Ikbkb Cas9-CKO Strategy

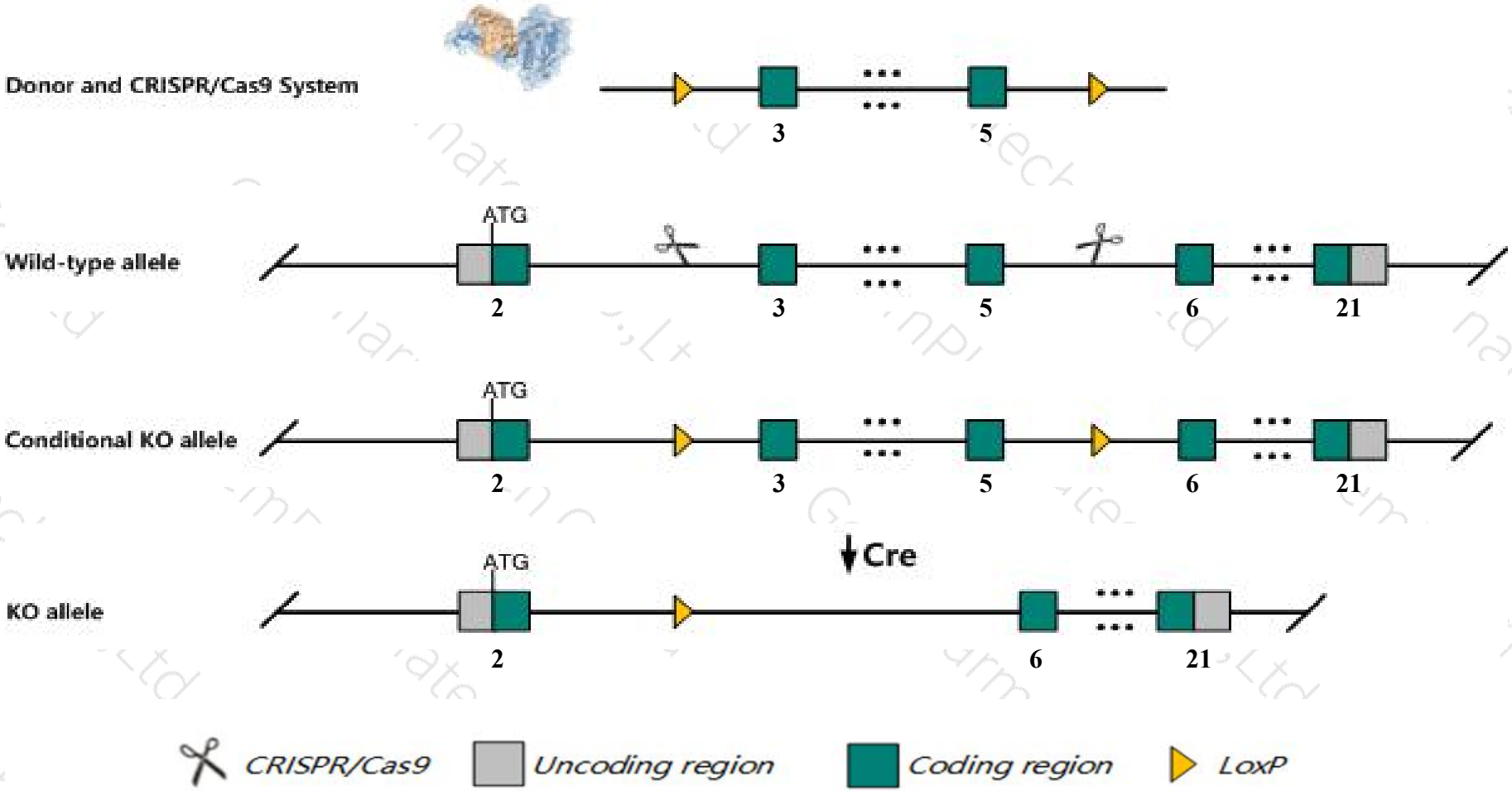
Designer: Huan Wang
Reviewer: Huan Fan
Design Date: 2020-2-18

Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Ikbkb* gene has 14 transcripts. According to the structure of *Ikbkb* gene, exon3-exon5 of *Ikbkb-202* (ENSMUST00000063401.9) transcript is recommended as the knockout region.The region contains 283bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Ikbkb* 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,Homozygotes for targeted null mutations exhibit liver degeneration and die in midgestation. Conditional mutations that lack gene expression in lymphoid cells or epidermal keratinocytes exhibit B and T cell deficits and skin inflammation, respectively.

➢ The KO region contains functional region of the *Gm15346* gene.Knockout the region may affect the function of *Gm15346* gene

➢ The *Ikbkb* gene is located on the Chr8. 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

Ikbkb inhibitor of kappaB kinase beta [Mus musculus (house mouse)]

Gene ID: 16150, updated on 9-Apr-2019

Summary

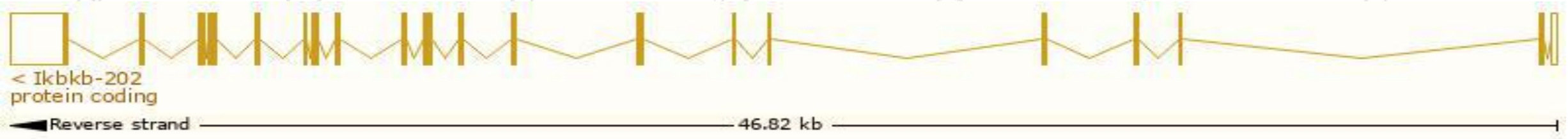
Official Symbol	Ikbkb provided by MGI
Official Full Name	inhibitor of kappaB kinase beta provided by MGI
Primary source	MGI:MGI:1338071
See related	Ensembl:ENSMUSG00000031537
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI132552, IKK-2, IKK-beta, IKK2, IKK[b], IKKbeta
Expression	Ubiquitous expression in spleen adult (RPKM 15.9), thymus adult (RPKM 12.5) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

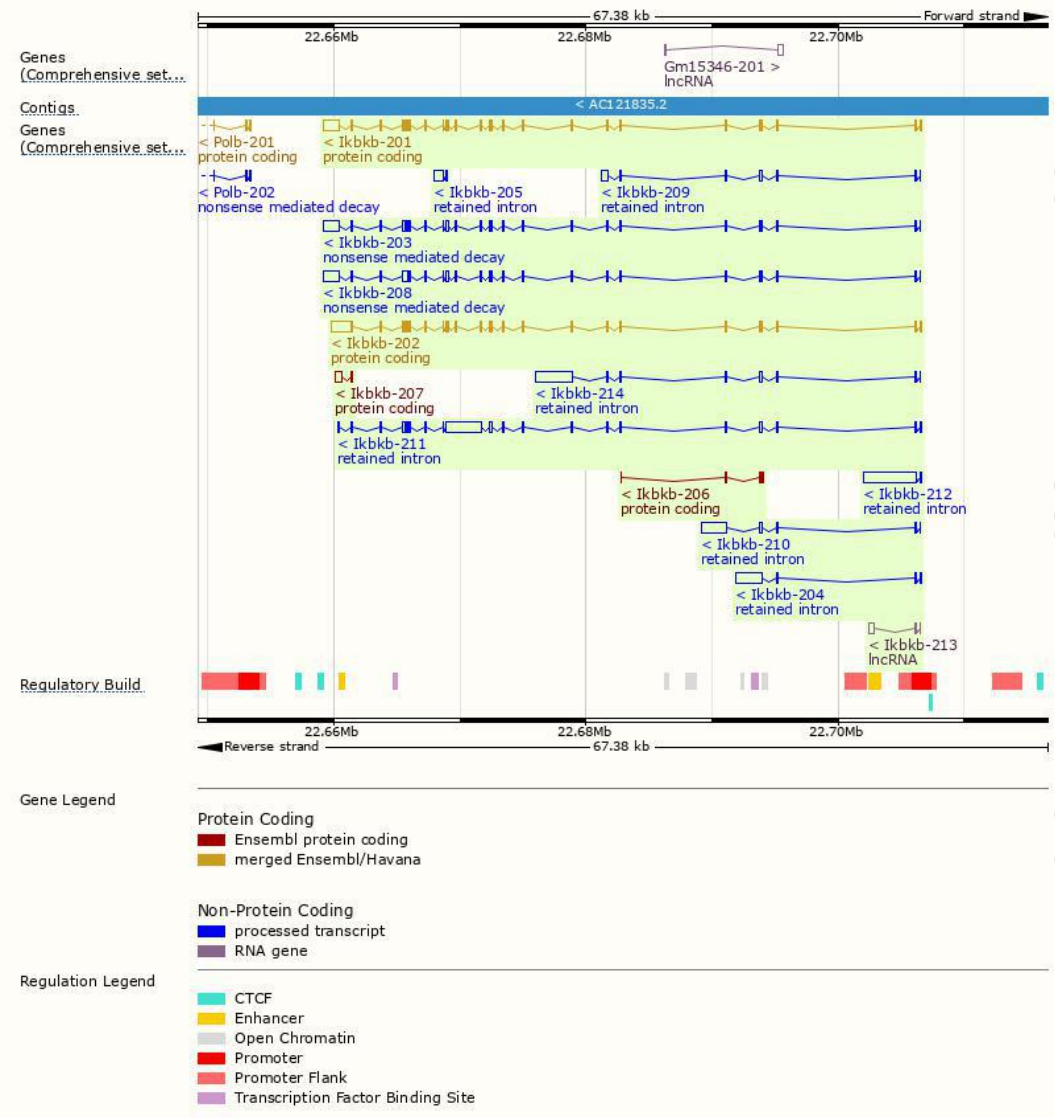
The gene has 14 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ikbkb-202	ENSMUST00000063401.9	3977	738aa	Protein coding	CCDS22182	A0A0R4J0T4	TSL:1 GENCODE basic
Ikbkb-201	ENSMUST00000033939.12	3580	757aa	Protein coding	CCDS52522	Q5D0E0	TSL:1 GENCODE basic APPRIS P1
Ikbkb-207	ENSMUST00000132279.1	636	35aa	Protein coding	-	F6XPL0	CDS 5' incomplete TSL:2
Ikbkb-206	ENSMUST00000131767.1	300	100aa	Protein coding	-	F6ULQ4	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Ikbkb-208	ENSMUST00000135326.7	3705	442aa	Nonsense mediated decay	-	Q3U141	TSL:1
Ikbkb-203	ENSMUST00000125314.7	3536	442aa	Nonsense mediated decay	-	Q3U141	TSL:1
Ikbkb-213	ENSMUST00000150214.1	601	No protein	Processed transcript	-	-	TSL:1
Ikbkb-211	ENSMUST00000146212.7	4845	No protein	Retained intron	-	-	TSL:5
Ikbkb-212	ENSMUST00000149093.1	4291	No protein	Retained intron	-	-	TSL:1
Ikbkb-214	ENSMUST00000150259.7	3587	No protein	Retained intron	-	-	TSL:1
Ikbkb-210	ENSMUST00000144895.7	2423	No protein	Retained intron	-	-	TSL:1
Ikbkb-204	ENSMUST00000126439.7	2416	No protein	Retained intron	-	-	TSL:1
Ikbkb-209	ENSMUST00000144583.7	1106	No protein	Retained intron	-	-	TSL:1
Ikbkb-205	ENSMUST00000126496.1	817	No protein	Retained intron	-	-	TSL:3

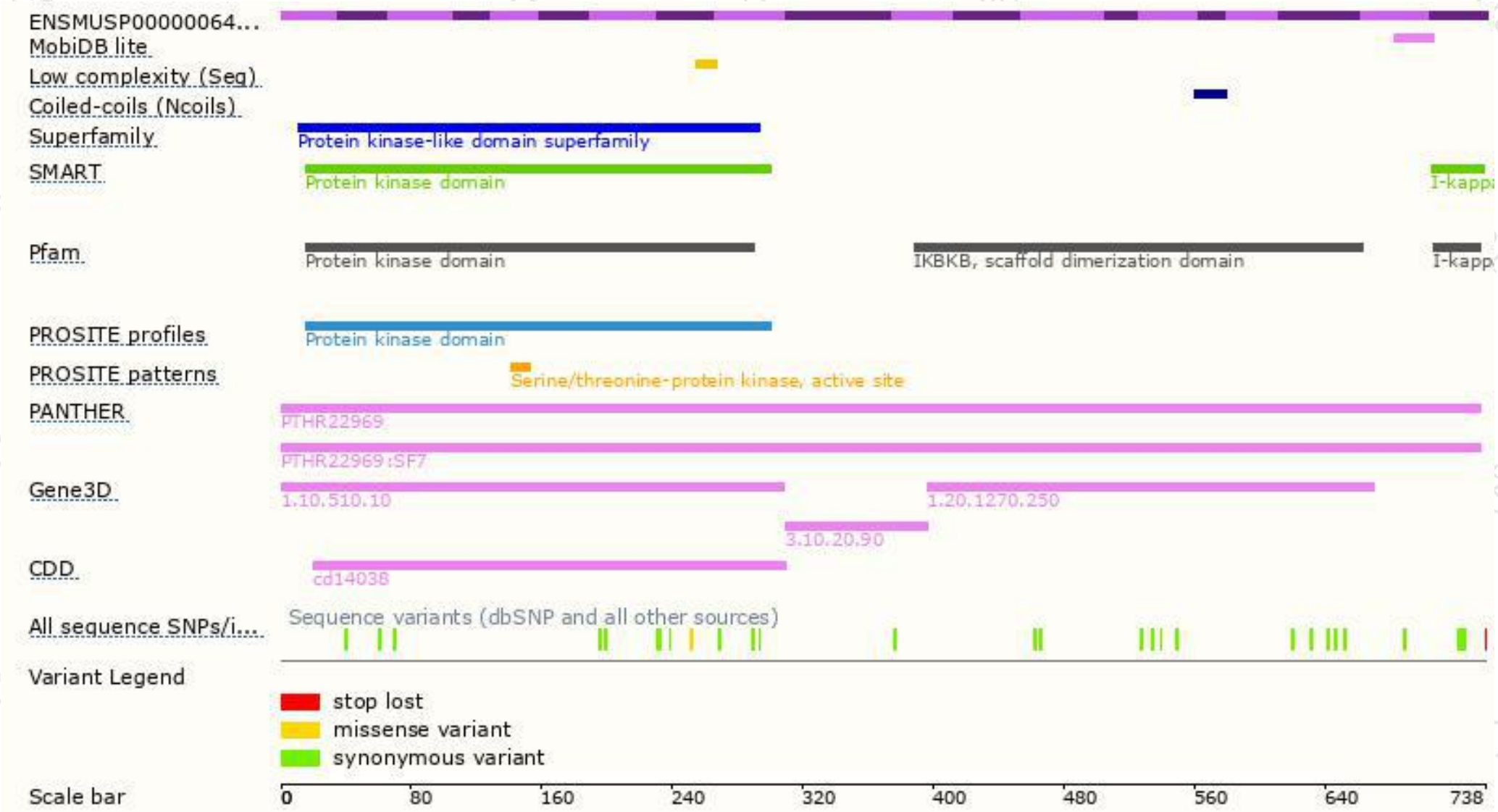
The strategy is based on the design of *Ikbkb-202* transcript,The transcription is shown below



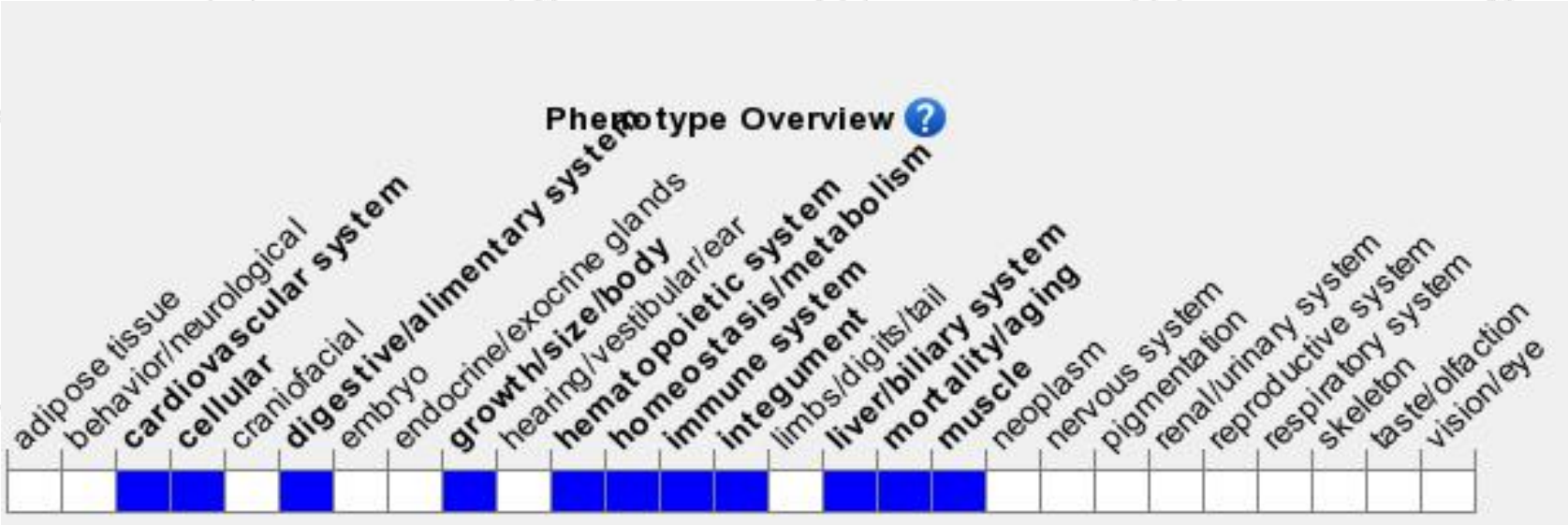
Genomic location distribution



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,Homozygotes for targeted null mutations exhibit liver degeneration and die in midgestation. Conditional mutations that lack gene expression in lymphoid cells or epidermal keratinocytes exhibit B and T cell deficits and skin inflammation, respectively.

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