Cftr Cas9-CKO Strategy

Designer: Huan Wang

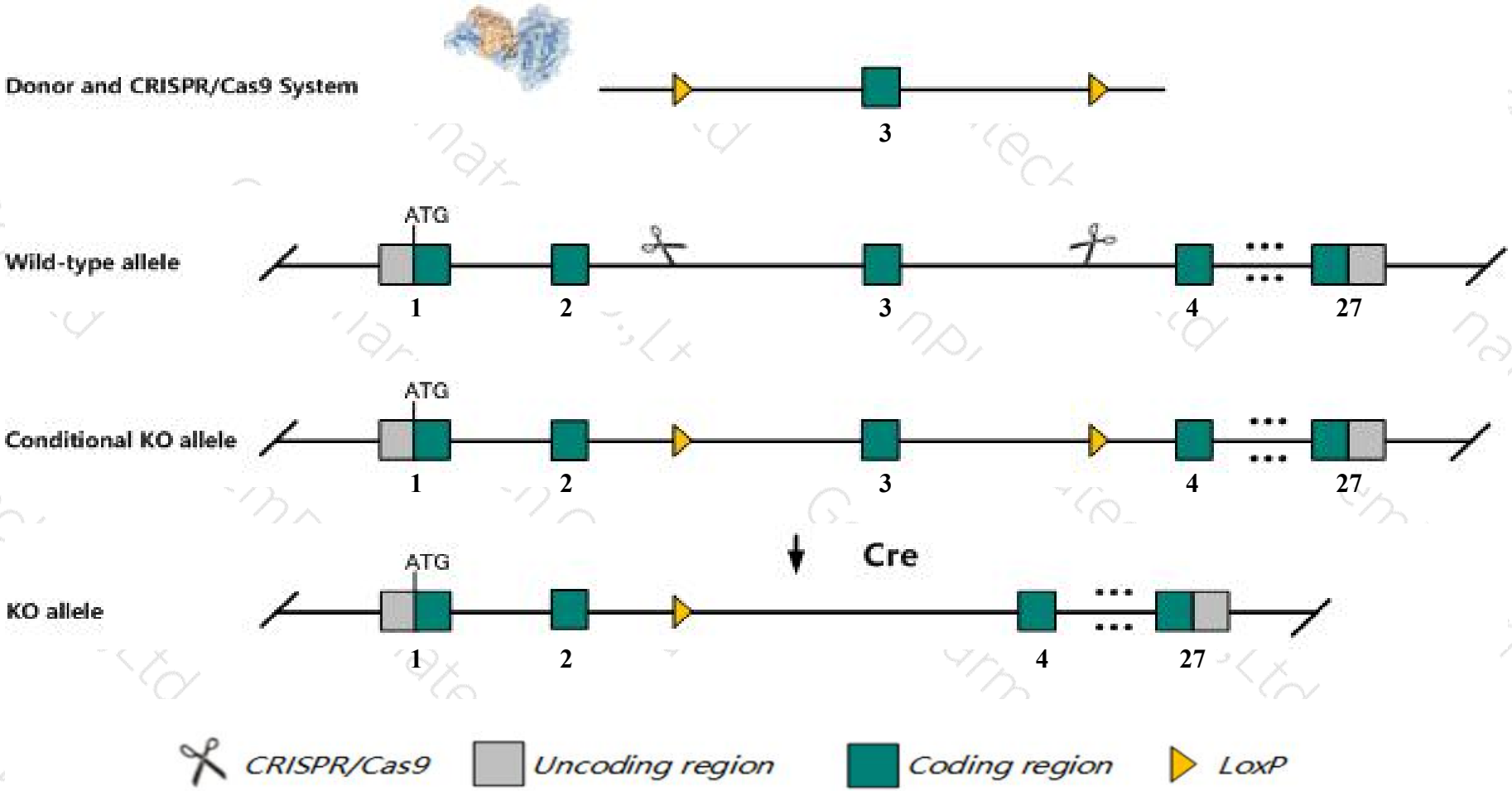
Design Date: 2019-7-24

Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Cftr* gene has 6 transcripts. According to the structure of *Cftr* gene, exon3 of *Cftr-201* (ENSMUST00000045706.11) transcript is recommended as the knockout region.The region contains 109bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Cftr* 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,Homozygotes for targeted null mutations exhibit high mortality associated with intestinal obstruction, and altered mucous and serous glands. Mutants, like humans with cystic fibrosis, also exhibit defective epithelial chloride transport.

- The *Cftr* gene is located on the Chr6. 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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Cftr cystic fibrosis transmembrane conductance regulator [Mus musculus (house mouse)]

Gene ID: 12638, updated on 9-Apr-2019

Summary

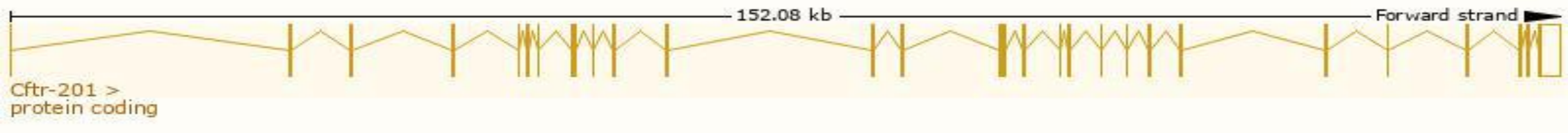
Official Symbol	Cftr provided by MGI
Official Full Name	cystic fibrosis transmembrane conductance regulator provided by MGI
Primary source	MGI:MGI:88388
See related	Ensembl:ENSMUSG00000041301
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW495489, Abcc7
Summary	The membrane-associated protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intra-cellular membranes. ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White). This protein is a member of the MRP subfamily which is involved in multi-drug resistance. This gene encodes the cystic fibrosis transmembrane regulator and a chloride channel that controls the regulation of other transport pathways. Mutations in this gene have been associated with autosomal recessive disorders such as cystic fibrosis and congenital bilateral aplasia of the vas deferens. Alternative splicing of exons 4, 5, and 11 have been observed, but full-length transcripts have not yet been fully described. [provided by RefSeq, Jul 2008]
Expression	Biased expression in large intestine adult (RPKM 4.3), testis adult (RPKM 3.6) and 9 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

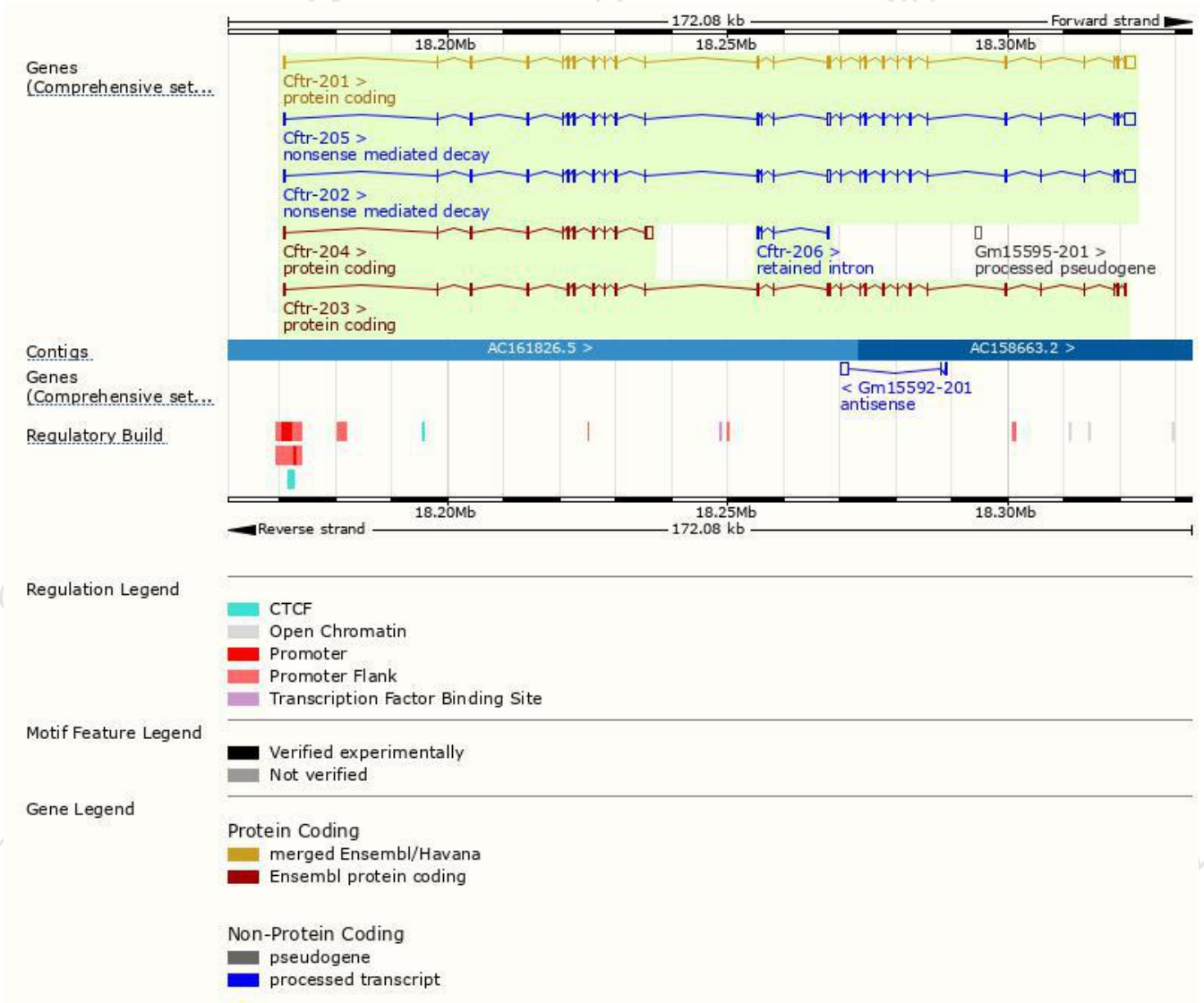
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cftr-201	ENSMUST00000045706.11	6303	1476aa	Protein coding	CCDS19930	P26361	TSL:1 GENCODE basic APPRIS P2
Cftr-203	ENSMUST00000115406.1	4341	1446aa	Protein coding	-	E9PVD7	TSL:5 GENCODE basic APPRIS ALT2
Cftr-204	ENSMUST00000129452.7	2835	529aa	Protein coding	-	F6U9G7	TSL:1 GENCODE basic
Cftr-205	ENSMUST00000140407.7	6410	576aa	Nonsense mediated decay	-	P26361	TSL:5
Cftr-202	ENSMUST00000115405.8	6391	600aa	Nonsense mediated decay	-	P26361	TSL:5
Cftr-206	ENSMUST00000140532.1	838	No protein	Retained intron	-	-	TSL:1

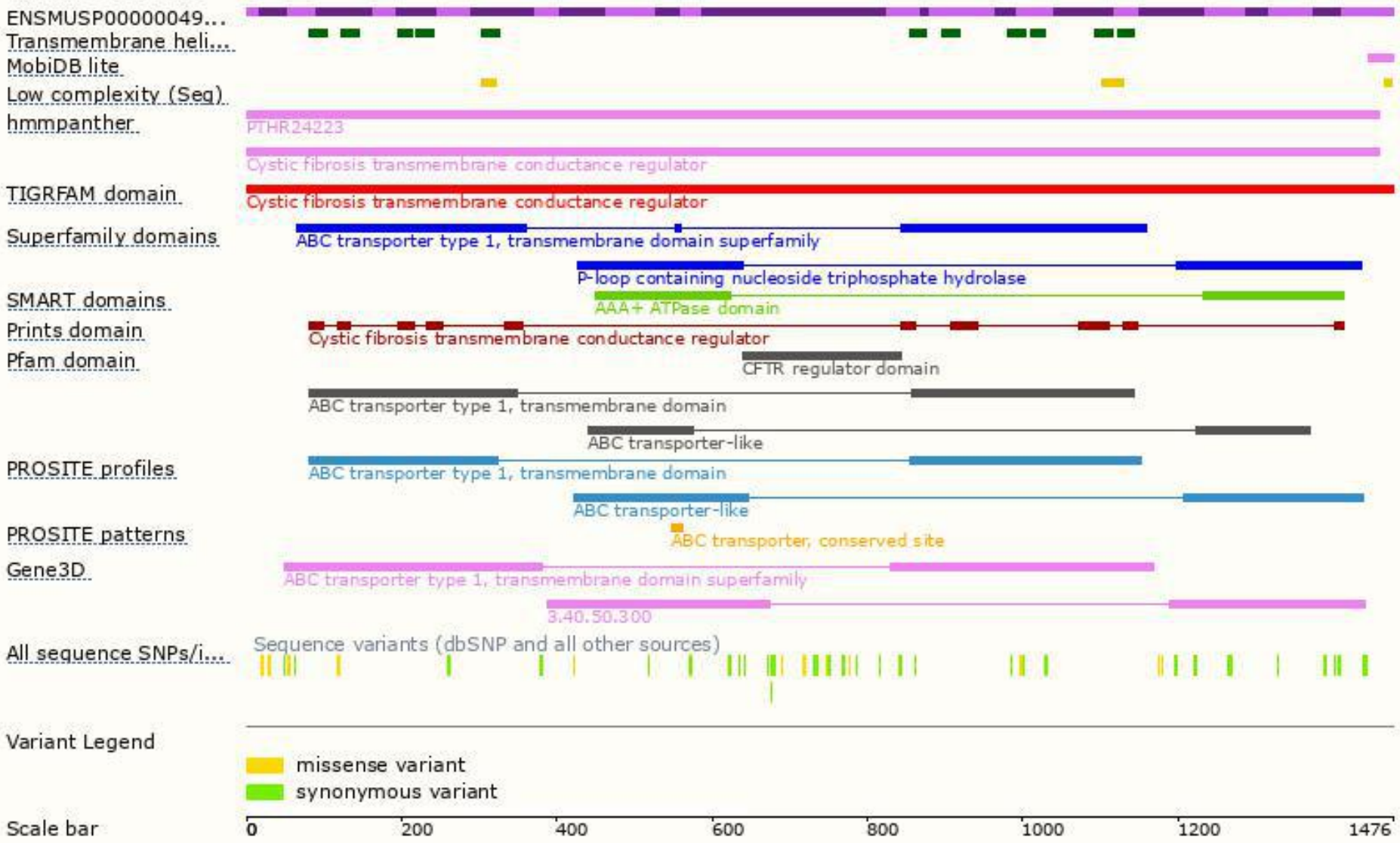
The strategy is based on the design of *Cftr-201* transcript,The transcription is shown below



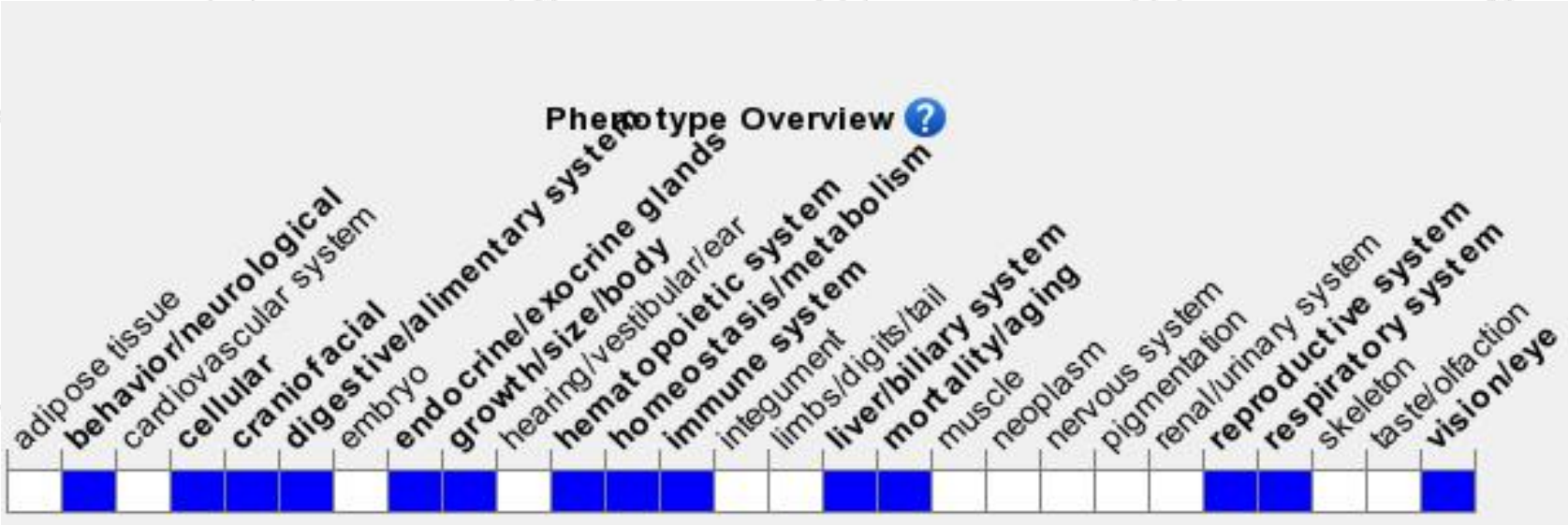
Genomic location distribution



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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集萃药康
GemPharmatech

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

Tel: 400-9660890

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GemPharmatech Co.,Ltd