Itpr2 Cas9-CKO Strategy

Designer:Xueting Zhang
Reviewer:Yanhua Shen
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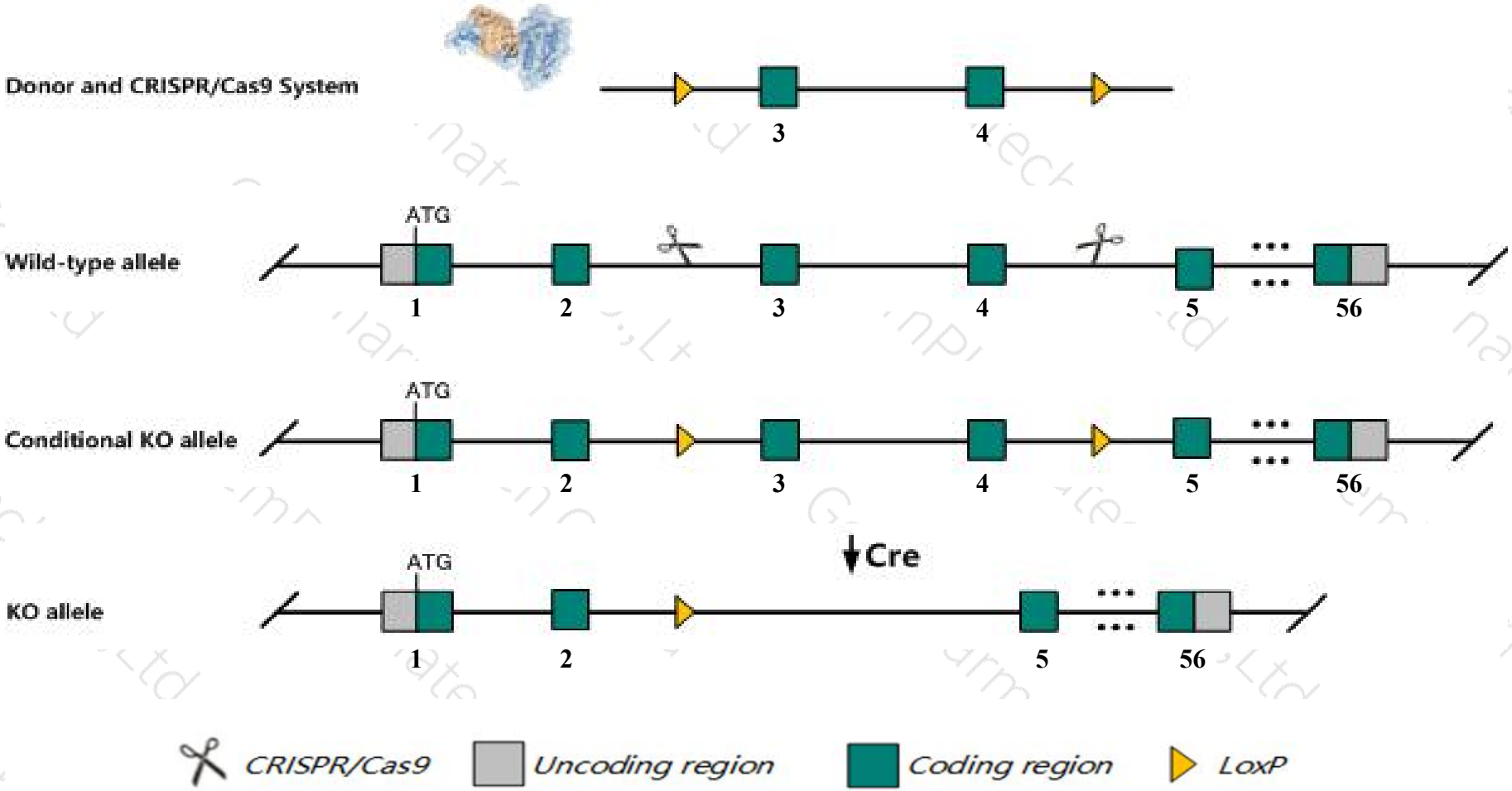
Project Overview

Project Name	***Itpr2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Itpr2* gene. The schematic diagram is as follows:



Technical routes

- The *Itpr2* gene has 11 transcripts. According to the structure of *Itpr2* gene, exon3-exon4 of *Itpr2-202* (ENSMUST00000079573.12) transcript is recommended as the knockout region.The region contains 203bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Itpr2* 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 a knock-out allele are viable and fertile but show decreased sweating and disturbed calcium signaling in sweat glands. Mice homozygous for a different knock-out allele have atrial myocytes that are significantly less prone to develop proarrhythmic disturbances in calcium signaling.
- Transcript *Itpr2*-205&207&209&211 may not be affected.
- The effect on transcript *Itpr2*-204&210 is unknown.
- The *Itpr2* 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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Itpr2 inositol 1,4,5-triphosphate receptor 2 [*Mus musculus* (house mouse)]

Gene ID: 16439, updated on 11-Nov-2019

Summary

Official Symbol	Itpr2 provided by MGI
Official Full Name	inositol 1,4,5-triphosphate receptor 2 provided by MGI
Primary source	MGI:MGI:99418
See related	Ensembl:ENSMUSG00000030287
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	Ip3r2; Itpr5; insP3R2; AI649341; InsP3R-2; InsP3R-5
Expression	Broad expression in thymus adult (RPKM 9.6), kidney adult (RPKM 6.8) and 23 other tissues See more
Orthologs	human all

Genomic context

Location: 6 G3; 6 77.7 cM

See Itpr2 in Genome Data Viewer

Exon count: 59

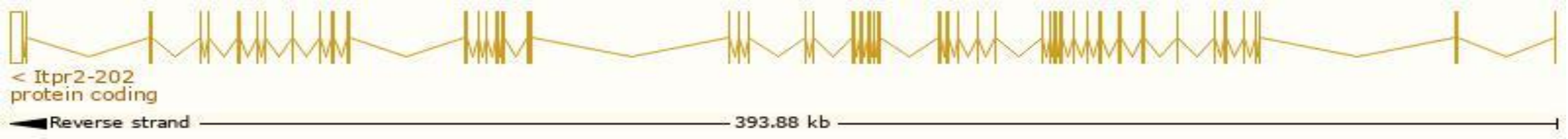
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	6	NC_000072.6 (146108299..146502258, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	6	NC_000072.5 (146060002..146450434, complement)

Transcript information（Ensembl）

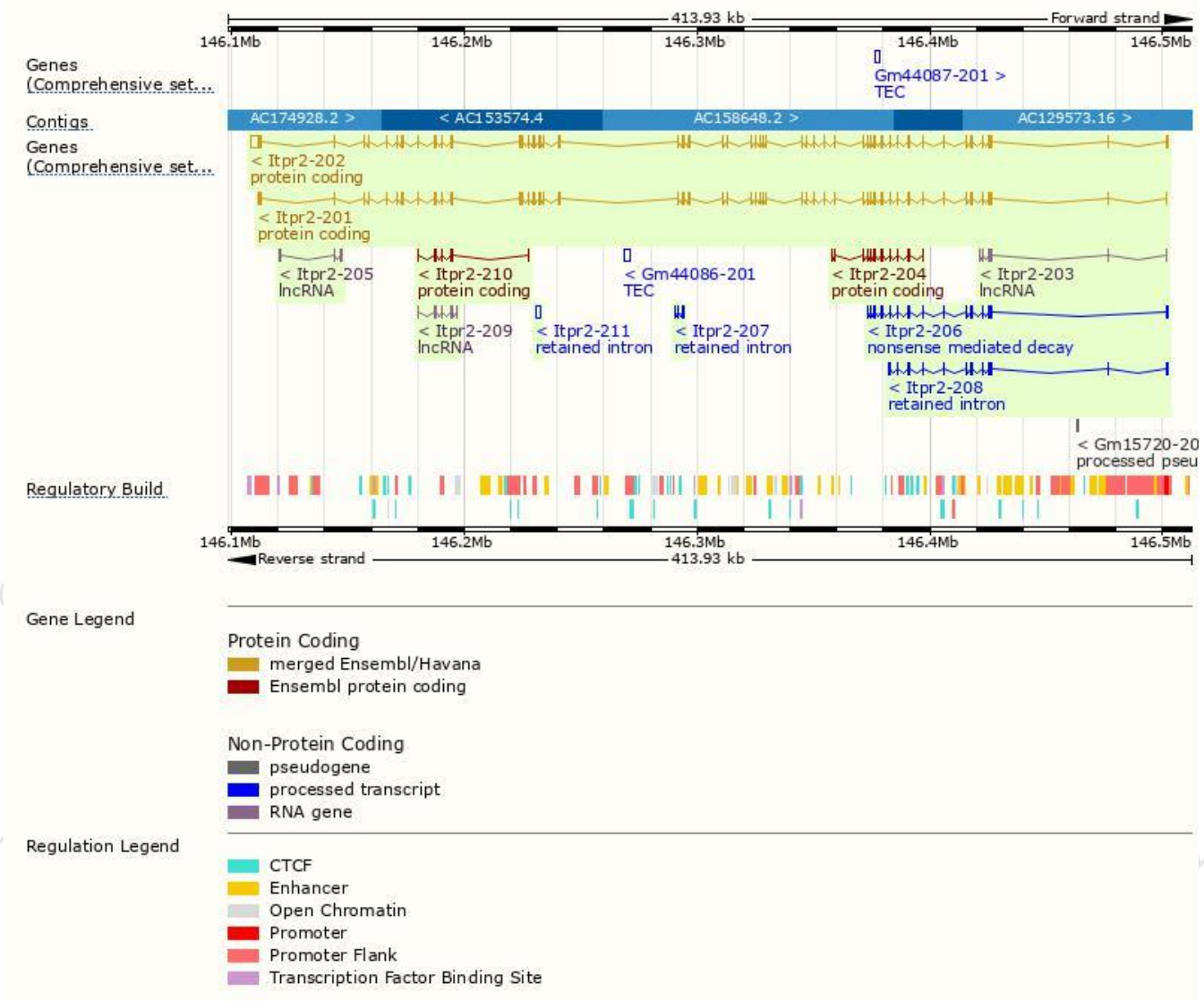
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Itpr2-202	ENSMUST00000079573.12	11679	2668aa	Protein coding	CCDS39708	Q9Z329	TSL:1 GENCODE basic
Itpr2-201	ENSMUST00000053273.14	8331	2701aa	Protein coding	CCDS39709	Q9Z329	TSL:1 GENCODE basic APPRIS P1
Itpr2-204	ENSMUST00000131890.5	1978	633aa	Protein coding	-	B2KF89	CDS 5' incomplete TSL:1
Itpr2-210	ENSMUST00000203292.1	575	192aa	Protein coding	-	A0A0N4SUL2	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Itpr2-206	ENSMUST00000139732.3	2834	79aa	Nonsense mediated decay	-	B2KF92	TSL:1
Itpr2-208	ENSMUST00000145418.7	2464	No protein	Retained intron	-	-	TSL:1
Itpr2-211	ENSMUST00000204901.1	1504	No protein	Retained intron	-	-	TSL:NA
Itpr2-207	ENSMUST00000144557.1	480	No protein	Retained intron	-	-	TSL:2
Itpr2-205	ENSMUST00000137938.1	760	No protein	lncRNA	-	-	TSL:2
Itpr2-203	ENSMUST00000111673.1	582	No protein	lncRNA	-	-	TSL:1
Itpr2-209	ENSMUST00000155339.1	556	No protein	lncRNA	-	-	TSL:5

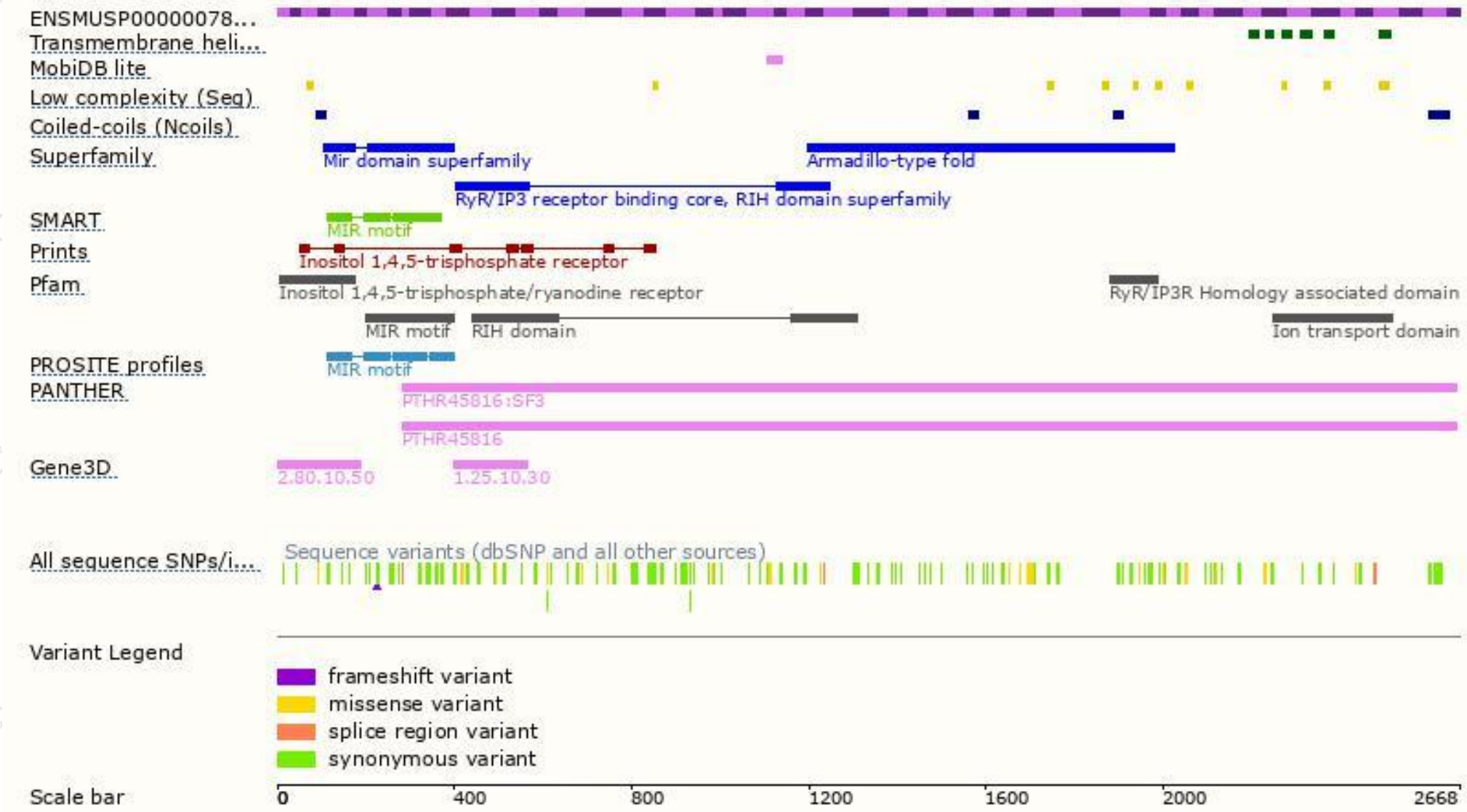
The strategy is based on the design of *Itpr2-202* transcript,The transcription is shown below



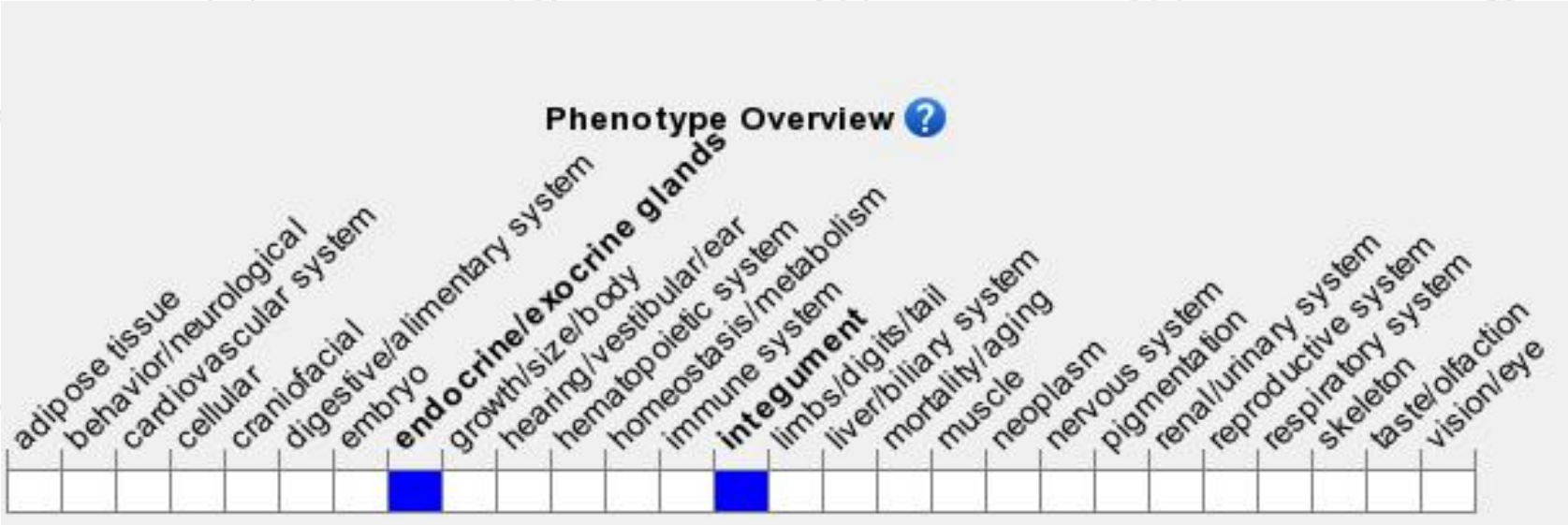
Genomic location distribution



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/).

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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