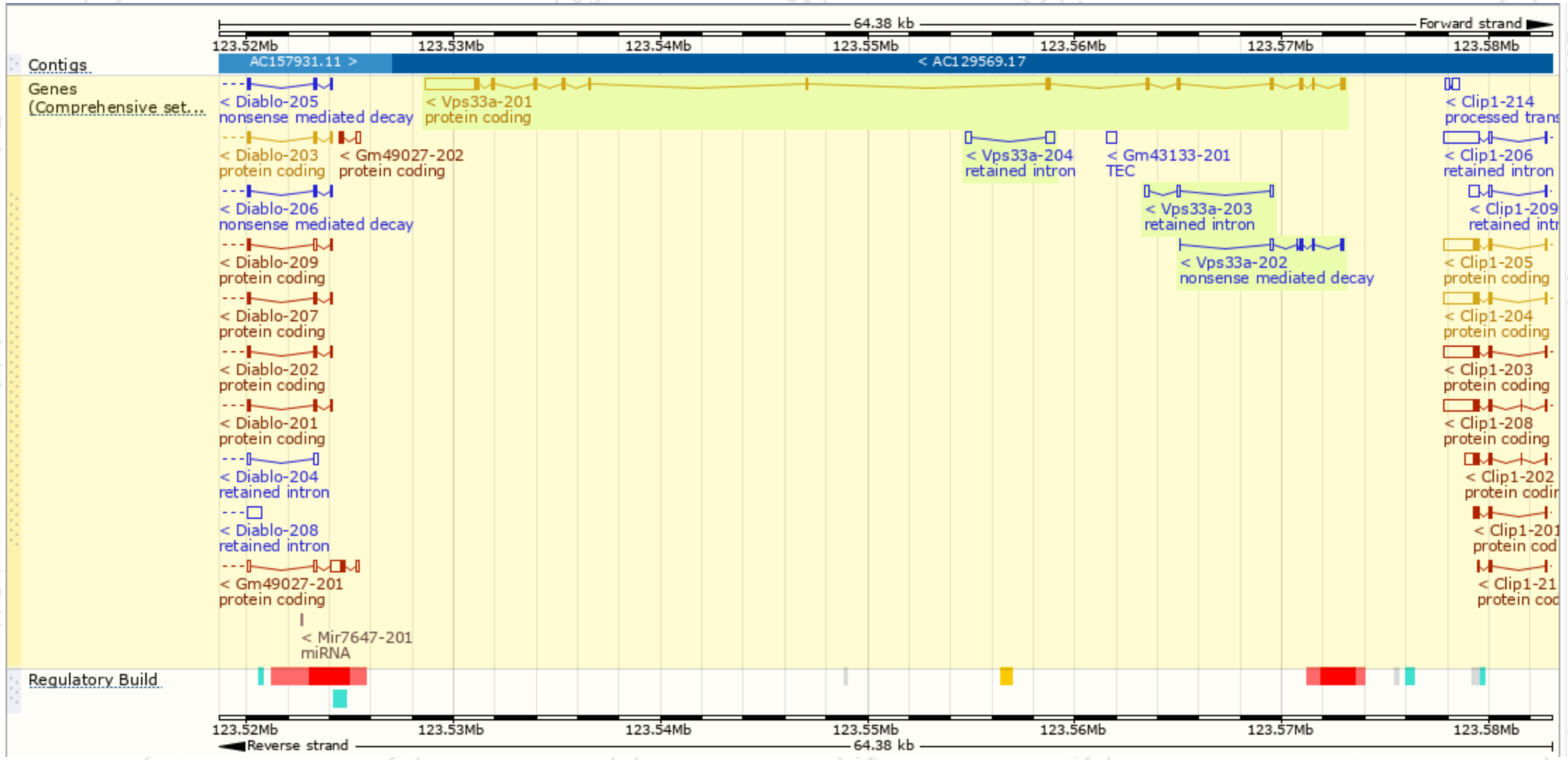
Show/hide columns (1 hidden) Filter

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags
Vps33a-201	ENSMUST00000031388.12	4235	598aa	Protein coding	CCDS19666	Q9D2N9	NM_029929 NP_084205	TSL:1 GENCODE basic APPRIS P1
Vps33a-202	ENSMUST00000197467.1	532	111aa	Nonsense mediated decay	-	A0A0G2JEL2	-	CDS 5' incomplete TSL:5
Vps33a-204	ENSMUST00000200325.1	652	No protein	Retained intron	-	-	-	TSL:2
Vps33a-203	ENSMUST00000198900.1	520	No protein	Retained intron	-	-	-	TSL:3

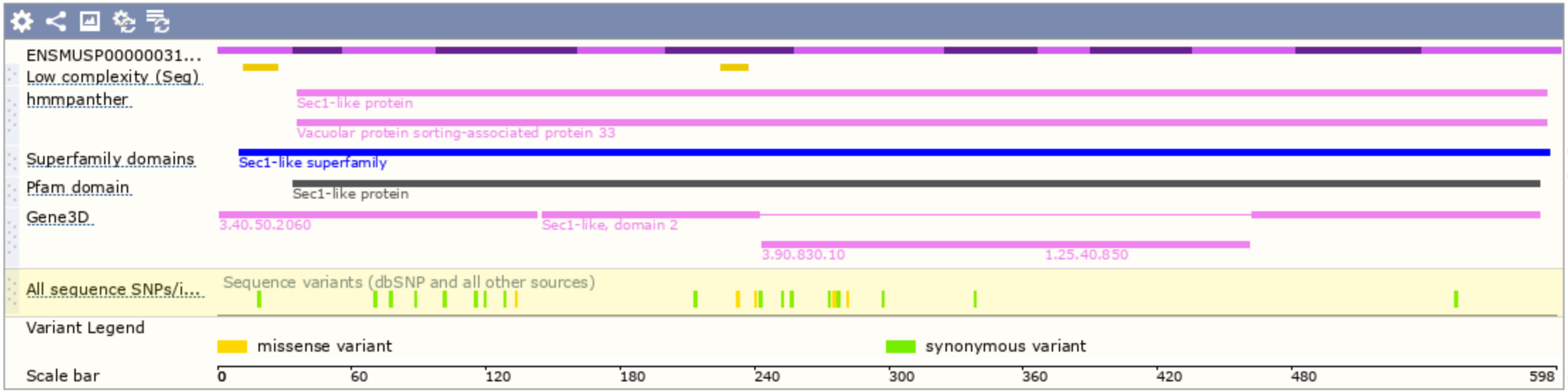
The strategy is based on the design of *Vps33a*-201 transcript,The transcription is shown below



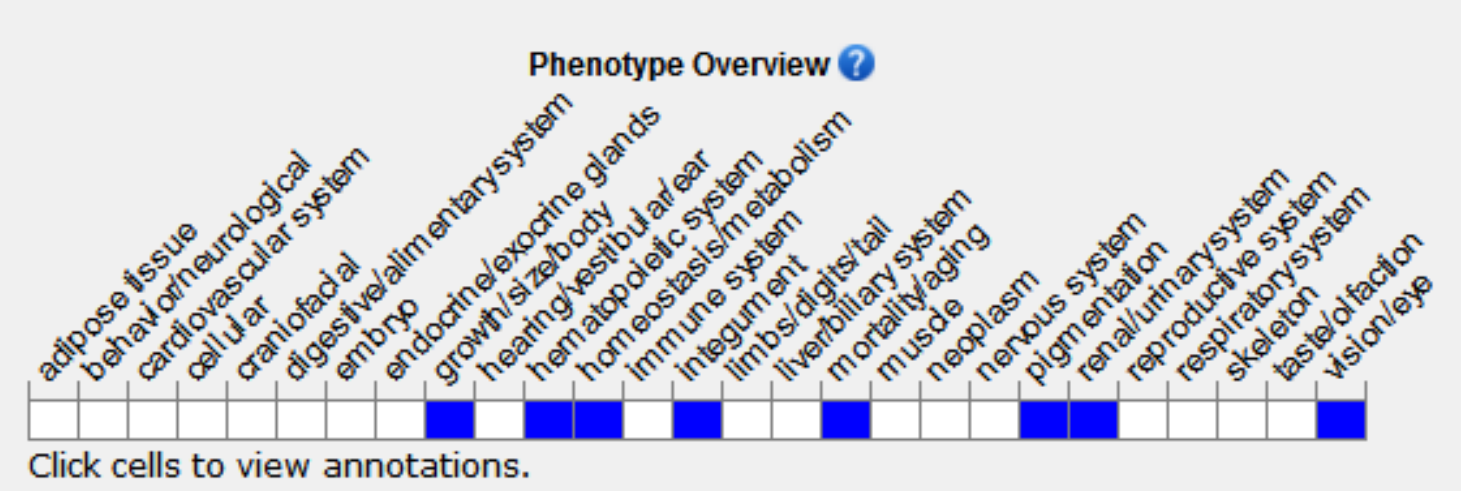
Genomic location distribution



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/) .

According to the existing MGI data,Mutations in this gene produce hypopigmentation, an extended bleeeding time and abnormal kidney function.

If you have any questions, you are welcome to inquire.

Tel: 025-5864 1534

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