Map3k8 Cas9-CKO Strategy

Designer: Yang Zeng
Reviewer: Xueting Zhang

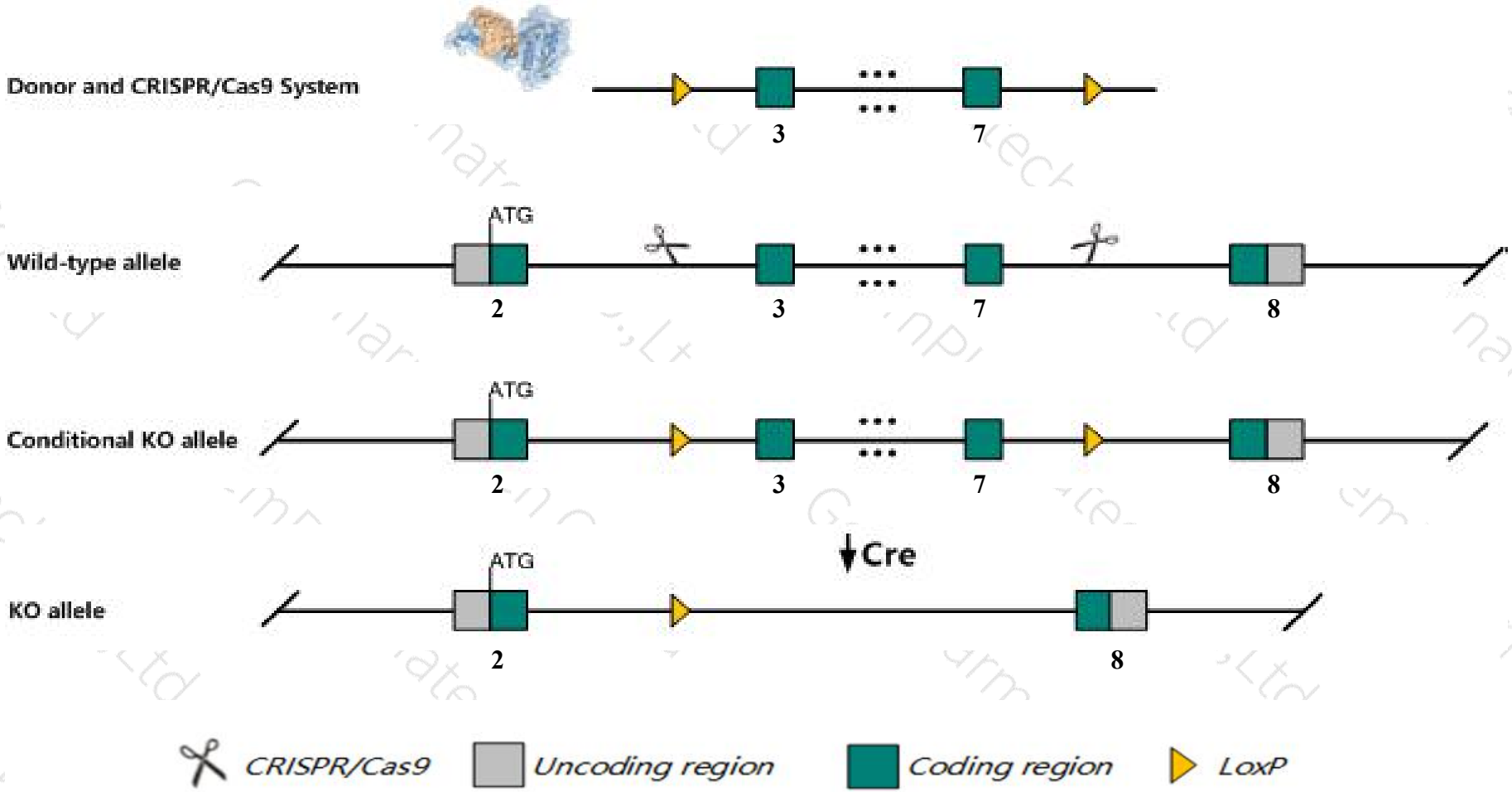
Design Date: 2019-10-28

Project Overview

Project Name	***Map3k8***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Map3k8* gene. The schematic diagram is as follows:



Technical routes

- The *Map3k8* gene has 5 transcripts. According to the structure of *Map3k8* gene, exon3-exon7 of *Map3k8-201* (ENSMUST00000025078.9) transcript is recommended as the knockout region.The region contains 937bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Map3k8* 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,Mutant mice resist endotoxic shock. Their MHC II expression is enhanced. Macrophages TNF-alpha response to viruses and to all TLR ligands is impaired. Macrophage and T-cell secretion of other cytokines in response to various TLR ligands or OVA is aberrant. Anti-OVA Ig classes are abnormally skewed.

➢ The *Map3k8* gene is located on the Chr18. 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

Map3k8 mitogen-activated protein kinase kinase kinase 8 [*Mus musculus* (house mouse)]

Gene ID: 26410, updated on 10-Oct-2019

Summary

Official Symbol	Map3k8 provided by MGI
Official Full Name	mitogen-activated protein kinase kinase kinase 8 provided by MGI
Primary source	MGI:MGI:1346878
See related	Ensembl:ENSMUSG00000024235
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	Cot; Est; Estf; Tpl2; Tpl-2; c-COT; Cot/Tpl2
Expression	Ubiquitous expression in spleen adult (RPKM 3.1), lung adult (RPKM 2.9) and 27 other tissues See more
Orthologs	human all

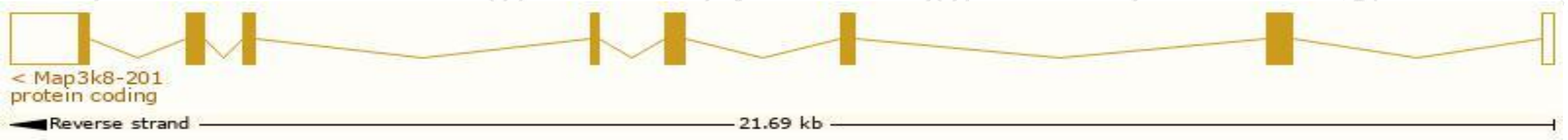
Chromosome 18 - NC_000084.6

Transcript information（Ensembl）

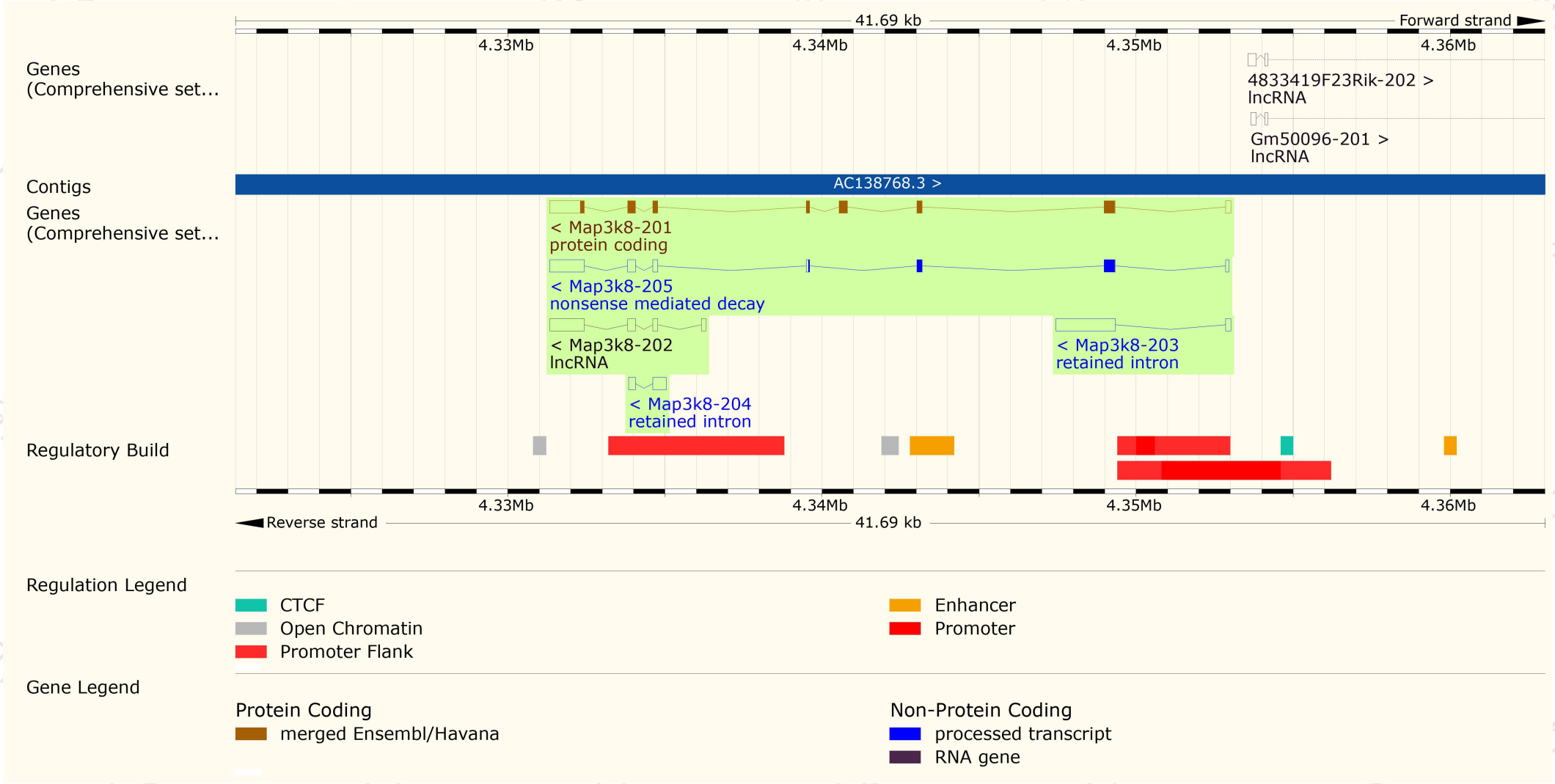
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Map3k8-201	ENSMUST00000025078.9	2569	467aa	ENSMUSP00000025078.2	Protein coding	CCDS29036	Q07174 Q3UEB8	TSL:1 GENCODE basic APPRIS P1
Map3k8-205	ENSMUST00000173930.7	2236	181aa	ENSMUSP00000133469.1	Nonsense mediated decay	-	G3UWY2	TSL:1
Map3k8-203	ENSMUST00000172805.1	2048	No protein	-	Retained intron	-	-	TSL:1
Map3k8-204	ENSMUST00000173708.1	652	No protein	-	Retained intron	-	-	TSL:3
Map3k8-202	ENSMUST00000105472.2	1626	No protein	-	lncRNA	-	-	TSL:1

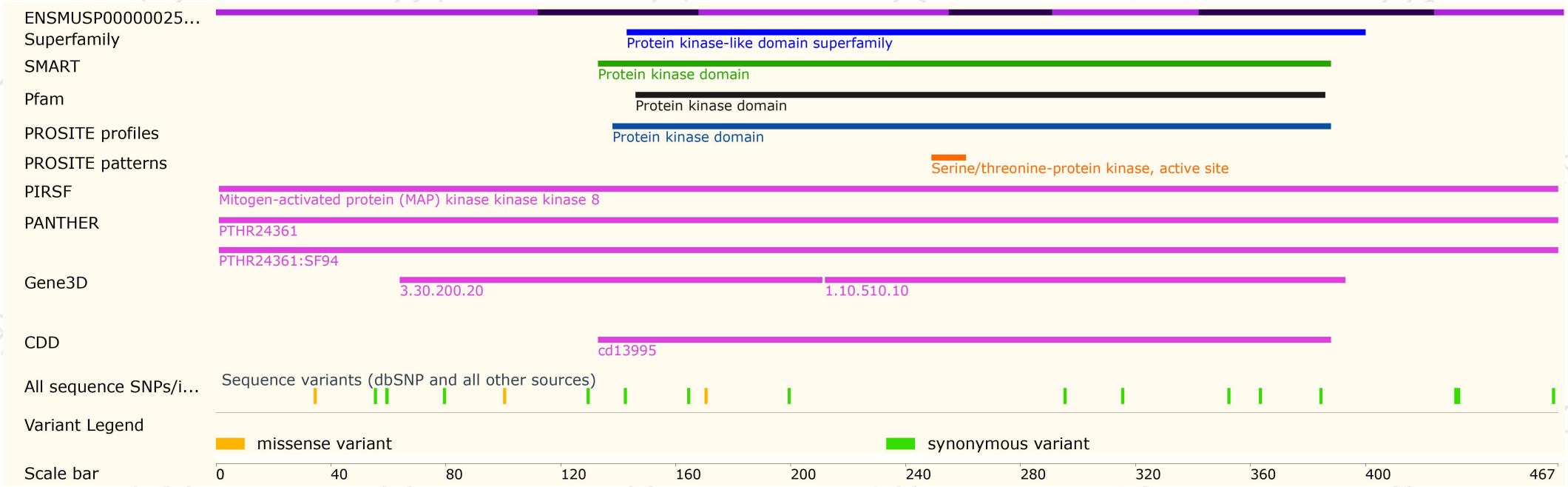
The strategy is based on the design of *Map3k8-201* transcript,The transcription is shown below



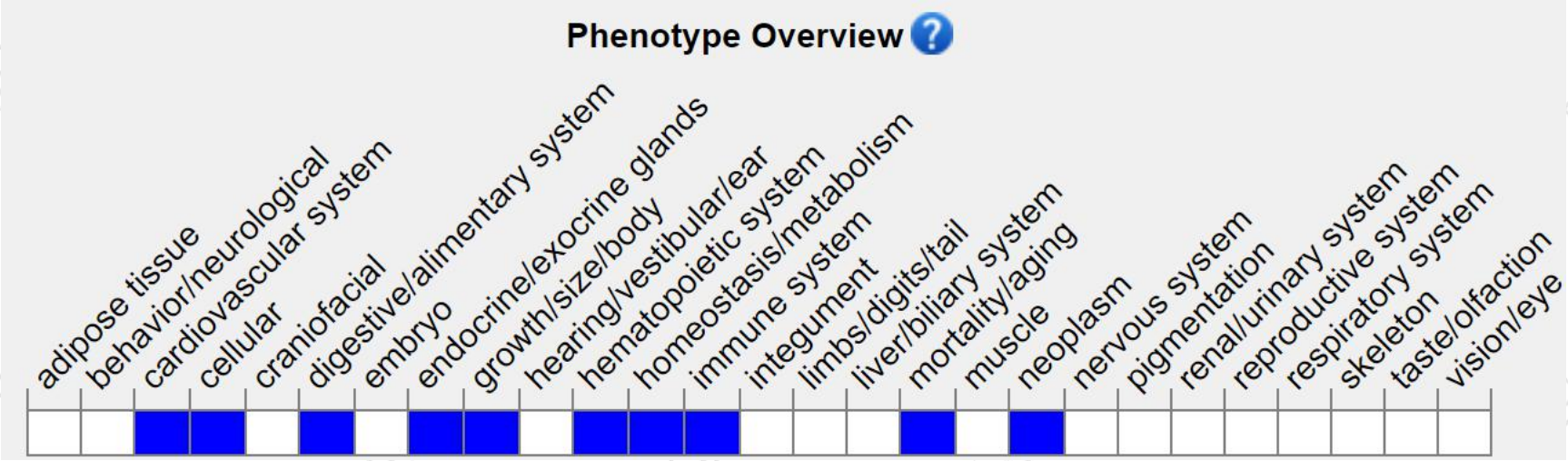
Genomic location distribution



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,Mutant mice resist endotoxic shock. Their MHC II expression is enhanced. Macrophages TNF-alpha response to viruses and to all TLR ligands is impaired. Macrophage and T-cell secretion of other cytokines in response to various TLR ligands or OVA is aberrant. Anti-OVA Ig classes are abnormally skewed.

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