Col17a1 Cas9-CKO Strategy

Designer: Daohua Xu
Reviewer: Huimin Su

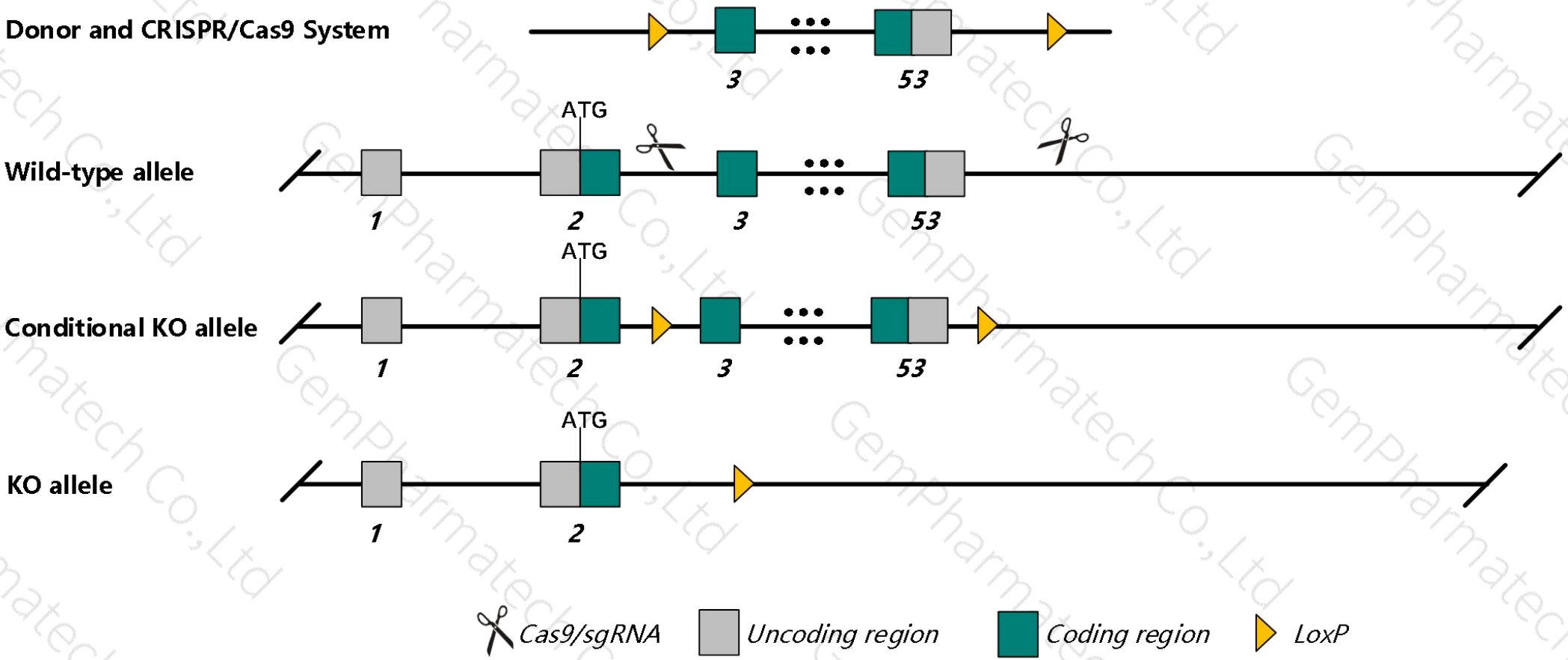
Design Date: 2020-4-7

Project Overview

Project Name	***Col17a1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Col17a1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Col17a1* gene has 5 transcripts. According to the structure of *Col17a1* gene, exon3-exon53 of *Col17a1-202* (ENSMUST00000086923.5) transcript is recommended as the knockout region.The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Col17a1* 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 are unable to reproduce and display postnatal growth retardation, blisters and erosion at sites of trauma, nonpigmented hair growth associated with hair loss, subepidermal blistering associated with poorly formed hemidesmosomes, and high postnatal lethality.

➢ The *Col17a1* gene is located on the Chr19. 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）

集萃药康 GemPharmatech

Col17a1 collagen, type XVII, alpha 1 [*Mus musculus* (house mouse)]

Gene ID: 12821, updated on 15-Mar-2020

Summary

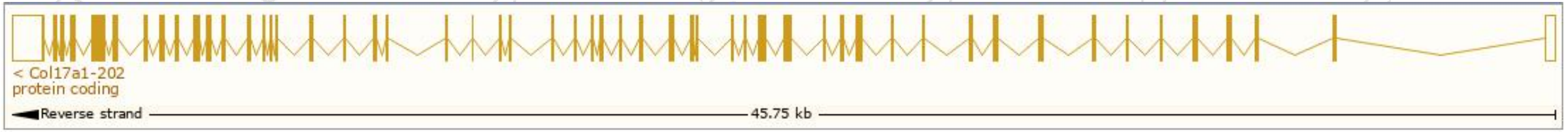
Official Symbol	Col17a1 provided by MGI
Official Full Name	collagen, type XVII, alpha 1 provided by MGI
Primary source	MGI:MGI:88450
See related	Ensembl:ENSMUSG00000025064
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Bpag; BP180; Bpag2
Expression	Biased expression in limb E14.5 (RPKM 10.4), mammary gland adult (RPKM 7.6) and 7 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

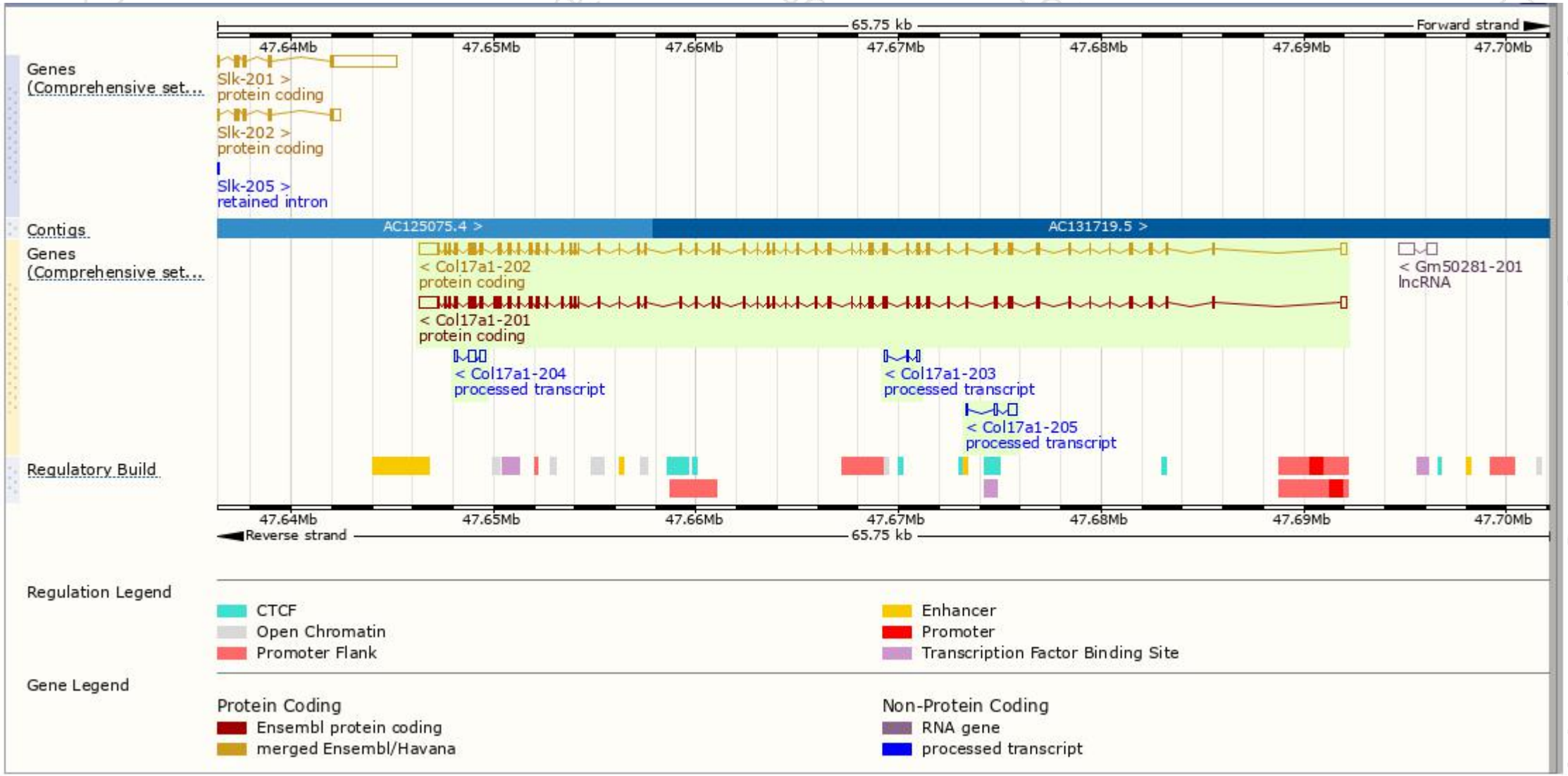
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Col17a1-201	ENSMUST00000026045.13	5588	1470aa	Protein coding	CCDS70959	Q07563	TSL:1 GENCODE basic APPRIS ALT2
Col17a1-202	ENSMUST00000086923.5	5477	1433aa	Protein coding	CCDS38018	Q07563	TSL:1 GENCODE basic APPRIS P3
Col17a1-204	ENSMUST00000151102.1	758	No protein	Processed transcript	-	-	TSL:5
Col17a1-205	ENSMUST00000235883.1	549	No protein	Processed transcript	-	-	-
Col17a1-203	ENSMUST00000145254.1	357	No protein	Processed transcript	-	-	TSL:2

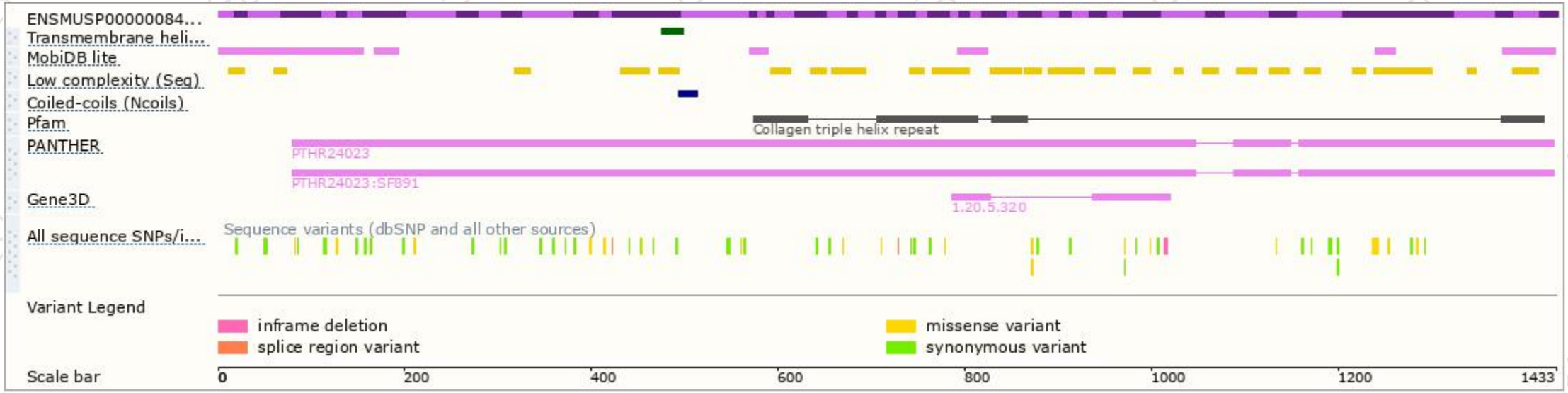
The strategy is based on the design of *Col17a1-202* transcript,The transcription is shown below



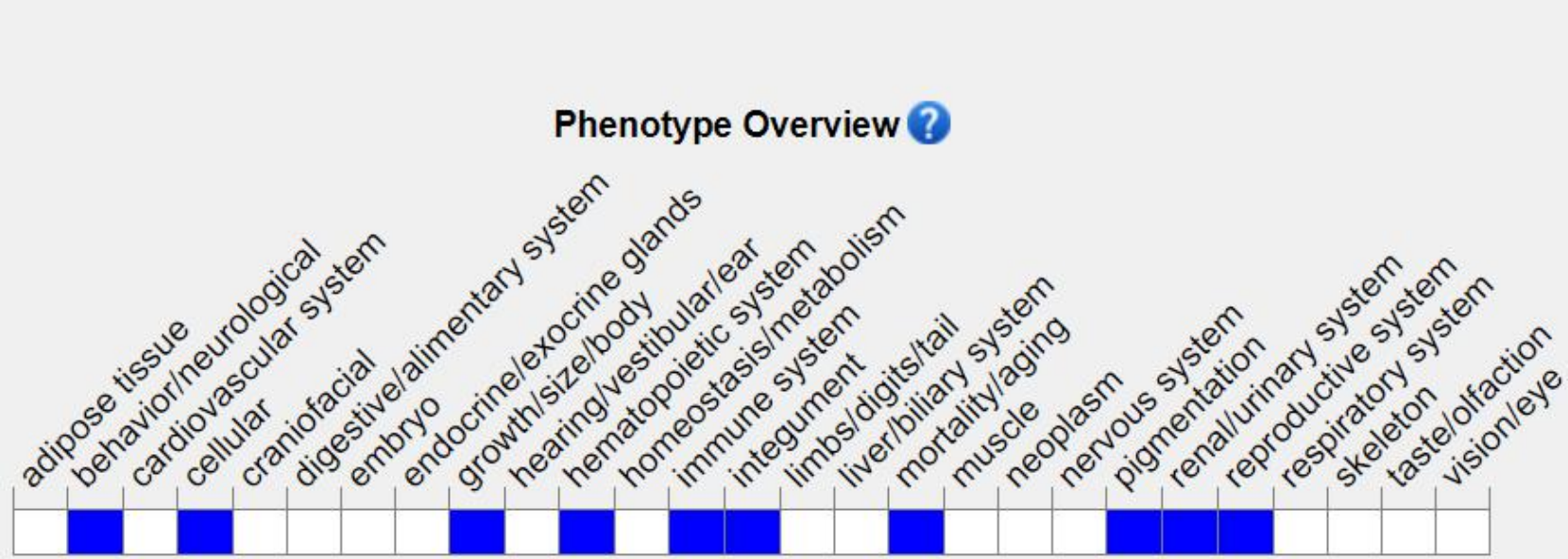
Genomic location distribution



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,Mice homozygous for a knock-out allele are unable to reproduce and display postnatal growth retardation, blisters and erosion at sites of trauma, nonpigmented hair growth associated with hair loss, subepidermal blistering associated with poorly formed hemidesmosomes, and high postnatal lethality.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd