Col1a1 Cas9-CKO Strategy

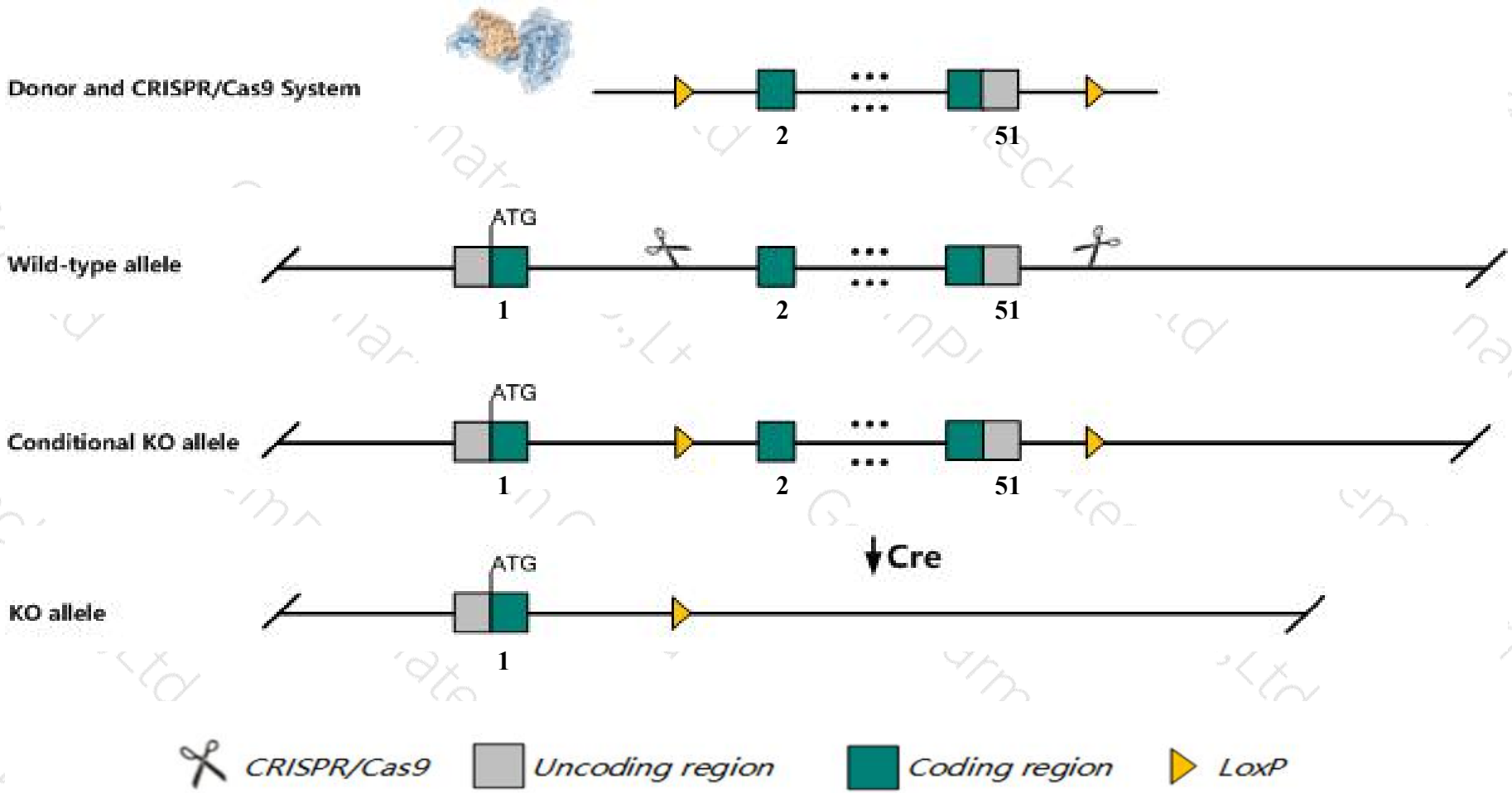
Designer: Daohua Xu
Reviewer: Huimin Su
Design Date: 2019-10-17

Project Overview

Project Name	***Col1a1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Col1a1* gene. The schematic diagram is as follows:



Technical routes

- The *Col1a1* gene has 4 transcripts. According to the structure of *Col1a1* gene, exon2-exon51 of *Col1a1-201* (ENSMUST00000001547.7) transcript is recommended as the knockout region.The region contains 4286bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Col1a1* 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,Mutations in this locus cause variable phenotype, from embryonic lethal to viable/fertile with altered fibrillogenesis. Homozygotes can show impaired bone formation and fragility, osteoporosis, dermal fibrosis, impaired uterine postpartum involution, andaortic dissection.

- The *Col1a1* 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 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

Col1a1 collagen, type I, alpha 1 [Mus musculus (house mouse)]

Gene ID: 12842, updated on 9-Apr-2019

Summary

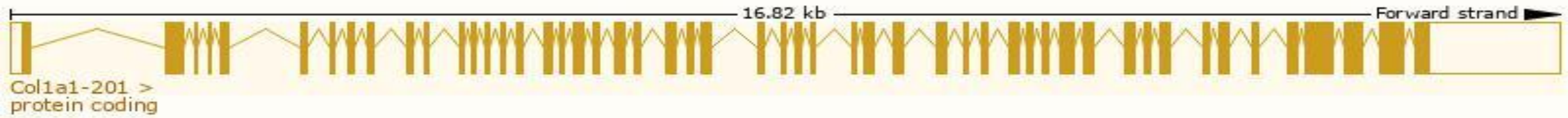
Official Symbol	Col1a1 provided by MGI
Official Full Name	collagen, type I, alpha 1 provided by MGI
Primary source	MGI:MGI:88467
See related	Ensembl:ENSMUSG00000001506
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Col1a-1, Cola-1, Cola1, Mov-13, Mov13
Summary	This gene encodes the alpha-1 subunit of the fibril-forming type I collagen, the most abundant protein of bone, skin and tendon extracellular matrices. The encoded protein, in association with alpha-2 subunit, forms heterotrimeric type I procollagen that undergoes proteolytic processing during fibril formation. Mice lacking the encoded protein die in utero caused by the rupture of a major blood vessel. Transgenic mice expressing significantly lower levels of this gene exhibit morphological and functional defects in mineralized and non-mineralized connective tissue and, progressive loss of hearing. [provided by RefSeq, Nov 2015]
Expression	Biased expression in limb E14.5 (RPKM 527.2), bladder adult (RPKM 493.7) and 12 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

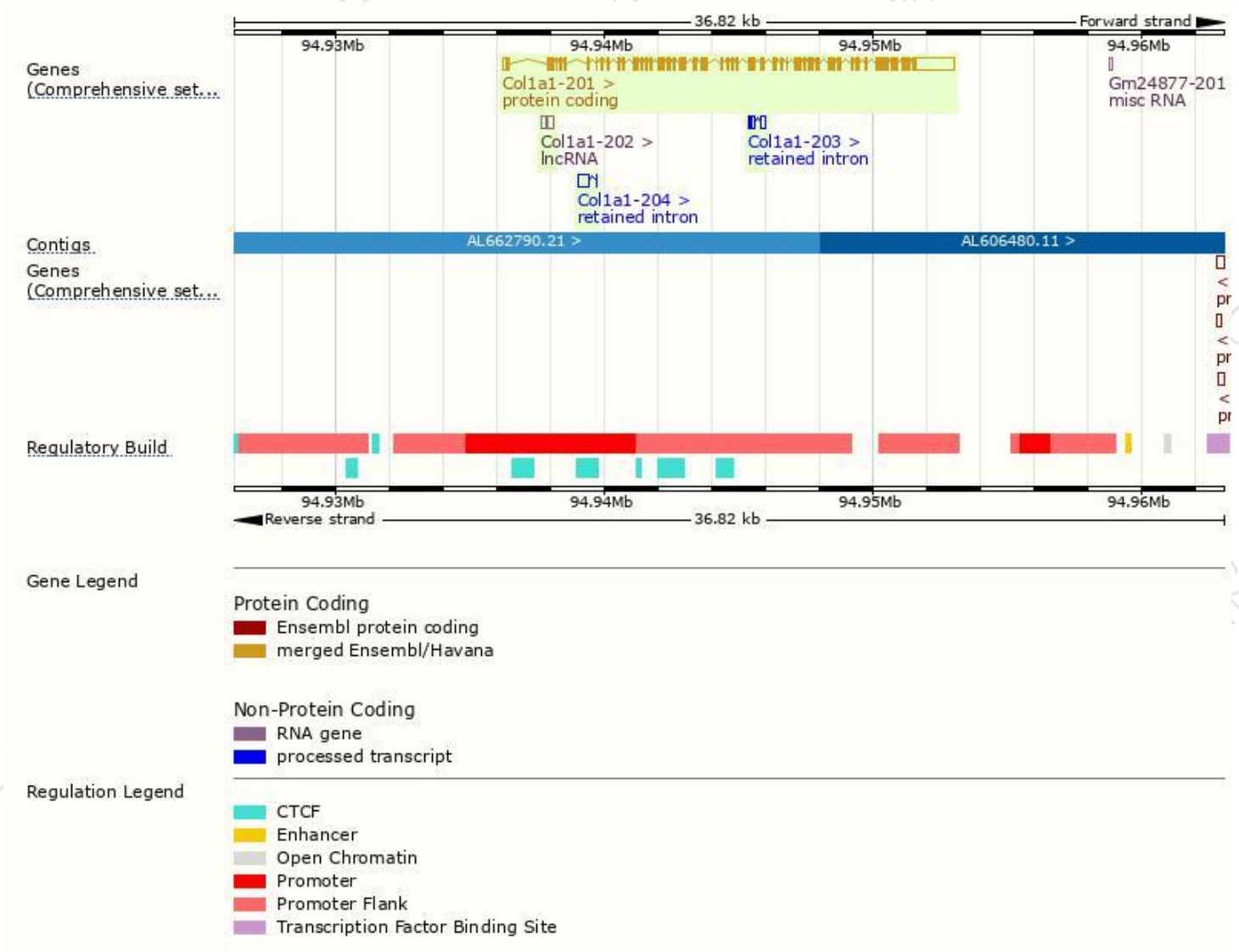
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Col1a1-201	ENSMUST00000001547.7	5930	1453aa	Protein coding	CCDS25265	P11087	TSL:1 GENCODE basic APPRIS P1
Col1a1-202	ENSMUST00000139974.1	337	No protein	Processed transcript	-	-	TSL:3
Col1a1-204	ENSMUST00000148593.1	493	No protein	Retained intron	-	-	TSL:3
Col1a1-203	ENSMUST00000148046.1	363	No protein	Retained intron	-	-	TSL:3

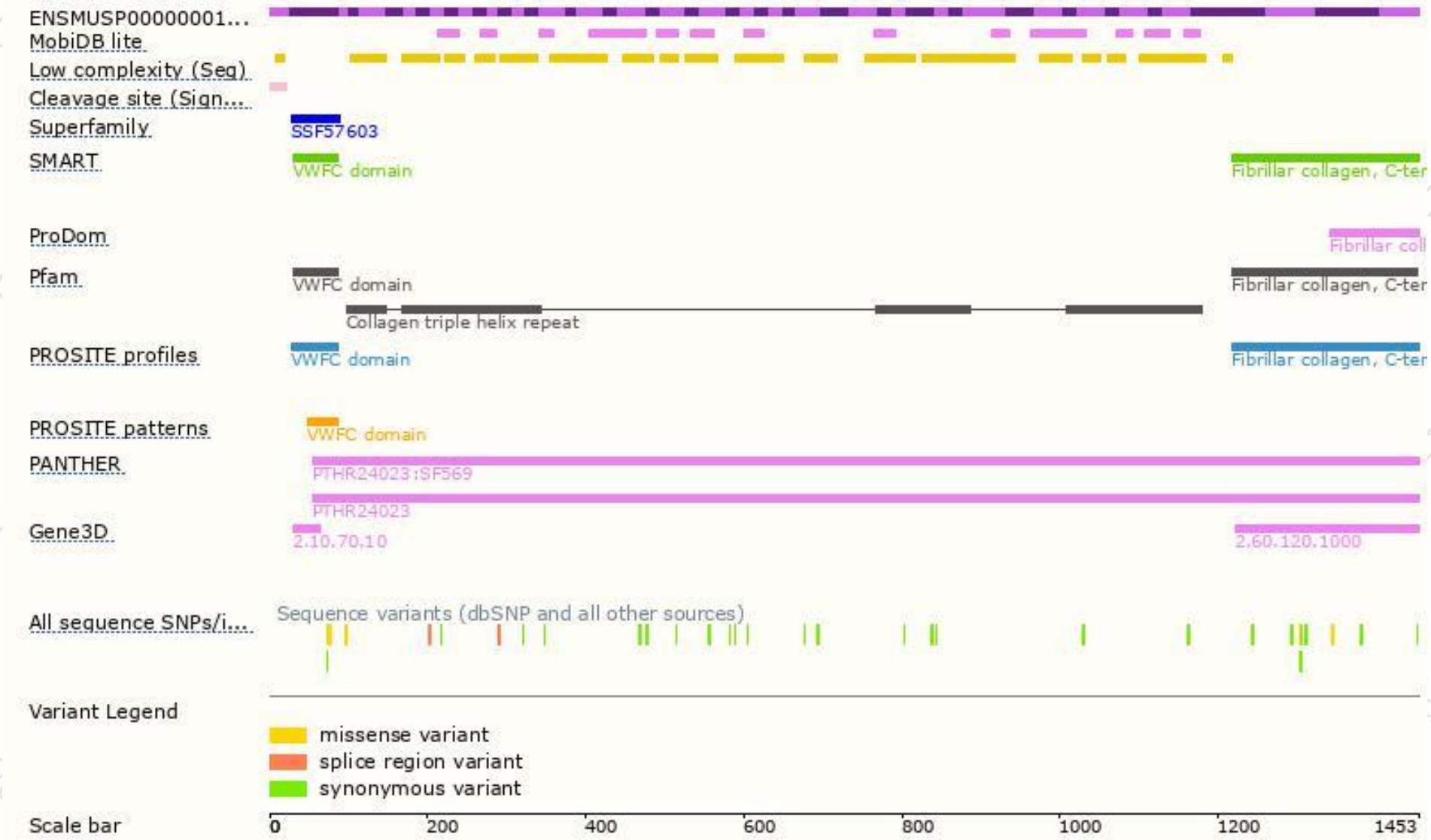
The strategy is based on the design of *Col1a1-201* transcript,The transcription is shown below



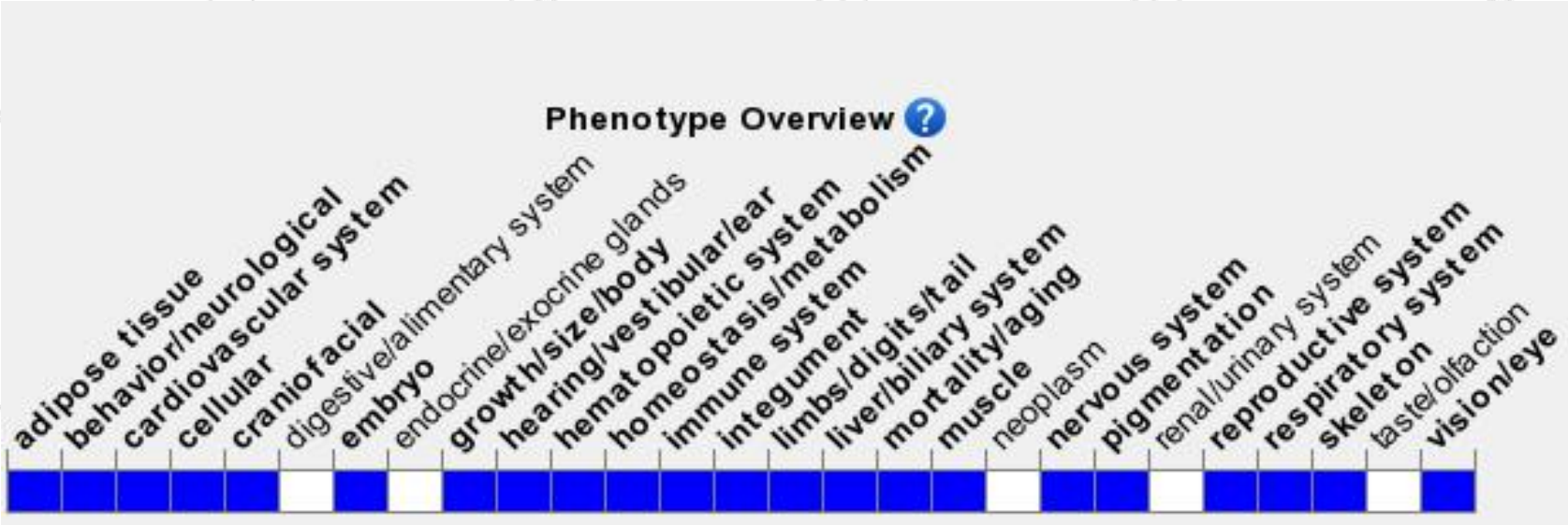
Genomic location distribution



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 locus cause variable phenotype, from embryonic lethal to viable/fertile with altered fibrillogenesis. Homozygotes can show impaired bone formation and fragility, osteoporosis, dermal fibrosis, impaired uterine postpartum involution, andaortic dissection.

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