Timp3 Cas9-CKO Strategy

Designer: Xiaojing Li
Design Date: 2019-9-11
Reviewer: JiaYu

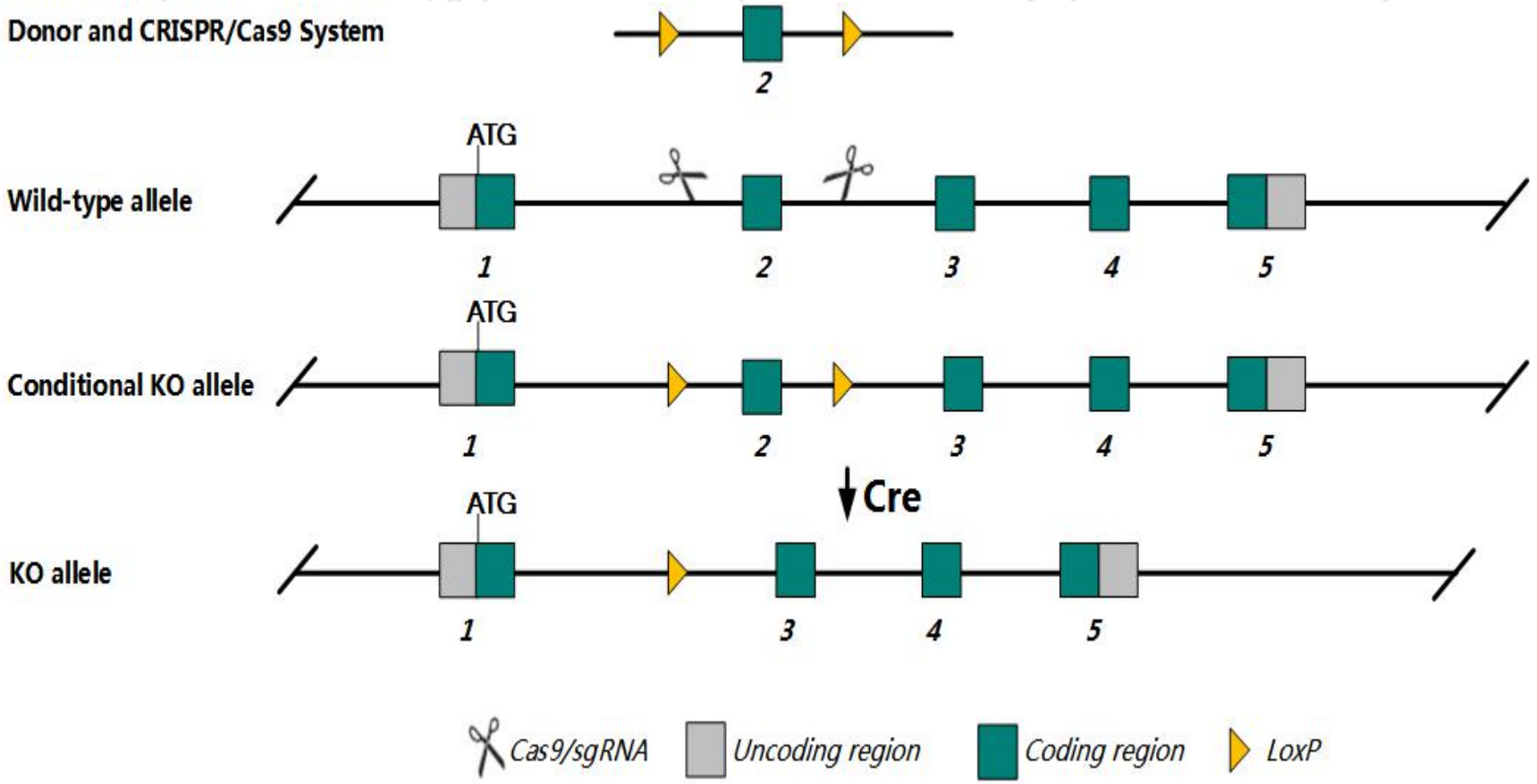
Project Overview

Project Name	***Timp3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

集萃药康 GemPharmatech

This model will use CRISPR/Cas9 technology to edit the *Timp3* gene. The schematic diagram is as follows:



Technical routes

- The *Timp3* gene has 4 transcripts. According to the structure of *Timp3* gene, exon2 of *Timp3-201* (ENSMUST00000020234.13) transcript is recommended as the knockout region.The region contains 83bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Timp3* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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,Targeted null mice die prematurely with lethargy, ruffled hair, and a hunched posture, displaying impaired bronchiole branching, reduced alveologenesis and abnormal mammary gland involution. Knock-ins harboring a Ser156Cys missense mutation provide a mouse model for Sorsby fundus dystrophy.

➢ The knockout region overlaps the intron region of Syn3, which may affect the normal shear function of Syn3.

➢ The *Timp3* gene is located on the Chr10. 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

Timp3 tissue inhibitor of metalloproteinase 3 [*Mus musculus* (house mouse)]

Gene ID: 21859, updated on 13-Aug-2019

Summary

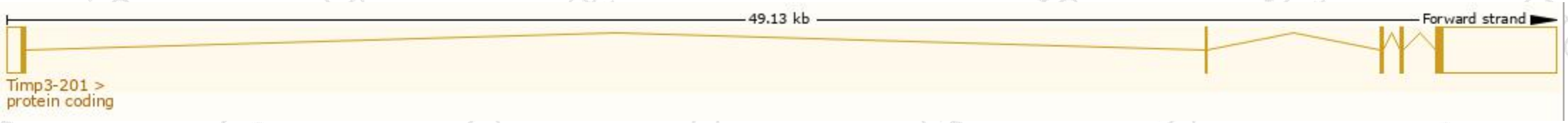
Official Symbol	Timp3 provided by MGI
Official Full Name	tissue inhibitor of metalloproteinase 3 provided by MGI
Primary source	MGI:MGI:98754
See related	Ensembl:ENSMUSG00000020044
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Timp-3
Expression	Biased expression in kidney adult (RPKM 234.0), lung adult (RPKM 92.0) and 12 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

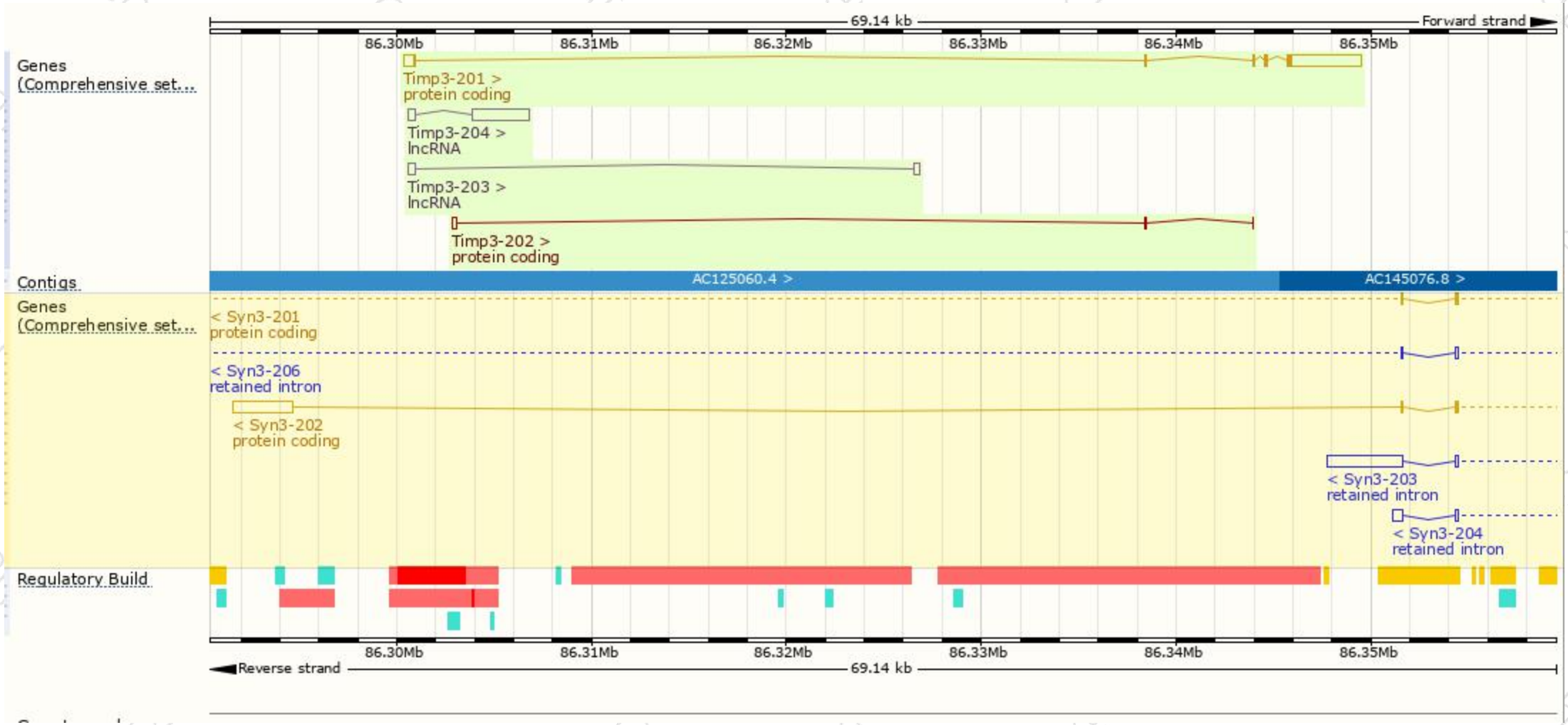
The gene has 4 transcripts,all transcripts are shown below:

Name ▲	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Timp3-201	ENSMUST00000020234.13	4722	211aa	Protein coding	CCDS24097	P39876 Q54AE5	TSL:1 GENCODE basic APPRIS P1
Timp3-202	ENSMUST00000132307.1	352	50aa	Protein coding	-	E9QAB2	CDS 3' incomplete TSL:3
Timp3-203	ENSMUST00000136045.1	674	No protein	lncRNA	-	-	TSL:2
Timp3-204	ENSMUST00000151134.1	3368	No protein	lncRNA	-	-	TSL:1

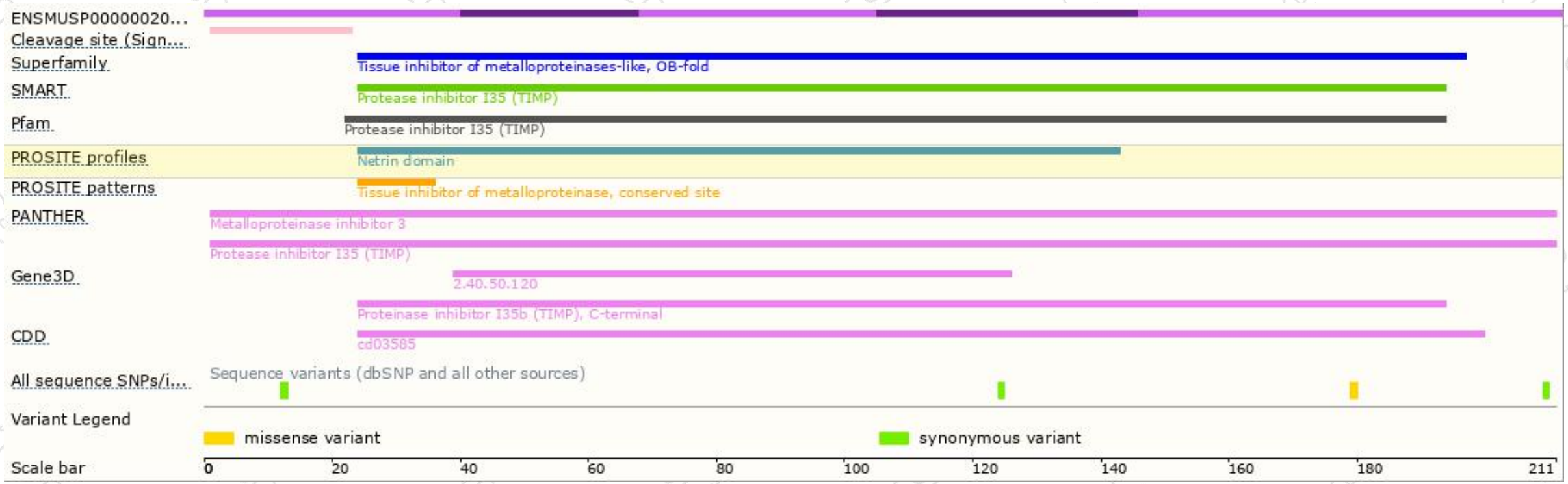
The strategy is based on the design of *Timp3-201* transcript,The transcription is shown below



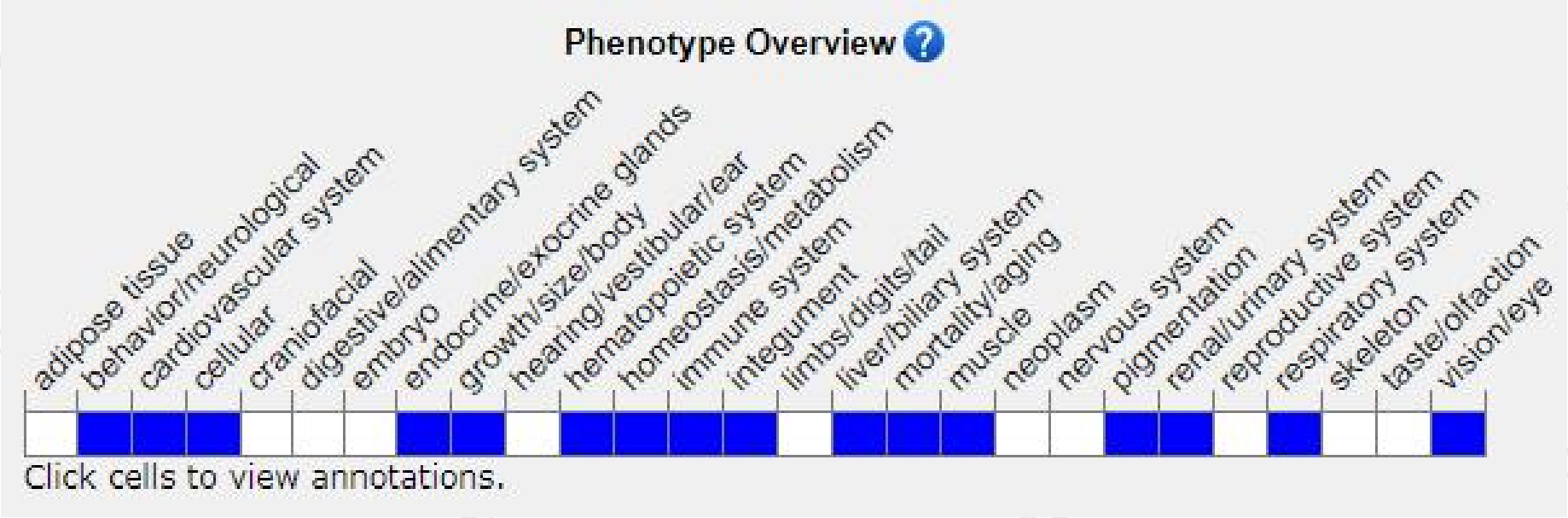
Genomic location distribution



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/).

Targeted null mice die prematurely with lethargy, ruffled hair, and a hunched posture, displaying impaired bronchiole branching, reduced alveologenesis and abnormal mammary gland involution. Knock-ins harboring a Ser156Cys missense mutation provide a mouse model for Sorsby fundus dystrophy.

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