Trpv1-IRES-EGFP Mouse Model Strategy -CRISPR/Cas9 technology

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Project Overview

Project Name	***Trpv1*-IRES-EGFP**
Project type	**Cas9-KI**
Strain background	**C57BL/6JGpt**

Technical Description

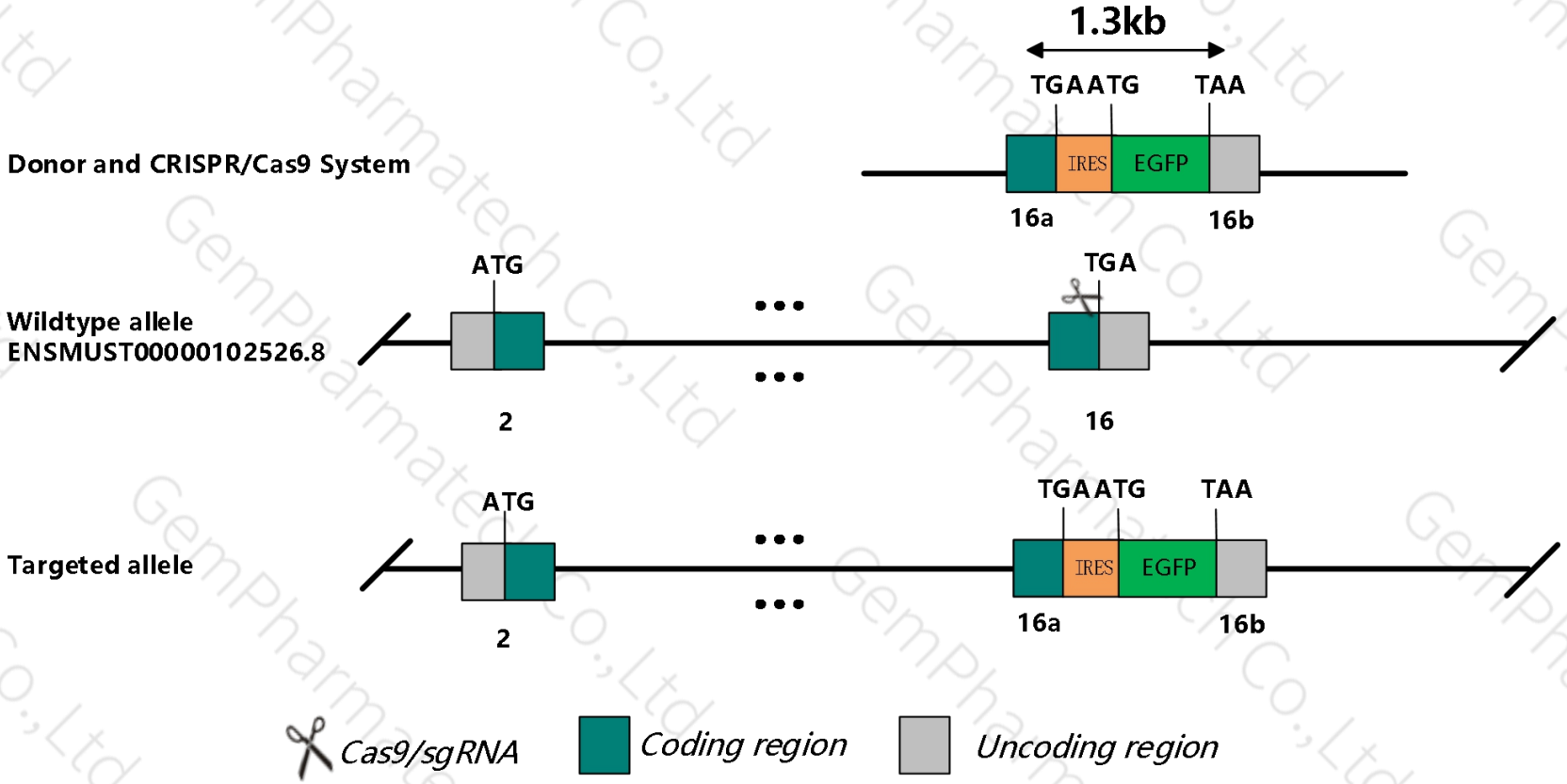
➢ The mouse *Trpv1* gene has 5 transcript.

➢ According to the structure of *Trpv1* gene, the element IRES-EGFP will be inserted at the translation stop codon of *Trpv1-202*(ENSMUST00000102526.8), the length of inserted fragment is about 1.3kb.

➢ In this project we use CRISPR/Cas9 technology to modify *Trpv1* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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.

Strategy

This model will use CRISPR/Cas9 technology to edit the *Trpv1* gene. The schematic diagram is as follows:



Notice

➢ According to the existing MGI data,homozygous mutant mice demonstrate abnormal nociception, abnormal anxiety- and conditioning-related behaviors, increased sensitivity to DOCA-salt-induced renal damage, resistance to diet-induced obesity, altered taste sensitivity, and impaired febrile response.

➢ It is necessary to introduce 1-2 synonymous mutation in exon16.

➢Transcript *Trpv1-205* may not be affected.

➢ The IERS-linked genes will be tarnscripted together and then be translated two protein separately, but the downstream protein is lower than the upstream protein.

➢ The *Trpv1* gene is located on the Chr11. 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.

➢ The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene between the 3'UTR and the gene coding region may affect the expression of endogenous and foreign genes. Due to the complexity of biological processes, it cannot be predicted completely at the present technology level.

Gene information （NCBI）

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Trpv1 transient receptor potential cation channel, subfamily V, member 1 [Mus musculus (house mouse)]

Gene ID: 193034, updated on 2-Apr-2019

Summary

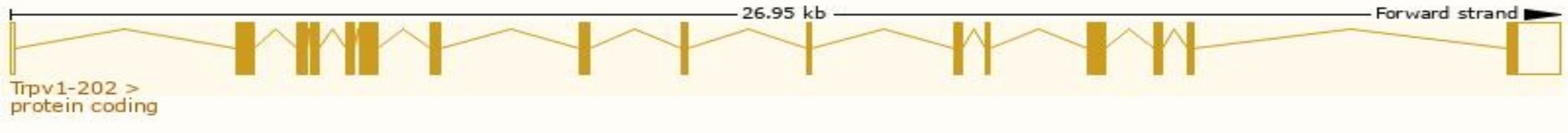
Official Symbol	Trpv1 provided by MGI
Official Full Name	transient receptor potential cation channel, subfamily V, member 1 provided by MGI
Primary source	MGI:MGI:1341787
See related	Ensembl:ENSMUSG00000005952
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	OTRPC1, TRPV1alpha, TRPV1beta, VR-1, Vr1
Expression	Biased expression in mammary gland adult (RPKM 1.7), subcutaneous fat pad adult (RPKM 1.4) and 9 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

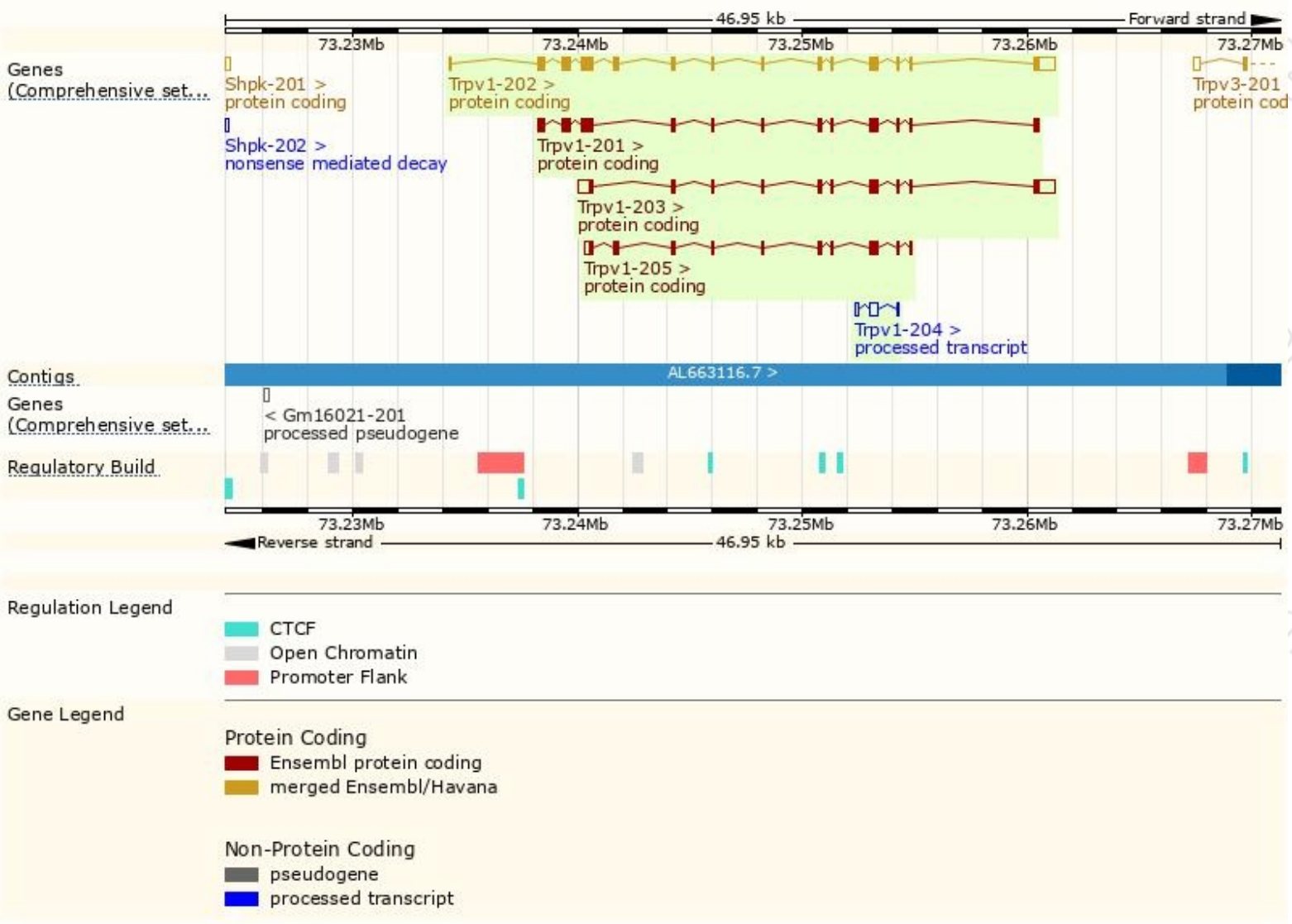
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Trpv1-202	ENSMUST00000102526.8	3361	839aa	Protein coding	CCDS25003	Q704Y3	TSL:1 GENCODE basic APPRIS P2
Trpv1-203	ENSMUST00000108470.7	2667	471aa	Protein coding	-	Z4YKR8	TSL:5 GENCODE basic
Trpv1-201	ENSMUST00000006106.13	2340	779aa	Protein coding	-	Z4YJD4	TSL:5 GENCODE basic APPRIS ALT2
Trpv1-205	ENSMUST00000138853.2	1675	474aa	Protein coding	-	Z4YLZ2	CDS 3' incomplete TSL:5
Trpv1-204	ENSMUST00000128113.1	533	No protein	Processed transcript	-	-	TSL:3

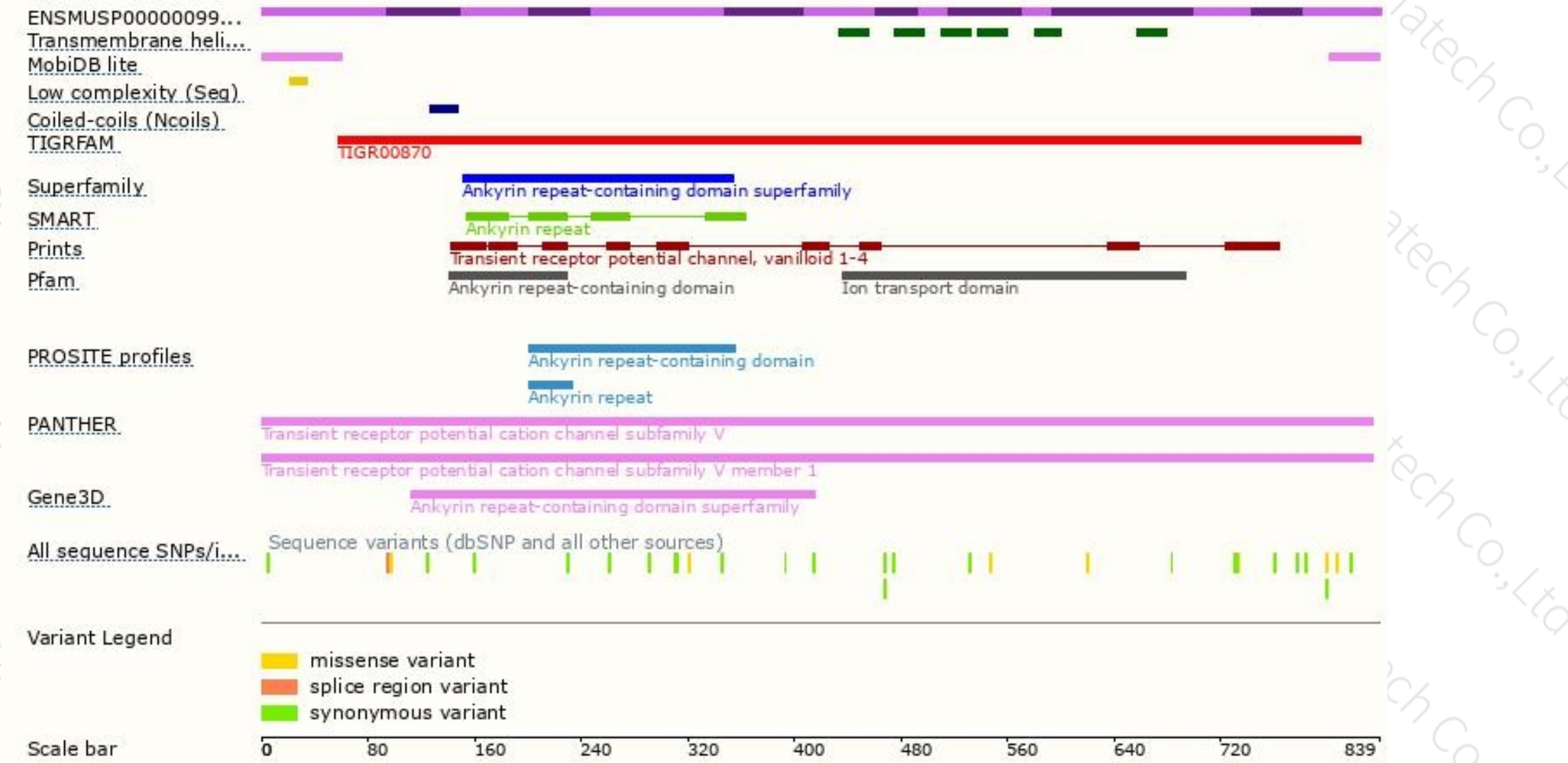
The strategy is based on the design of *Trpv1-202* transcript,the transcription is shown below:



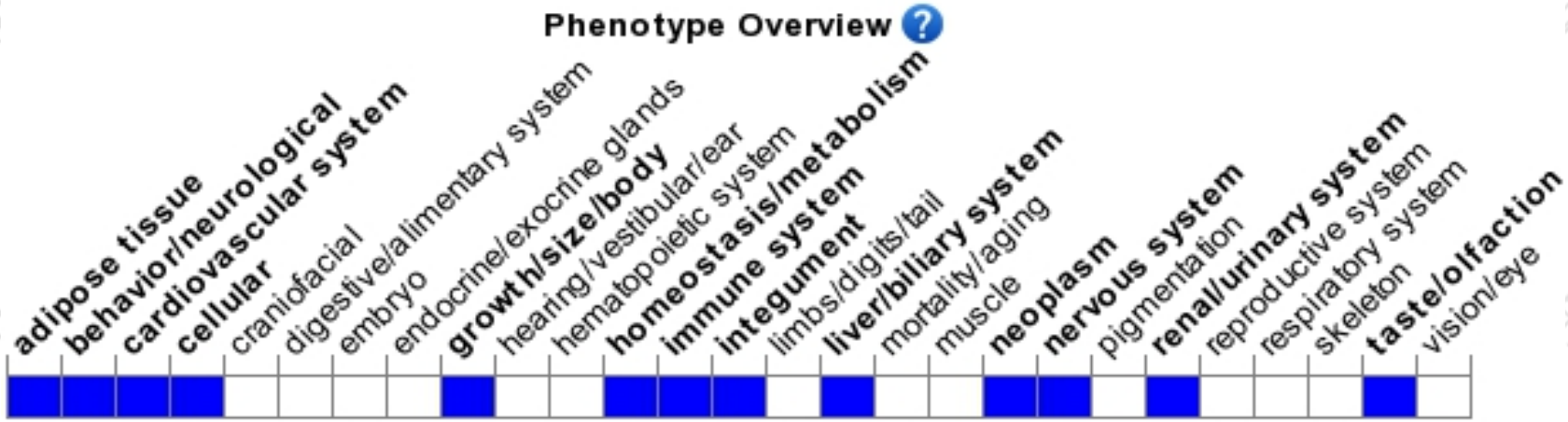
Genomic location distribution



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,homozygous mutant mice demonstrate abnormal nociception, abnormal anxiety- and conditioning-related behaviors, increased sensitivity to DOCA-salt-induced renal damage, resistance to diet-induced obesity, altered taste sensitivity, and impaired febrile response.

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

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