集萃药康
GemPharmatech

Fas Cas9-CKO Strategy

Designer: **Jinling Wang**

Design Date: **2019-7-17**

GemPharmatech Co.,Ltd

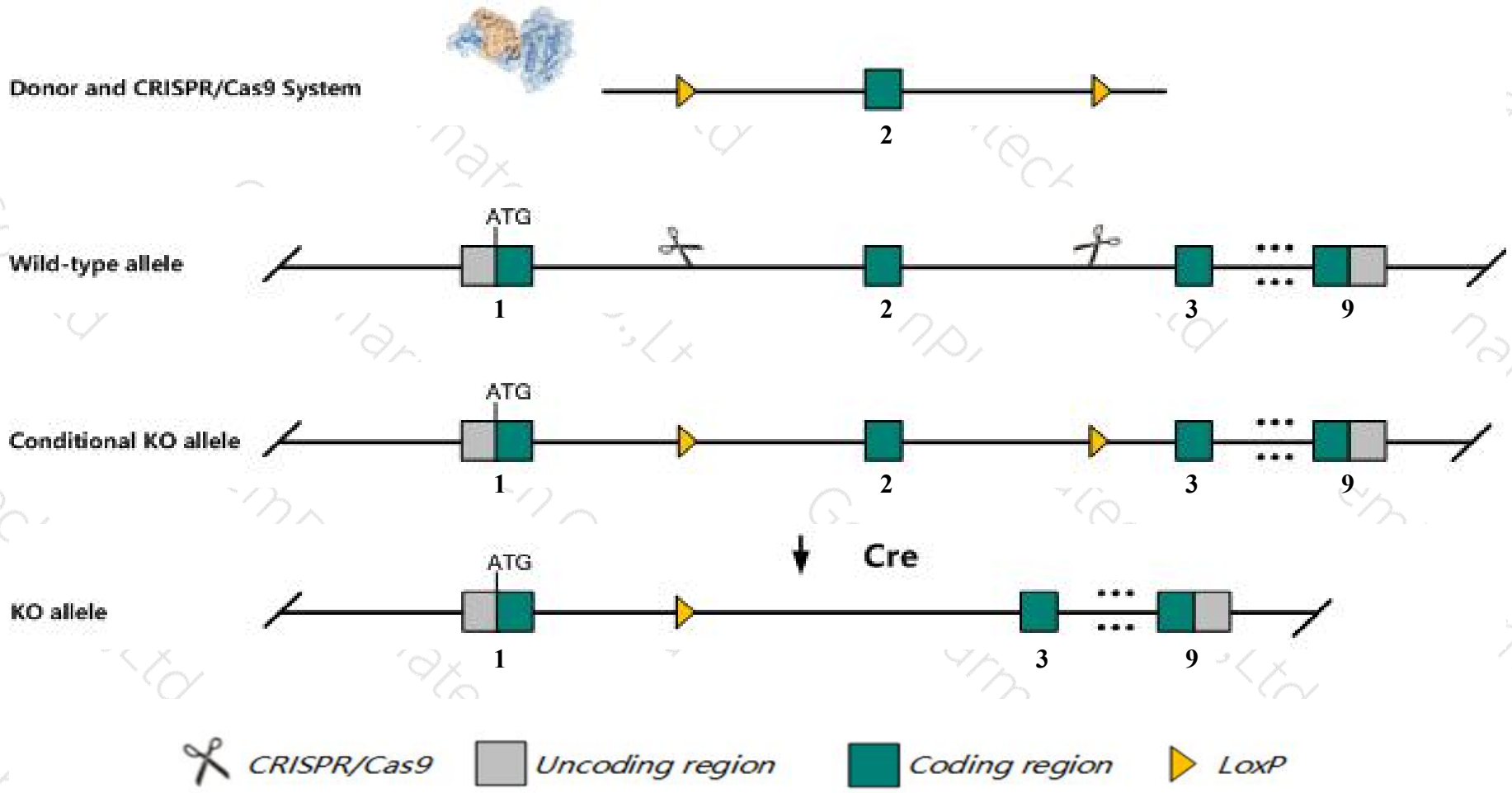
Project Overview

Project Name	***Fas***
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 *Fas* gene. The schematic diagram is as follows:



Technical routes

集萃药康 GemPharmatech

- The *Fas* gene has 5 transcripts. According to the structure of *Fas* gene, exon2 of *Fas-201* (ENSMUST00000025691.12) transcript is recommended as the knockout region.The region contains 154bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Fas* 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 affect immune function and homozygotes show varying severity of lymphadenopathy, splenomegaly, lymphocytic infiltrations, elevated immunoglobulin levels, autoantibodies, impaired clonal deletion of T cells, and lupus-like disease.

- The *Fas* 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

Fas Fas (TNF receptor superfamily member 6) [Mus musculus (house mouse)]

Gene ID: 14102, updated on 9-Apr-2019

Summary

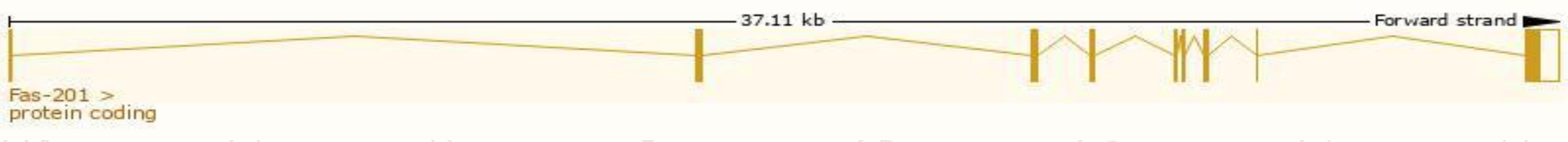
Official Symbol	Fas provided by MGI
Official Full Name	Fas (TNF receptor superfamily member 6) provided byMGI
Primary source	MGI:MGI:95484
See related	Ensembl:ENSMUSG00000024778
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI196731, APO1, APT1, CD95, TNFR6, Tnfrsf6, lpr
Expression	Broad expression in liver E18 (RPKM 4.7), bladder adult (RPKM 3.9) and 19 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

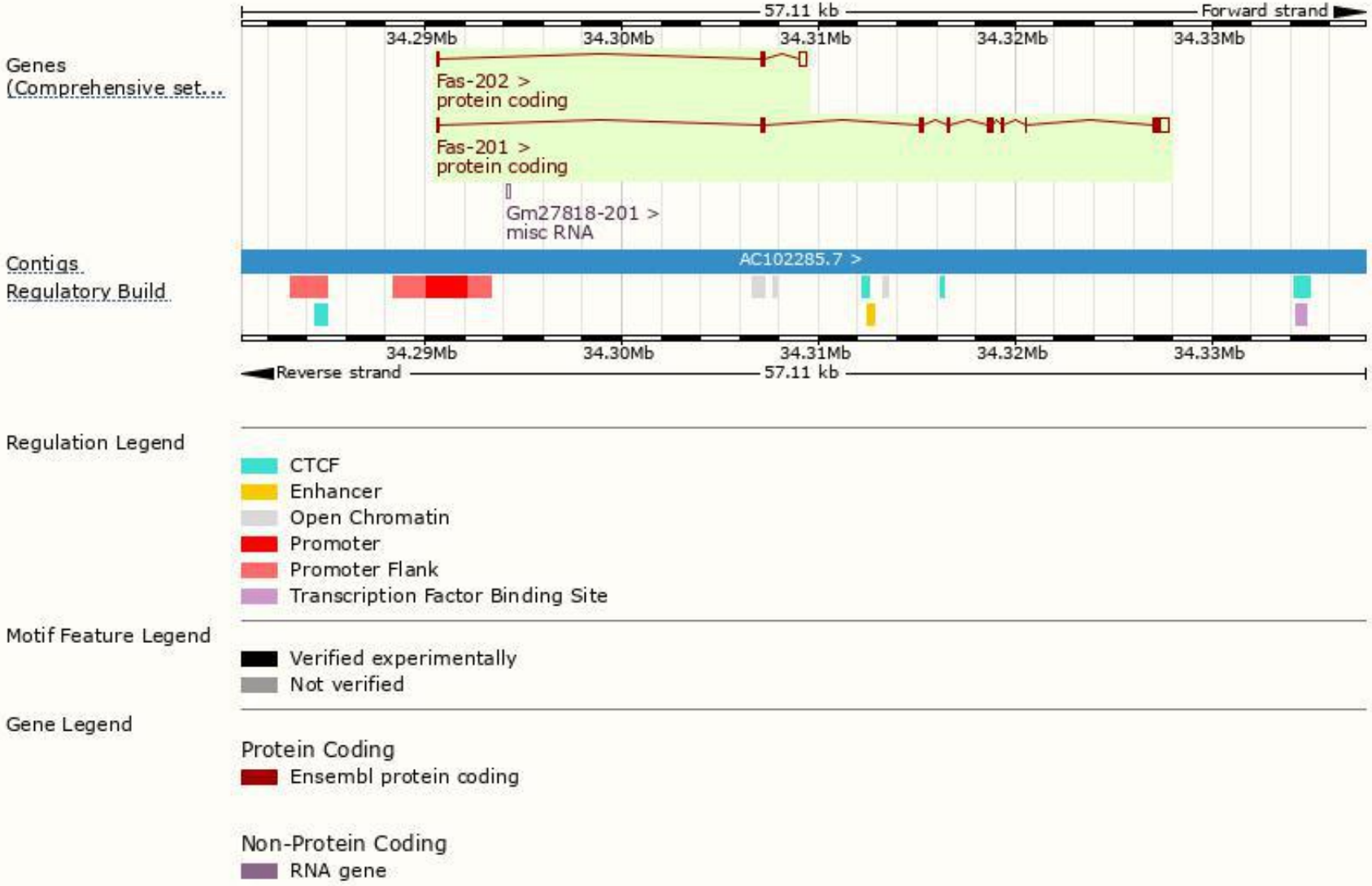
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fas-201	ENSMUST00000025691.12	1481	327aa	Protein coding	CCDS29758	P25446	TSL:1 GENCODE basic APPRIS P2
Fas-202	ENSMUST00000112472.3	532	63aa	Protein coding	CCDS50420	Q8C350	TSL:1 GENCODE basic
Fas-203	ENSMUST00000235232.1	1405	309aa	Protein coding	-	-	GENCODE basic APPRIS ALT2
Fas-204	ENSMUST00000235709.1	1625	123aa	Nonsense mediated decay	-	-	
Fas-205	ENSMUST00000236731.1	509	No protein	Processed transcript	-	-	

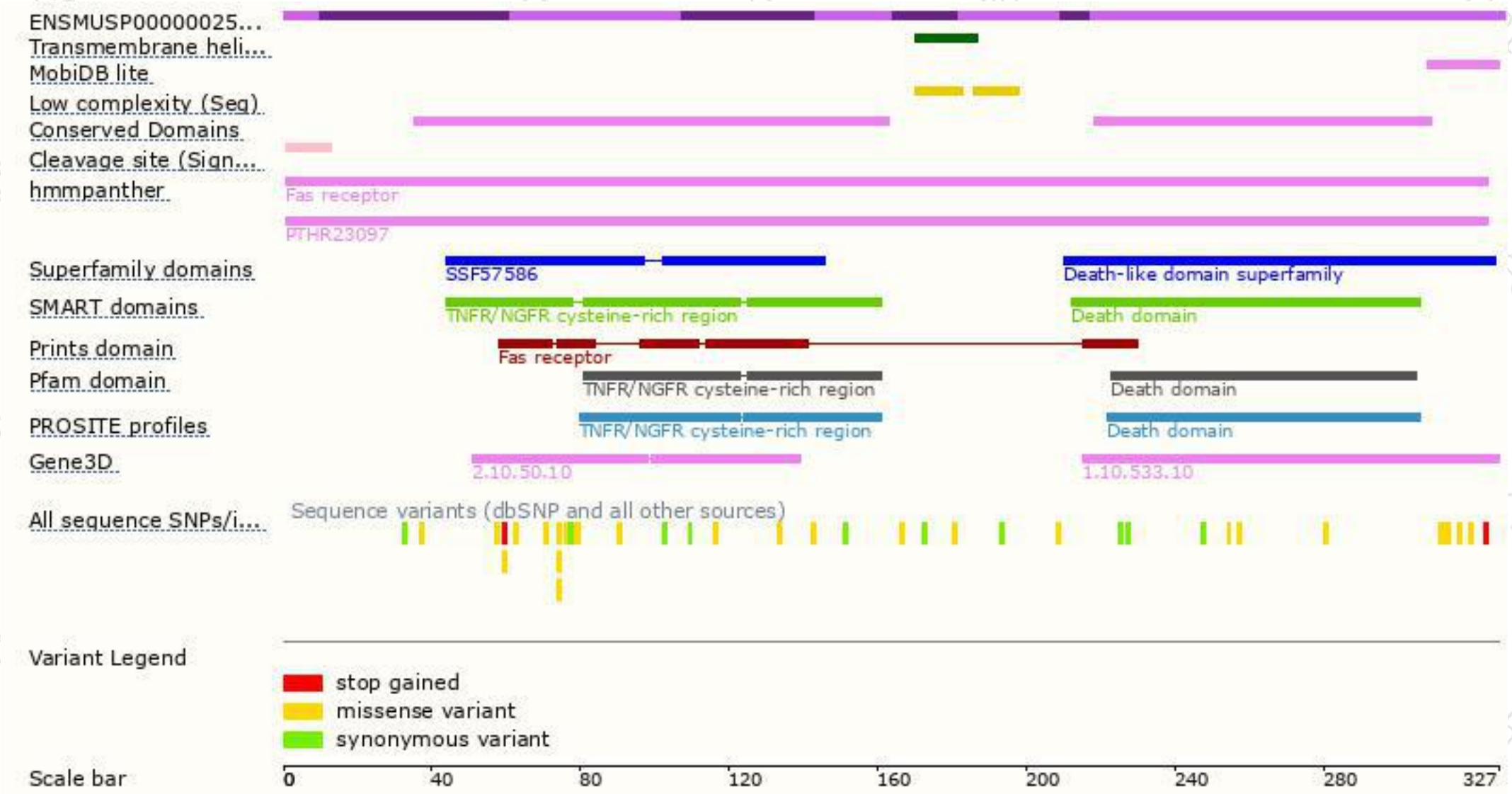
The strategy is based on the design of *Fas-201* transcript,The transcription is shown below



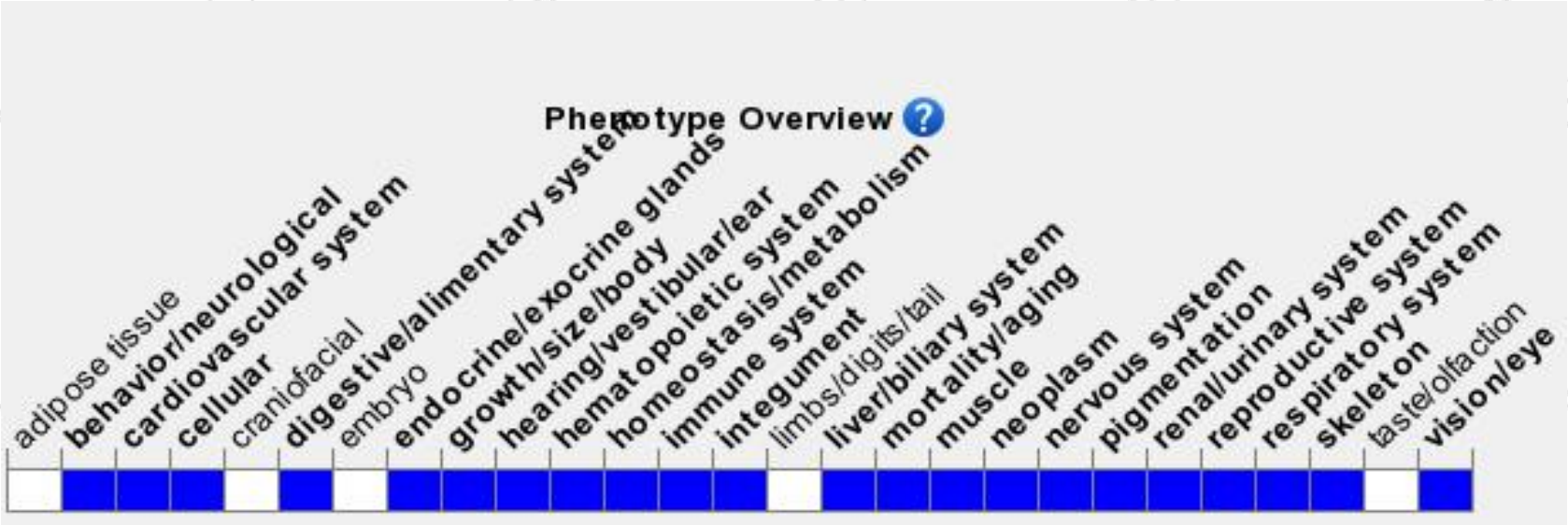
Genomic location distribution



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 affect immune function and homozygotes show varying severity of lymphadenopathy, splenomegaly, lymphocytic infiltrations, elevated immunoglobulin levels, autoantibodies, impaired clonal deletion of T cells, and lupus-like disease.

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