Krt14 Cas9-KO Strategy

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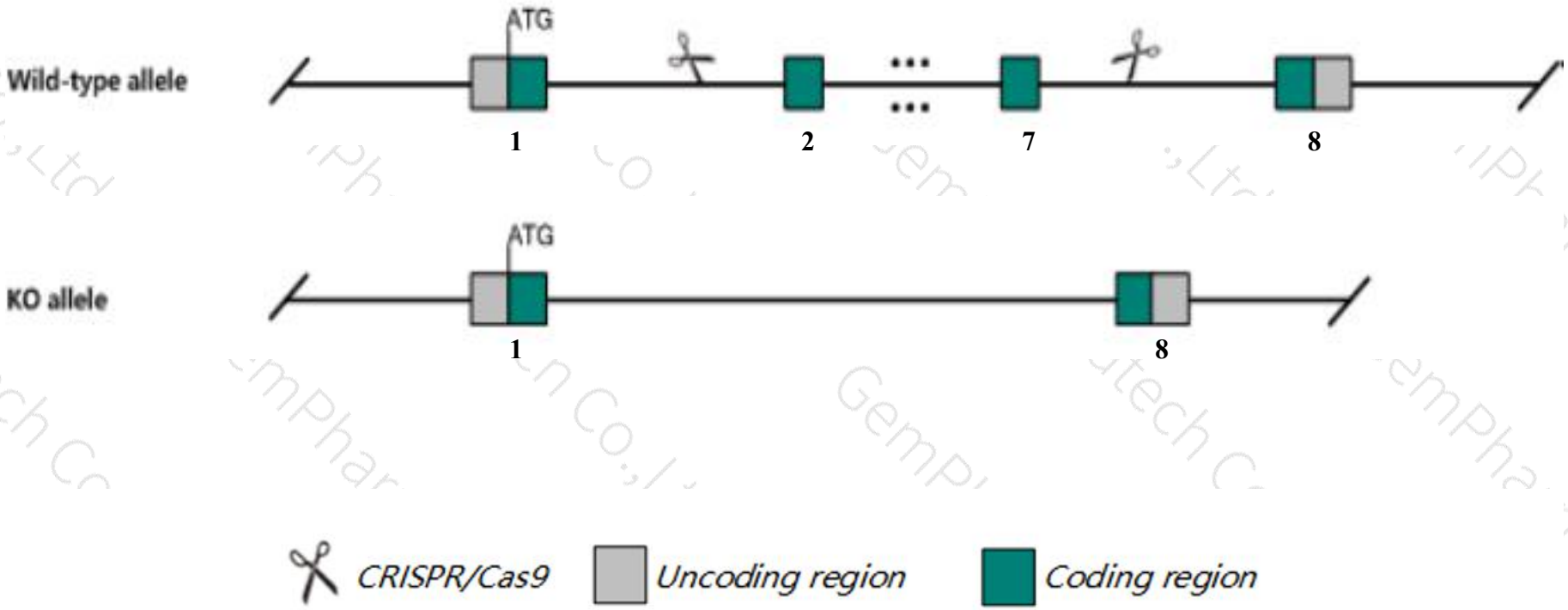
Design Date: 2019-1-16

Project Overview

Project Name	***Krt14***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Krt14* gene. The schematic diagram is as follows:



Technical routes

➢ The *Krt14* gene has 2 transcripts. According to the structure of *Krt14* gene, exon2-exon7 of *Krt14-201*(ENSMUST00000007272.7) transcript is recommended as the knockout region.The region contains 814bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Krt14* gene. The brief process is as follows: CRISPR/Cas9 system 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.

Notice

➢ According to the existing MGI data,homozygotes for targeted null mutations develop extensive skin blistering after birth and die by 2 days of age. If keratin 16 is also expressed in skin, development is normal but later alopecia, chronic skin ulcers and stratified epithelial defects occur.

➢ The Intron7 is only 546bp,loxp insertion may affect mRNA splicing.

➢ The *Krt14* gene is located on the Chr11. 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 gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

集萃药康 GemPharmatech

Krt14 keratin 14 [Mus musculus (house mouse)]

Gene ID: 16664, updated on 20-Mar-2020

Summary

Official Symbol	Krt14 provided by MGI
Official Full Name	keratin 14 provided by MGI
Primary source	MGI:MGI:96688
See related	Ensembl:ENSMUSG00000045545
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	AI626930, CK-14, K14, Krt-1.14, Krt1-14
Summary	This gene encodes a member of the keratin family, the most diverse group of intermediate filaments. This gene product, a type I keratin, is usually found as a heterotetramer with two keratin 5 molecules, a type II keratin. Together they form the cytoskeleton of epithelial cells. Three transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Sep 2015]
Expression	Biased expression in stomach adult (RPKM 89.1), limb E14.5 (RPKM 80.1) and 6 other tissuesSee more
Orthologs	human all

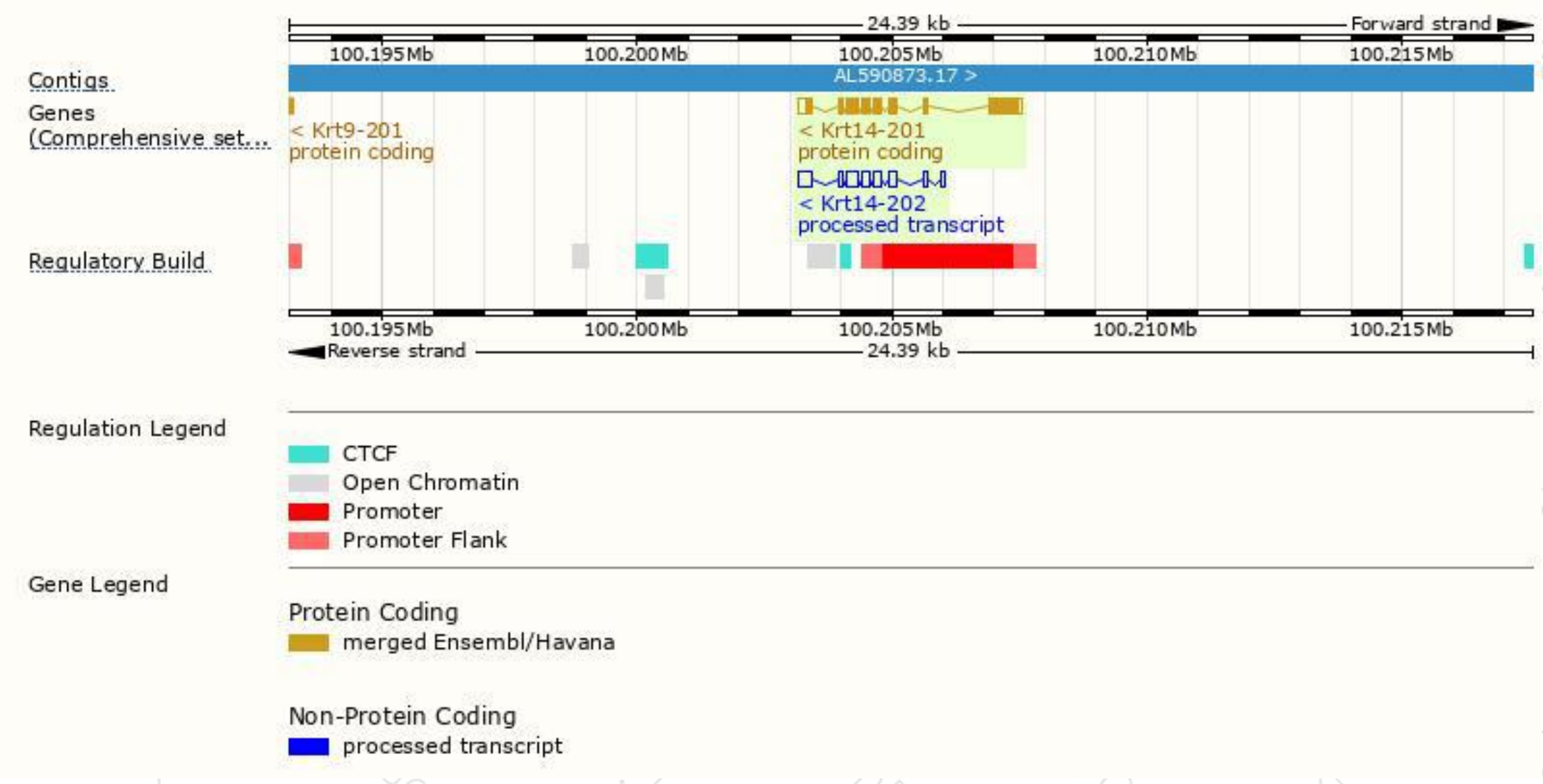
Transcript information（Ensembl）

The gene has 2 transcripts,all transcripts are shown below:

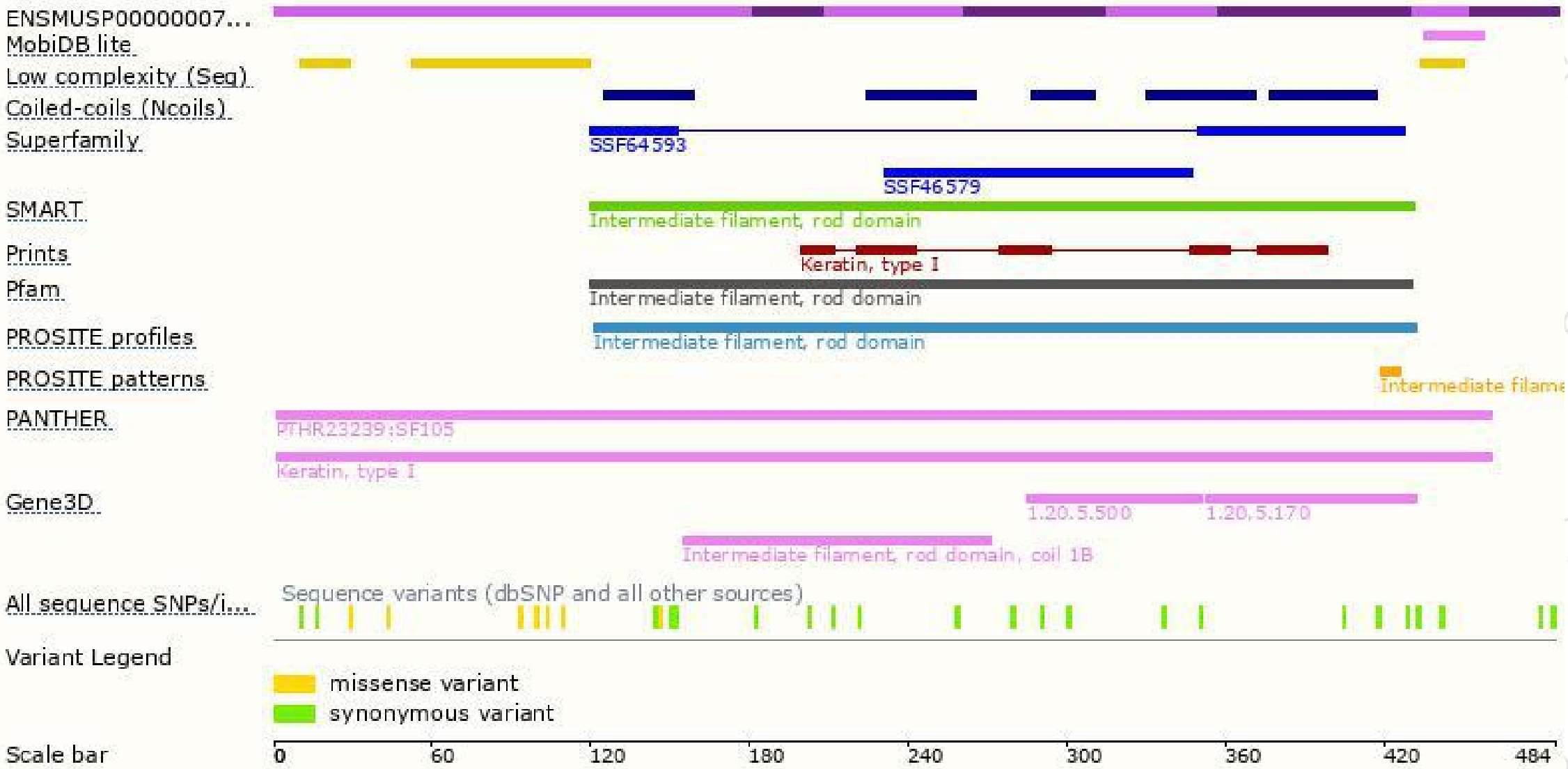
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Krt14-201	ENSMUST00000007272.7	1698	484aa	Protein coding	CCDS25413	Q61781	TSL:1 GENCODE basic APPRIS P1
Krt14-202	ENSMUST00000137265.1	1122	No protein	Processed transcript	-	-	TSL:1

The strategy is based on the design of *Krt14-201* transcript,the transcription is shown below:

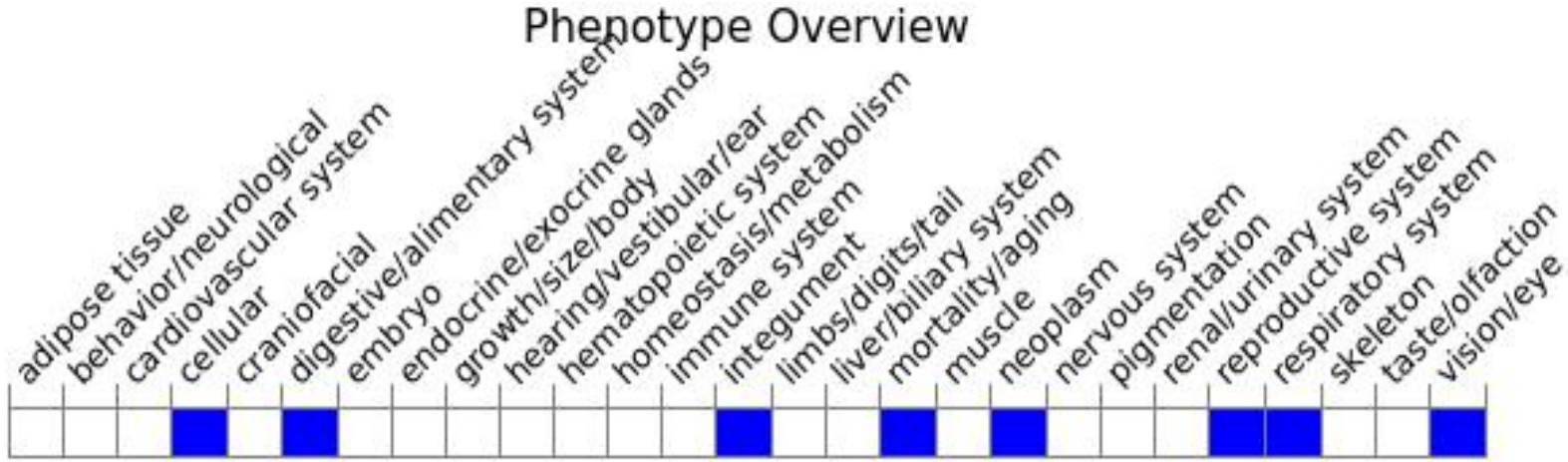
Genomic location distribution



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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