Trpc1 Cas9-CKO Strategy

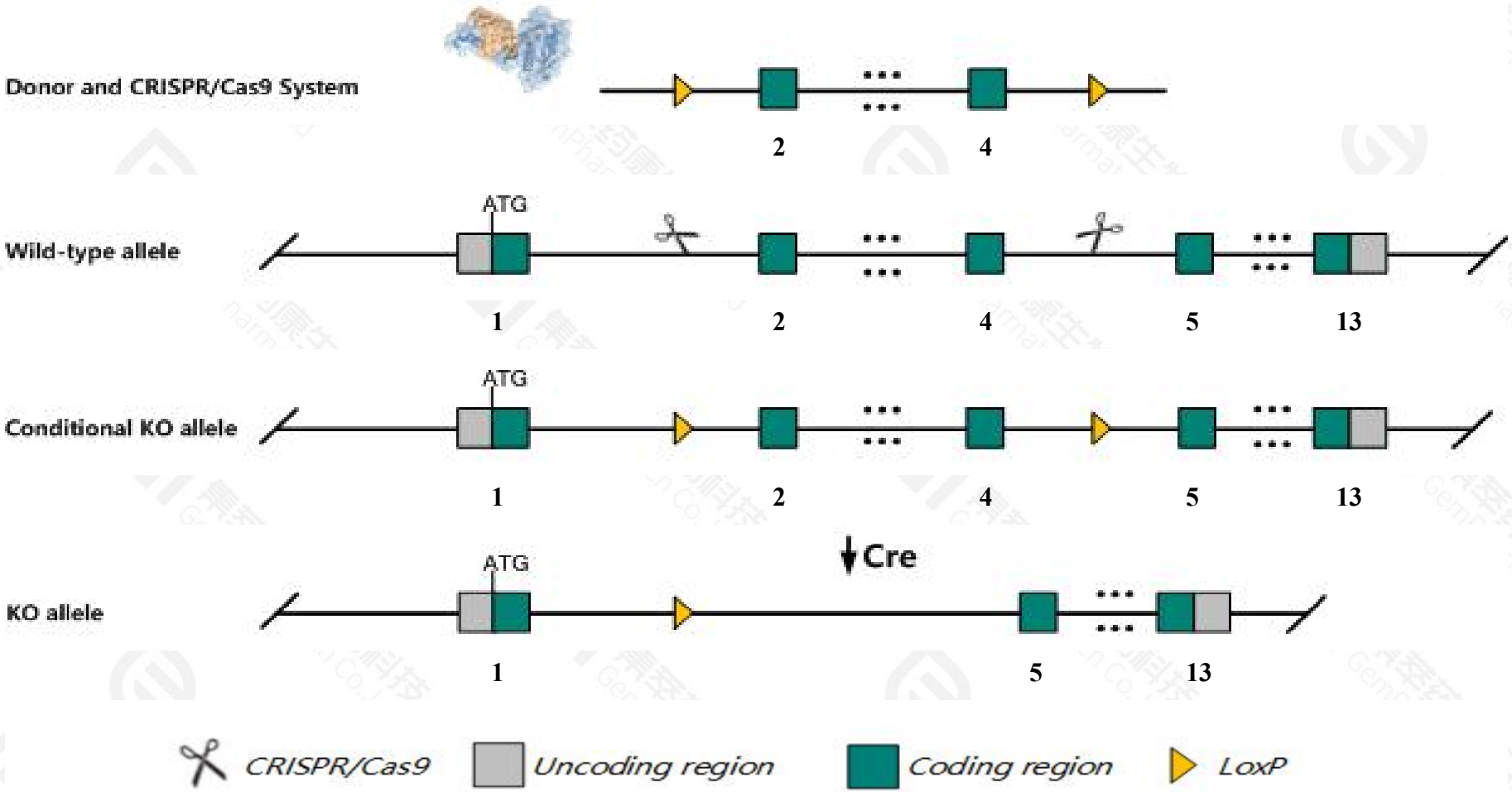
Designer: Daohua Xu

Project Overview

Project Name	***Trpc1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Trpc1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Trpc1* gene has 7 transcripts. According to the structure of *Trpc1* gene, exon2-exon4 of *Trpc1-204*(ENSMUST00000189137.7) transcript is recommended as the knockout region.The region contains 460bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Trpc1* 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 knock-out allele exhibit increased body weight and a severe loss of salivary gland fluid secretion due to attenuation of store-operated Ca2+ currents. Surprisingly, no abnormalities are seen in store-operated or mechanosensitive cation channels in vascular smooth muscle cells.

➢ The *Trpc1* gene is located on the Chr9. 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）

集萃药康 GemPharmatech

Trpc1 transient receptor potential cation channel, subfamily C, member 1 [Mus musculus (house mouse)]

Gene ID: 22063, updated on 13-Mar-2020

Summary

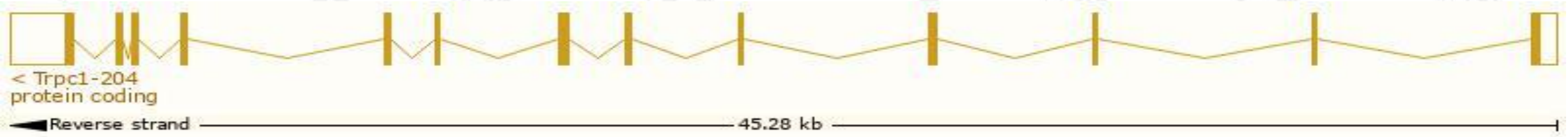
Official Symbol	Trpc1 provided by MGI
Official Full Name	transient receptor potential cation channel, subfamily C, member 1 provided byMGI
Primary source	MGI:MGI:109528
See related	Ensembl:ENSMUSG00000032839
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Mtrp1, Trp1, Trrp1
Expression	Broad expression in cortex adult (RPKM 5.3), frontal lobe adult (RPKM 4.6) and 25 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

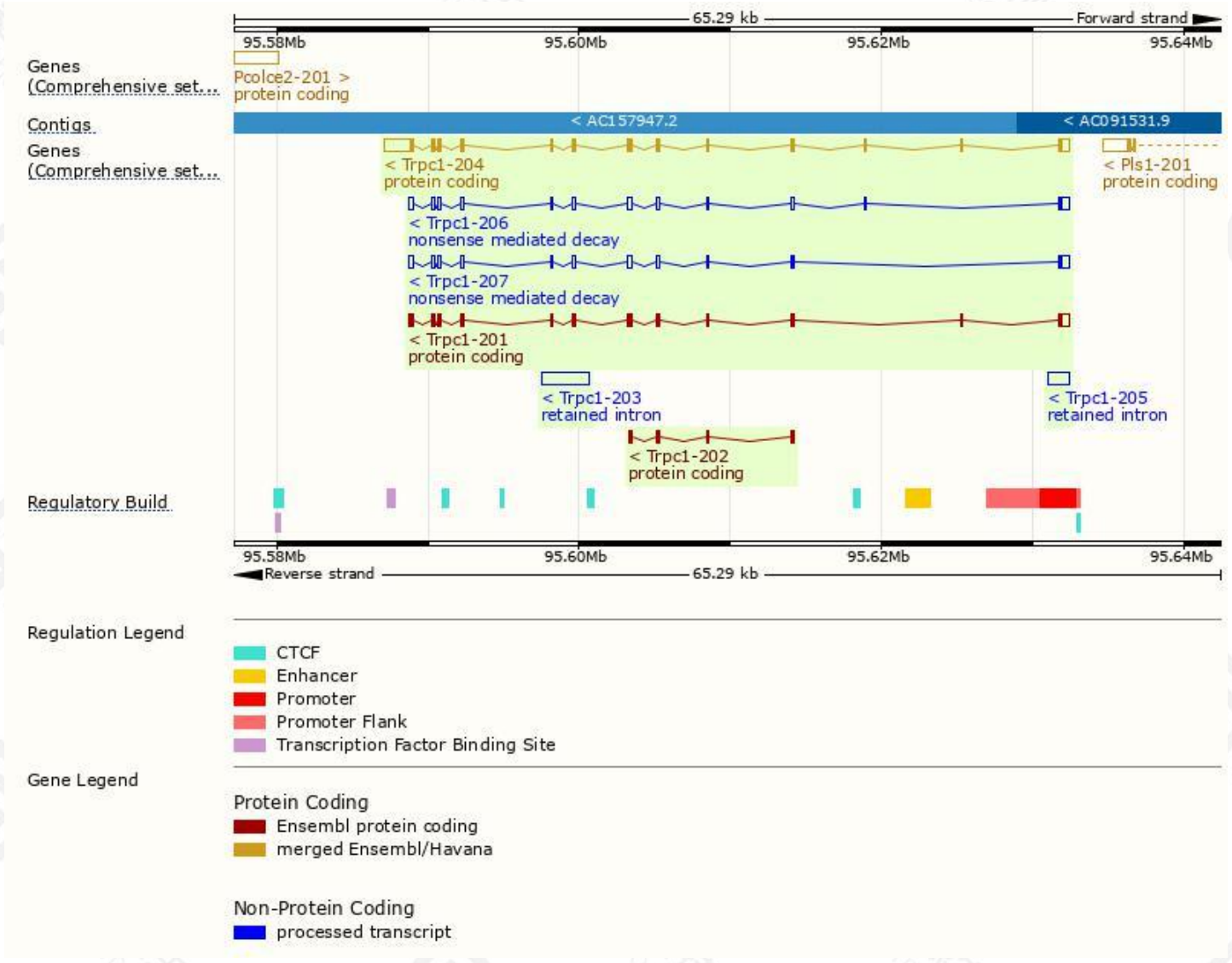
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Trpc1-204	ENSMUST00000189137.6	4559	809aa	Protein coding	CCDS23411	B2RPS7	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Trpc1-201	ENSMUST00000053785.9	2912	775aa	Protein coding	CCDS81052	B7ZMP6	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Trpc1-202	ENSMUST00000186235.1	765	255aa	Protein coding	-	A0A087WSD0	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
Trpc1-206	ENSMUST00000190497.1	2859	84aa	Nonsense mediated decay	-	A0A087WRB2	TSL:1
Trpc1-207	ENSMUST00000190604.6	2757	99aa	Nonsense mediated decay	-	A0A087WP07	TSL:1
Trpc1-203	ENSMUST00000188141.1	3108	No protein	Retained intron	-	-	TSL:NA
Trpc1-205	ENSMUST00000190205.1	1338	No protein	Retained intron	-	-	TSL:NA

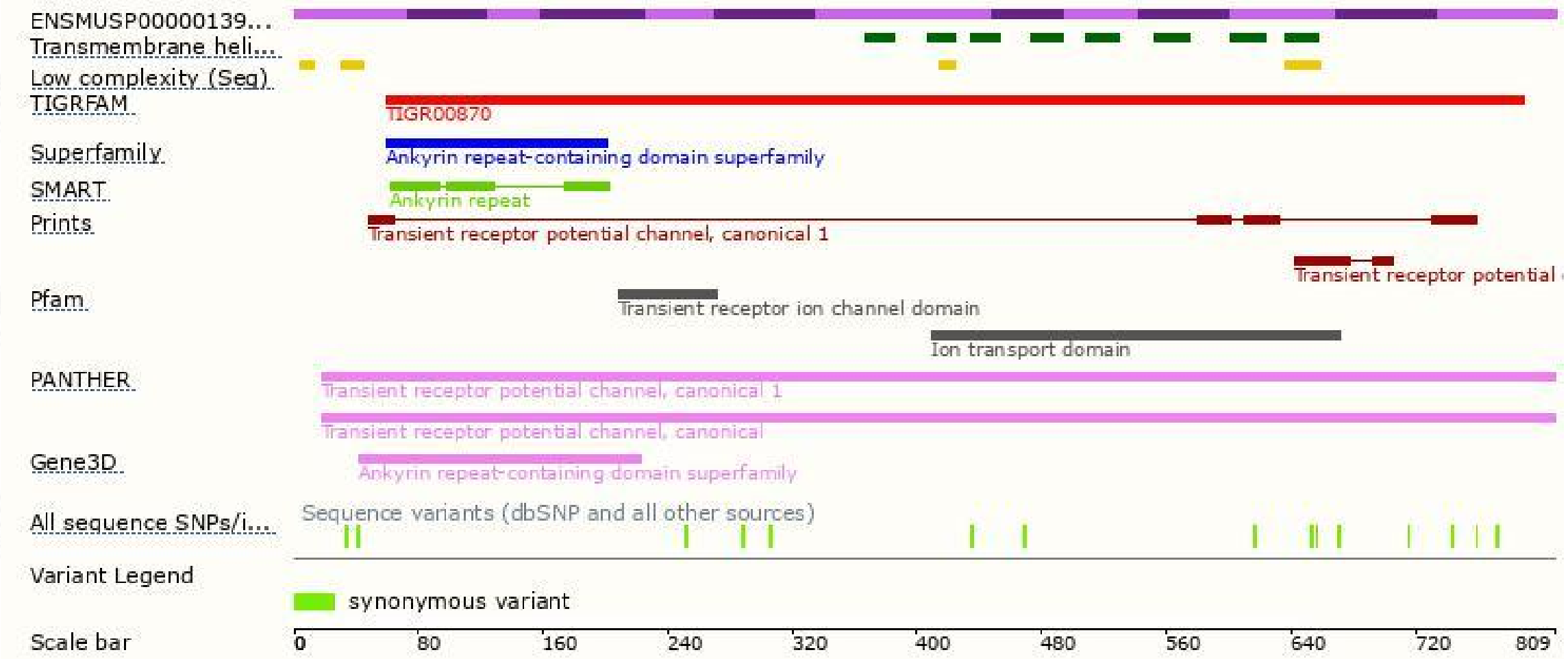
The strategy is based on the design of *Trpc1-204* transcript,the transcription is shown below:



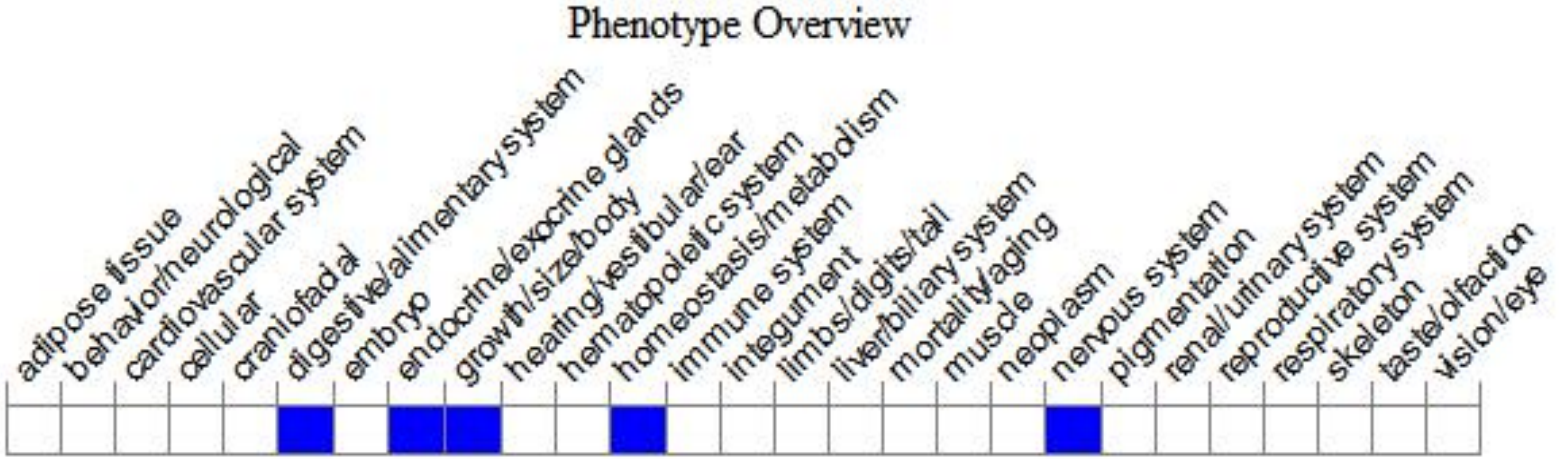
Genomic location distribution



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 knock-out allele exhibit increased body weight and a severe loss of salivary gland fluid secretion due to attenuation of store-operated Ca2+ currents. Surprisingly, no abnormalities are seen in store-operated or mechanosensitive cation channels in vascular smooth muscle cells.

集萃药康
GemPharmatech

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

集萃药康生物科技
GemPharmatech Co.,Ltd