Gtf2i Cas9-CKO Strategy

Designer: Rui Xiong

Reviewer: Longyun Hu

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Project Overview

Project Name	***Gtf2i***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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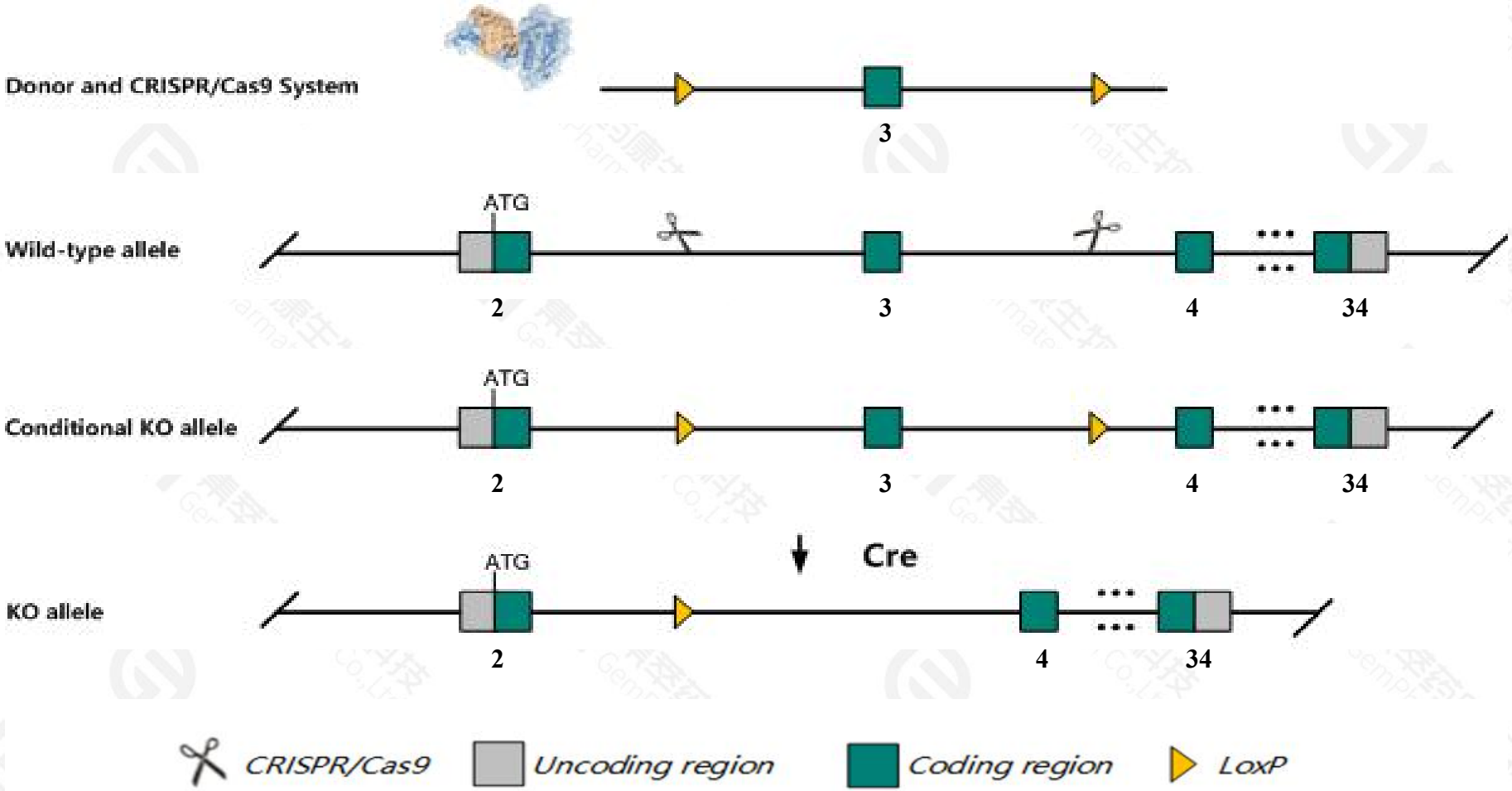
This model will use CRISPR/Cas9 technology to edit the *Gtf2i* gene. The schematic diagram is as follows:



Technical routes

The *Gtf2i* gene has 28 transcripts. According to the structure of *Gtf2i* gene, exon3 of *Gtf2i-201*(ENSMUST00000059042.15) transcript is recommended as the knockout region.The region contains 139bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Gtf2i* 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

Transcript *Gtf2i-211* may not be affected.

According to the existing MGI data,homozygotes for null allele is embryonic lethal, and show brain hemorrhage and neural tube defects. Although most heterozygote are normal and fertile, at low frequency, growth retardation and small head are also reported.

The *Gtf2i* gene is located on the Chr5. 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

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Gtf2i general transcription factor II I [Mus musculus (house mouse)]

Gene ID: 14886, updated on 17-Dec-2020

Summary

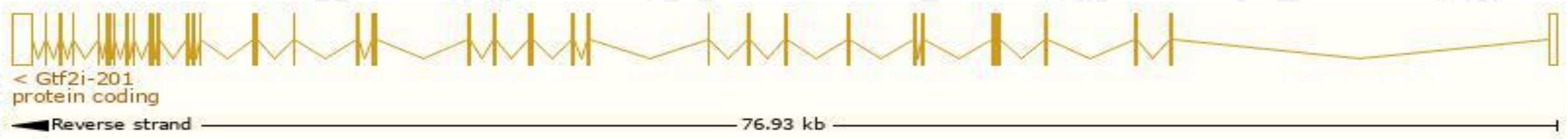
Official Symbol	Gtf2i provided by MGI
Official Full Name	general transcription factor II I provided byMGI
Primary source	MGI:MGI:1202722
See related	Ensembl:ENSMUSG00000060261
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	6030441I21Rik, BAP-1, BAP-135, Diws1t, GtfII-I, Spin, TFI, TFII-I, WBSCR6
Expression	Ubiquitous expression in CNS E11.5 (RPKM 36.6), limb E14.5 (RPKM 32.6) and 28 other tissuesSee more
Orthologs	human all

Transcript information Ensembl

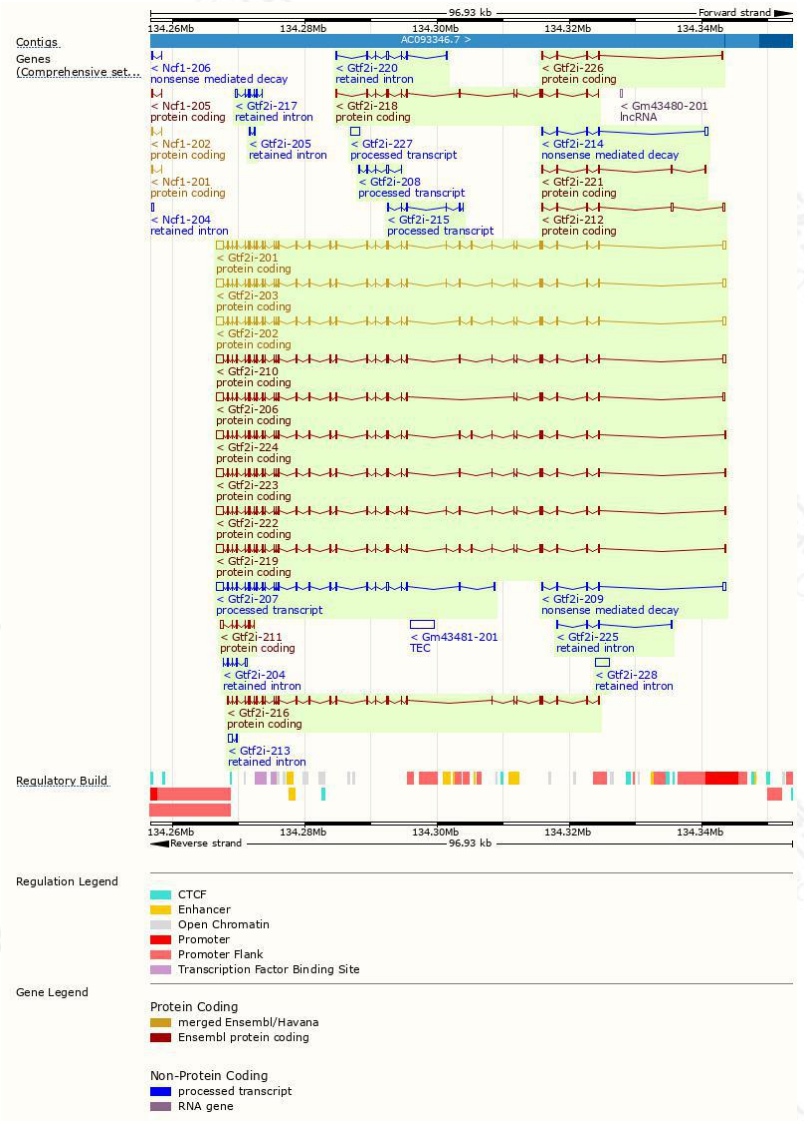
The gene has 28 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gtf2i-201	ENSMUST00000059042.15	4455	998aa	Protein coding	CCDS39301		TSL:1 , GENCODE basic , APPRIS P4 ,
Gtf2i-203	ENSMUST00000111261.12	4381	979aa	Protein coding	CCDS39299		TSL:5 , GENCODE basic , APPRIS ALT1 ,
Gtf2i-202	ENSMUST00000082057.10	4375	977aa	Protein coding	CCDS39300		TSL:5 , GENCODE basic , APPRIS ALT1 ,
Gtf2i-210	ENSMUST00000173341.9	4302	962aa	Protein coding	CCDS57387		TSL:1 , GENCODE basic ,
Gtf2i-219	ENSMUST00000174155.8	4141	998aa	Protein coding	CCDS39301		TSL:2 , GENCODE basic , APPRIS P4 ,
Gtf2i-222	ENSMUST00000174354.8	4084	979aa	Protein coding	CCDS39299		TSL:2 , GENCODE basic , APPRIS ALT1 ,
Gtf2i-224	ENSMUST00000174772.8	4078	977aa	Protein coding	CCDS39300		TSL:2 , GENCODE basic , APPRIS ALT1 ,
Gtf2i-223	ENSMUST00000174513.8	4021	958aa	Protein coding	CCDS57386		TSL:2 , GENCODE basic , APPRIS ALT1 ,
Gtf2i-206	ENSMUST00000172715.8	4114	913aa	Protein coding	-		TSL:5 , GENCODE basic ,
Gtf2i-216	ENSMUST00000173888.8	2820	939aa	Protein coding	-		TSL:5 , GENCODE basic ,
Gtf2i-218	ENSMUST00000174133.5	1310	437aa	Protein coding	-		CDS 5' and 3' incomplete , TSL:5 ,
Gtf2i-212	ENSMUST00000173504.8	950	150aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Gtf2i-211	ENSMUST00000173485.8	833	167aa	Protein coding	-		CDS 5' incomplete , TSL:3 ,
Gtf2i-221	ENSMUST00000174188.8	733	159aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Gtf2i-226	ENSMUST00000174867.8	704	169aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Gtf2i-214	ENSMUST00000173651.5	818	86aa	Nonsense mediated decay	-		TSL:5 ,
Gtf2i-209	ENSMUST00000173263.6	738	79aa	Nonsense mediated decay	-		TSL:5 ,
Gtf2i-207	ENSMUST00000172904.8	3274	No protein	Processed transcript	-		TSL:1 ,
Gtf2i-227	ENSMUST00000201371.2	1329	No protein	Processed transcript	-		TSL:NA ,
Gtf2i-208	ENSMUST00000172953.2	630	No protein	Processed transcript	-		TSL:3 ,
Gtf2i-215	ENSMUST00000173887.2	380	No protein	Processed transcript	-		TSL:2 ,
Gtf2i-228	ENSMUST00000202343.2	2164	No protein	Retained intron	-		TSL:NA ,
Gtf2i-213	ENSMUST00000173509.2	803	No protein	Retained intron	-		TSL:3 ,
Gtf2i-217	ENSMUST00000174003.8	771	No protein	Retained intron	-		TSL:2 ,
Gtf2i-220	ENSMUST00000174182.8	758	No protein	Retained intron	-		TSL:2 ,
Gtf2i-225	ENSMUST00000174773.3	517	No protein	Retained intron	-		TSL:1 ,
Gtf2i-204	ENSMUST00000172553.2	490	No protein	Retained intron	-		TSL:1 ,
Gtf2i-205	ENSMUST00000172600.2	358	No protein	Retained intron	-		TSL:2 ,

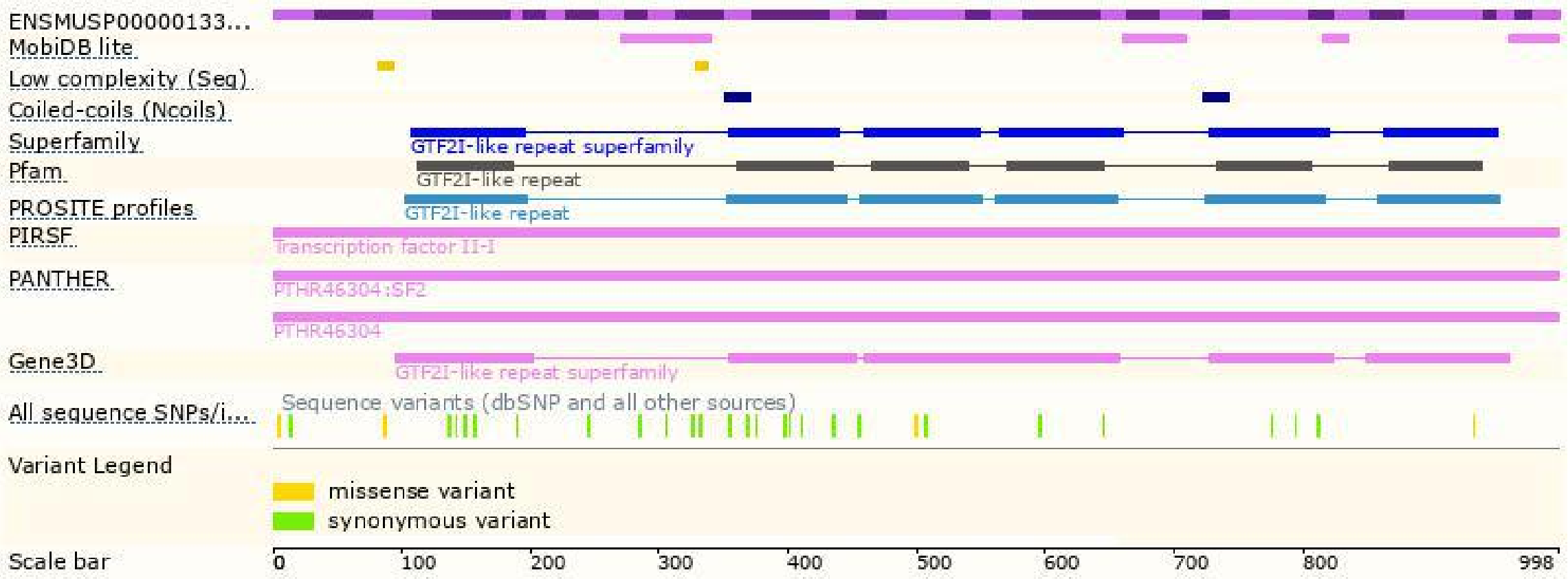
The strategy is based on the design of *Gtf2i-201* transcript,the transcription is shown below:



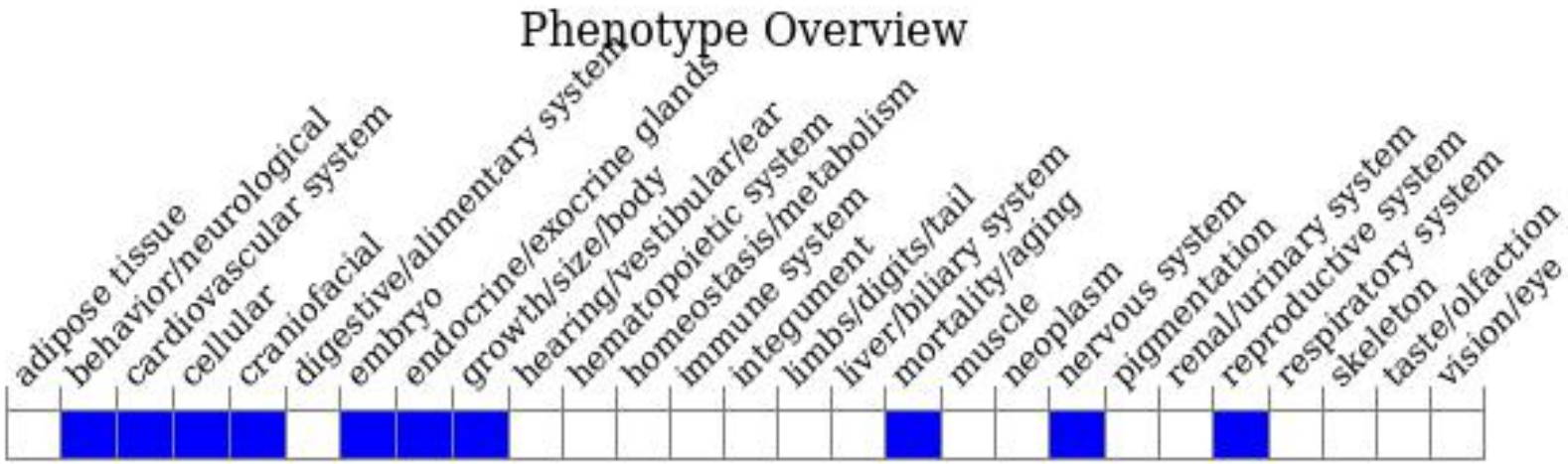
Genomic location distribution



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/).

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GemPharmatech

If you have any questions, you are welcome to inquire.
Tel: 400-9660890

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GemPharmatech Co.,Ltd