Dio3 Cas9-CKO Strategy

Designer: Miaomiao Cui

Reviewer: Rui Xiong

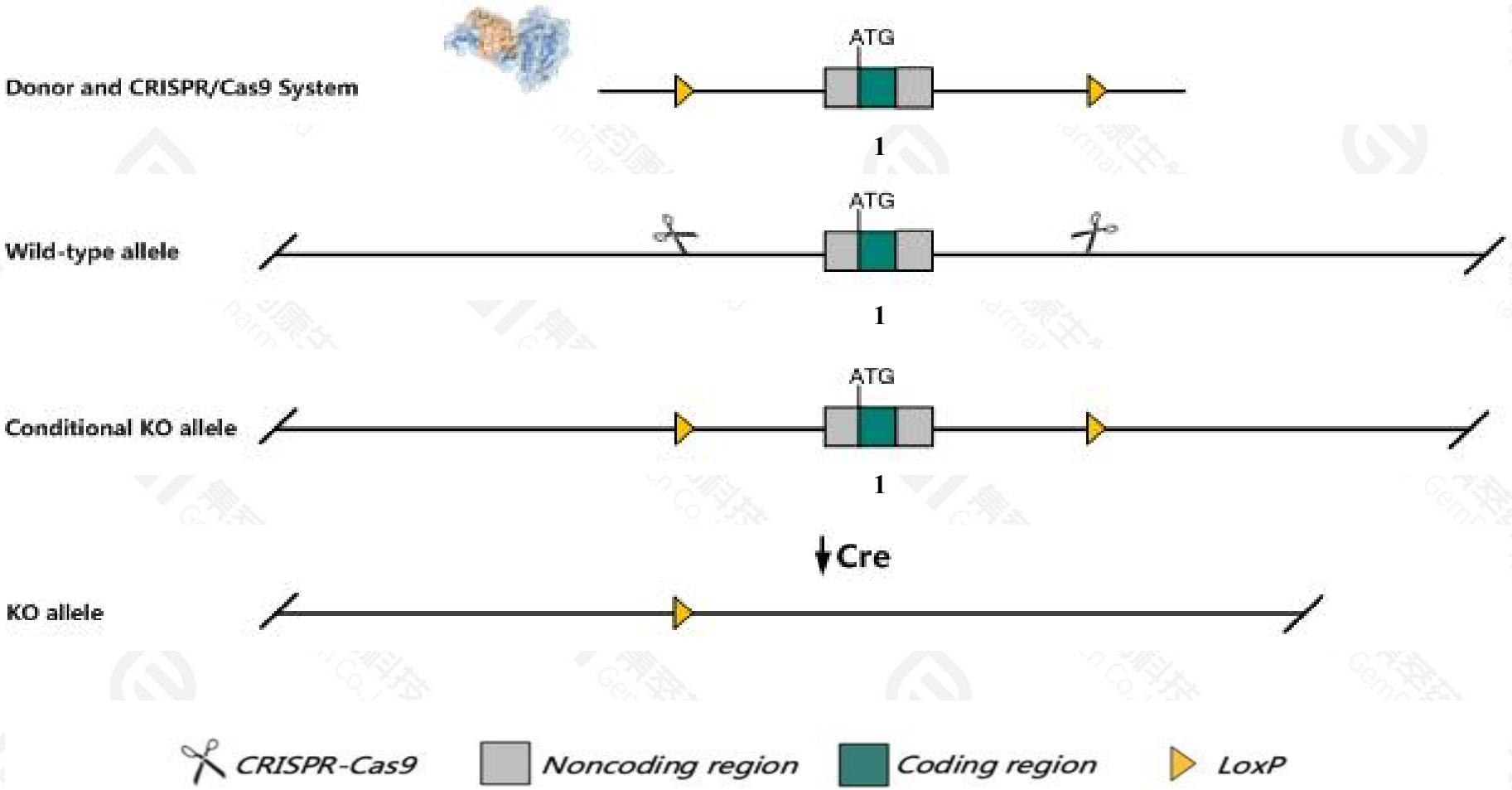
Design Date: 2022-7-22

Project Overview

Project Name	***Dio3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR-Cas9 technology to edit the *Dio3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Dio3* gene has 2 transcripts. According to the structure of *Dio3* gene, exon1 of *Dio3-202*(ENSMUST00000173014.3) transcript is recommended as the knockout region.The region contains all of the coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR-Cas9 technology to modify *Dio3* 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,mice homozygous for a null allele show partial embryonic or perinatal mortality, growth retardation, reduced fertility, and severe anomalies in thyroid status and physiology, including reduced T3 clearance and neonatal thyrotoxicosis followed by central hypothyroidism that persists throughout life.

➢ The KO region contains functional region of the Dio3os and Mir1247 gene.Knockout the region may affect the function of Dio3os and Mir1247 gene.

➢ The *Dio3* gene is located on the Chr12. 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

Dio3 deiodinase, iodothyronine type III [Mus musculus (house mouse)]

Gene ID: 107585, updated on 24-Apr-2022

Summary

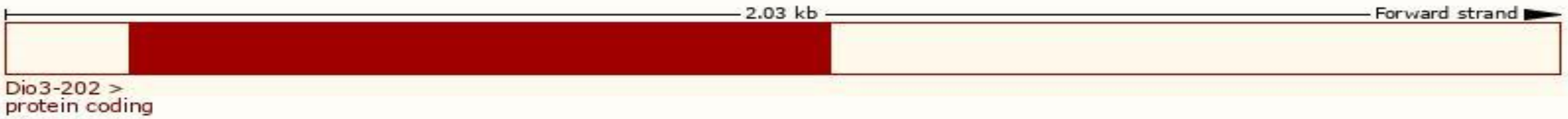
Official Symbol	Dio3 provided by MGI
Official Full Name	deiodinase, iodothyronine type III provided byMGI
Primary source	MGI:MGI:1306782
See related	Ensembl:ENSMUSG00000075707
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Summary	This is an intronless, imprinted gene that is preferentially expressed from the paternal allele in the mouse fetus. The encoded protein belongs to the iodothyronine deiodinase family, and catalyzes the inactivation of thyroid hormone by inner ring deiodination of the prohormone thyroxine (T4) and the bioactive hormone 3,3',5-triiodothyronine (T3) to inactive metabolites, 3,3',5' triiodothyronine (RT3) and 3,3'-diiodothyronine (T2), respectively. It is highly expressed in placenta, fetal and neonatal tissues, and thought to prevent premature exposure of developing fetal tissues to adult levels of thyroid hormones. It thus plays a critical role in mammalian development by regulating circulating fetal thyroid hormone concentration. Knockout mice lacking this gene exhibit severe abnormalities related to development and reproduction. This protein is a selenoprotein, containing the rare selenocysteine (Sec) amino acid at its active site. Sec is encoded by the UGA codon, which normally signals translation termination. The 3' UTRs of selenoprotein mRNAs contain a conserved stem-loop structure, designated the Sec insertion sequence (SECIS) element, that is necessary for the recognition of UGA as a Sec codon rather than as a stop signal. [provided by RefSeq, Jun 2016]
Orthologs	human all

Transcript information（Ensembl）

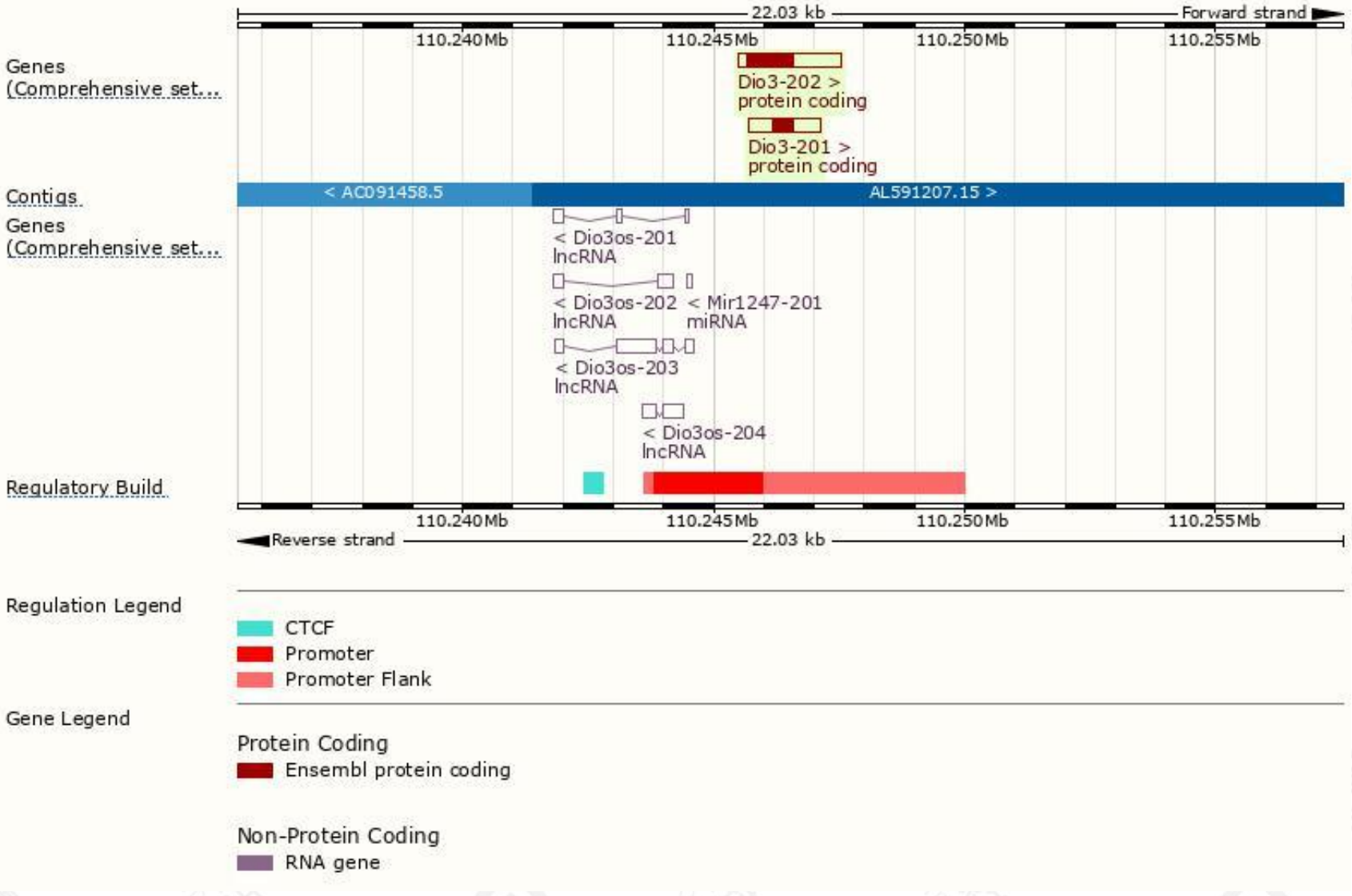
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dio3-202	ENSMUST00000173014.3	2030	304aa	Protein coding	CCDS26171		TSL:NA , GENCODE basic , APPRIS P1 ,
Dio3-201	ENSMUST00000097228.5	1449	131aa	Protein coding	-		TSL:NA , GENCODE basic ,

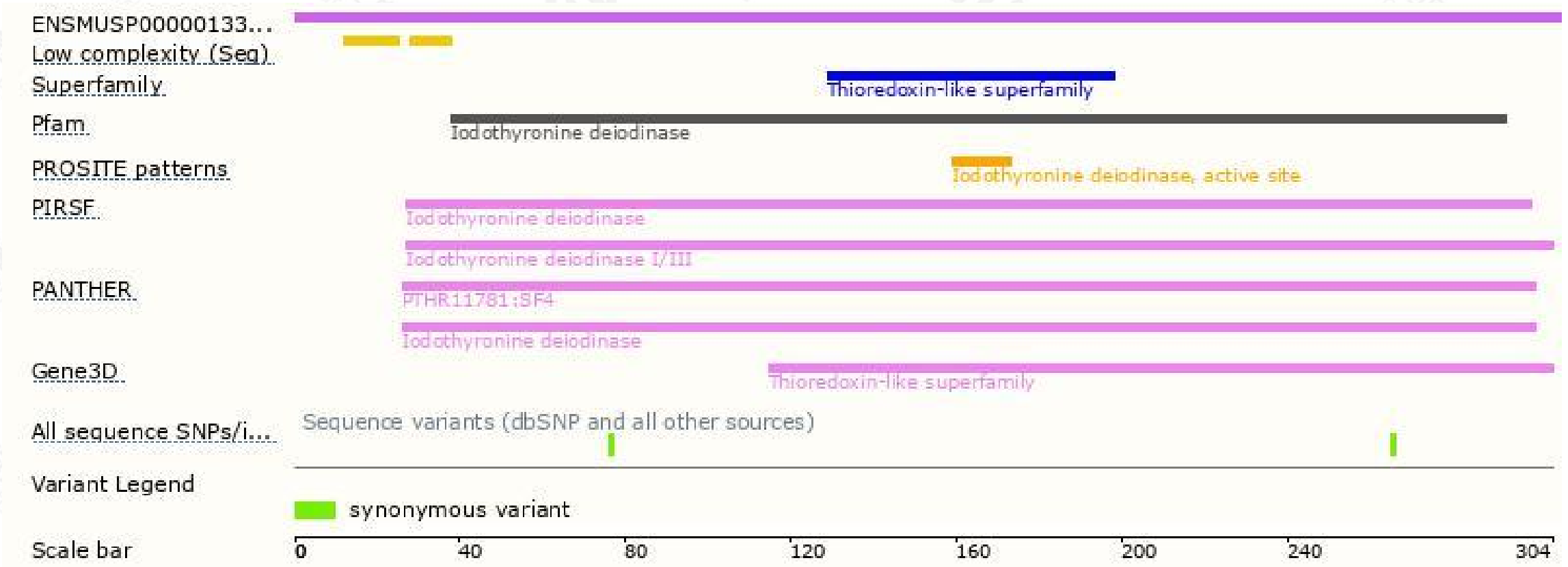
The strategy is based on the design of *Dio3-202* transcript,the transcription is shown below:



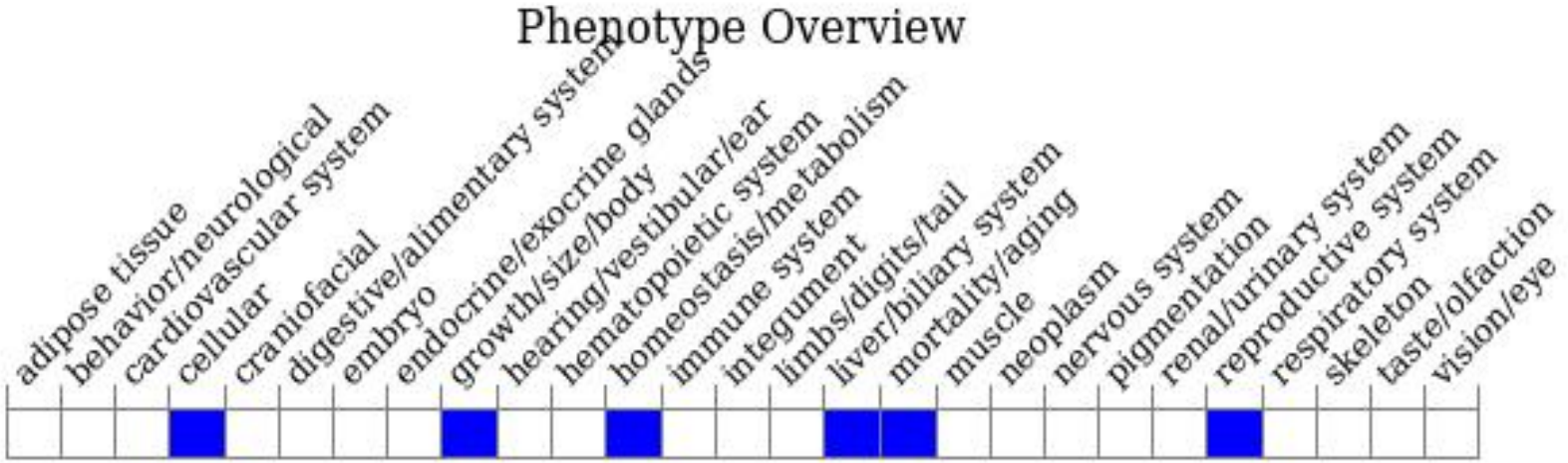
Genomic location distribution



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,mice homozygous for a null allele show partial embryonic or perinatal mortality, growth retardation, reduced fertility, and severe anomalies in thyroid status and physiology, including reduced T3 clearance and neonatal thyrotoxicosis followed by central hypothyroidism that persists throughout life.

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