Gtf2a1 Cas9-CKO Strategy

Designer: Yupeng Yang

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Project Overview

Project Name	***Gtf2a1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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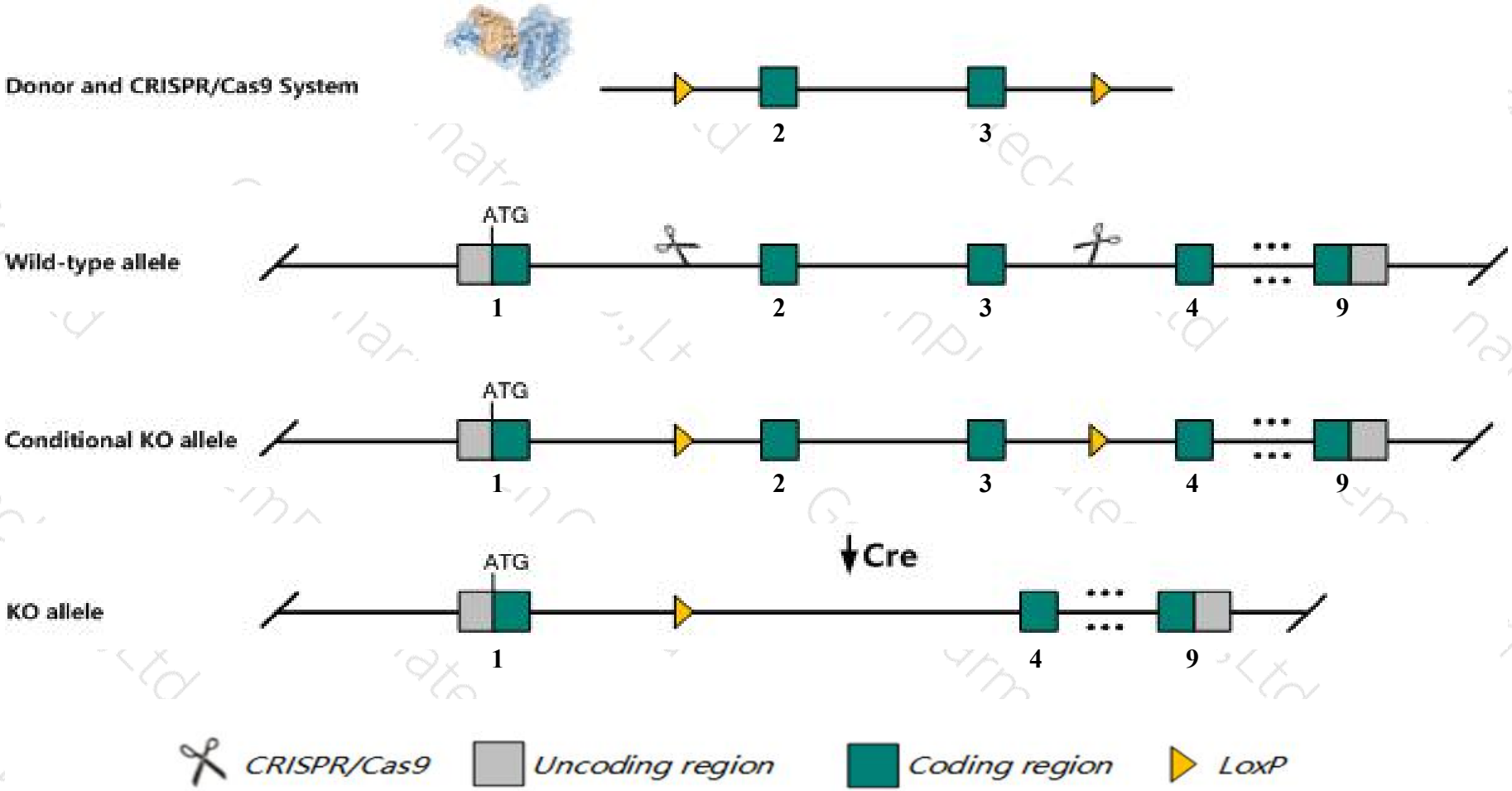
This model will use CRISPR/Cas9 technology to edit the *Gtf2a1* gene. The schematic diagram is as follows:



Technical routes

- The *Gtf2a1* gene has 3 transcripts. According to the structure of *Gtf2a1* gene, exon2-exon3 of *Gtf2a1-201* (ENSMUST00000021345.13) transcript is recommended as the knockout region.The region contains 310bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Gtf2a1* 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 hypomorphic allele where D/G cleavage residues are replaced with noncleavable A/A show neonatal lethality, feeding defects, low testis weight, and male infertility associated with azoospermia, small seminiferous tubules, lack of elongating spermatids, and increased apoptosis.

➢ The *Gtf2a1* 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）

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Gtf2a1 general transcription factor II A, 1 [Mus musculus (house mouse)]

Gene ID: 83602, updated on 31-Jan-2019

Summary

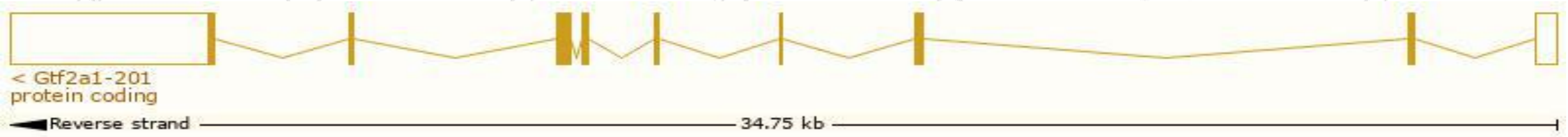
Official Symbol	Gtf2a1 provided by MGI
Official Full Name	general transcription factor II A, 1 provided by MGI
Primary source	MGI:MGI:1933277
See related	Ensembl:ENSMUSG00000020962
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	6330549H03Rik, AA536742, AA959775, AW060250, TfIIAa/b, Tfiia1
Expression	Ubiquitous expression in whole brain E14.5 (RPKM 9.2), CNS E11.5 (RPKM 8.9) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

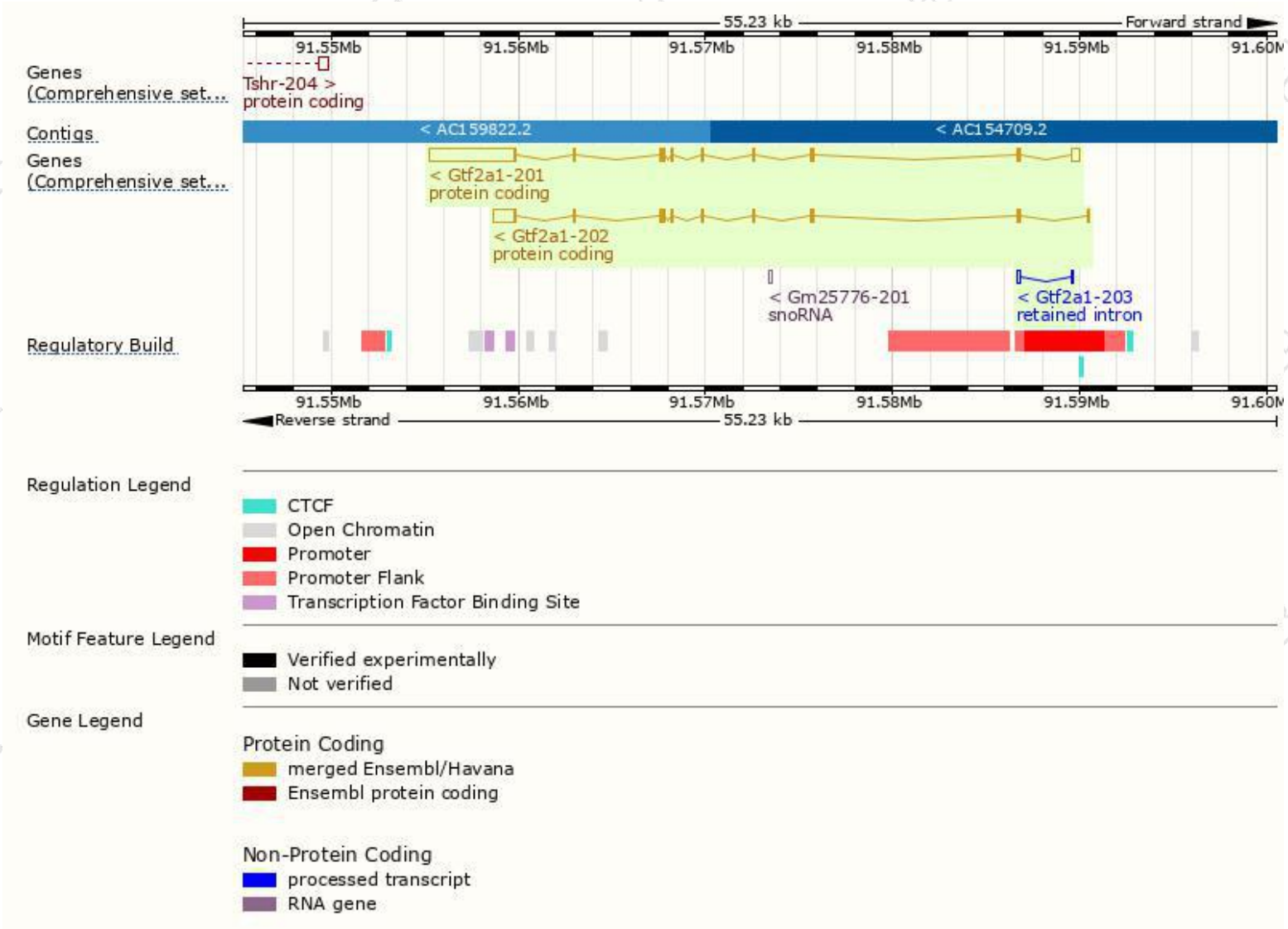
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gtf2a1-201	ENSMUST00000021345.13	6064	378aa	Protein coding	CCDS26089	Q99PM3	TSL:1 GENCODE basic APPRIS P1
Gtf2a1-202	ENSMUST00000063314.6	2265	339aa	Protein coding	CCDS49136	Q149E9	TSL:1 GENCODE basic
Gtf2a1-203	ENSMUST00000163693.1	282	No protein	Retained intron	-	-	TSL:3

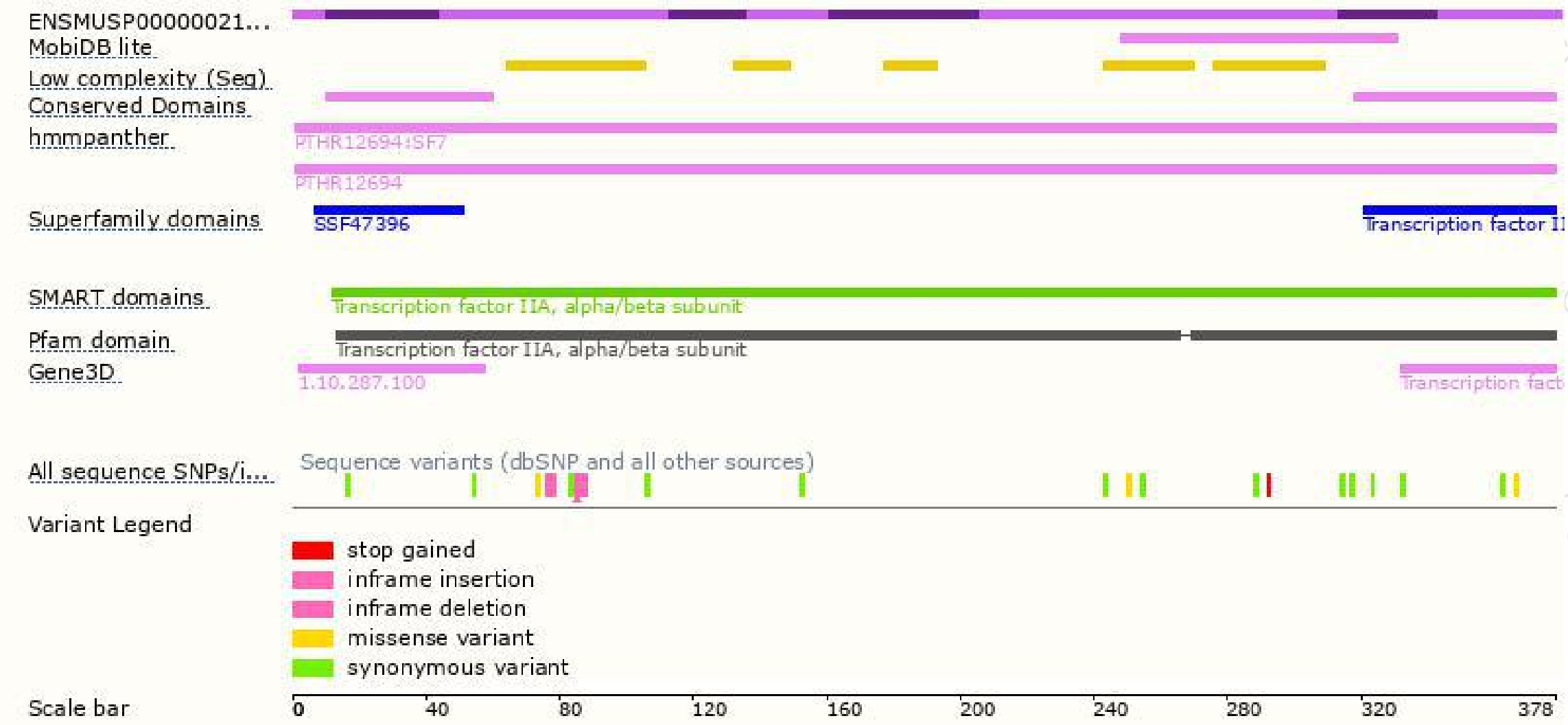
The strategy is based on the design of *Gtf2a1-201* transcript,The transcription is shown below



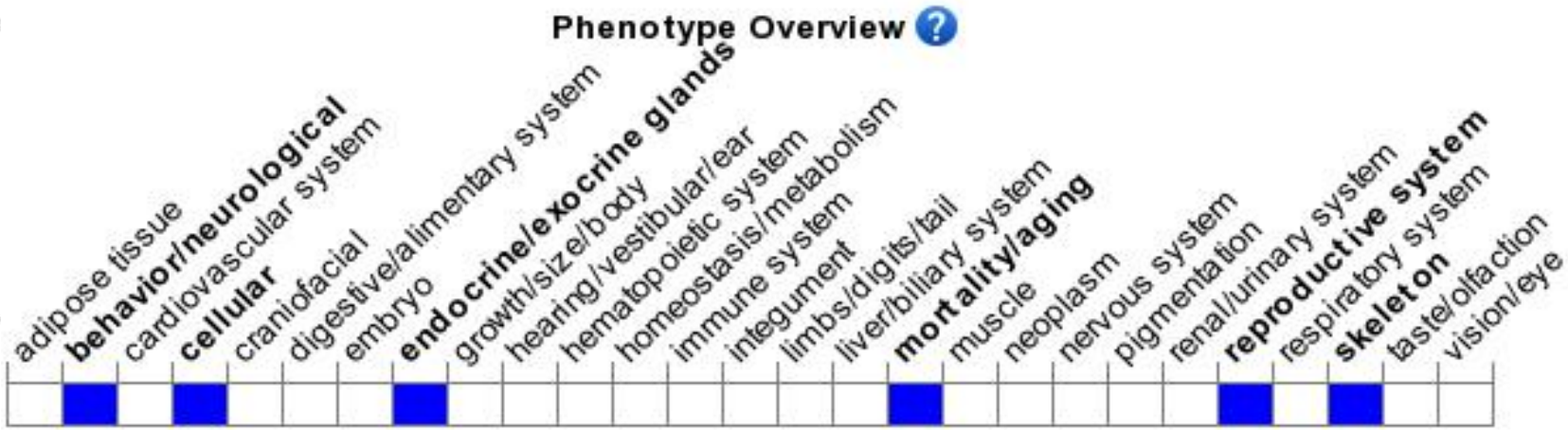
Genomic location distribution



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,Mice homozygous for a hypomorphic allele where D/G cleavage residues are replaced with noncleavable A/A show neonatal lethality, feeding defects, low testis weight, and male infertility associated with azoospermia, small seminiferous tubules, lack of elongating spermatids, and increased apoptosis.

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If you have any questions, you are welcome to inquire.

Tel: 400-9660890

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GemPharmatech Co.,Ltd