Msh5 Cas9-CKO Strategy

Designer: Xiaojing Li

Reviewer: JiaYu

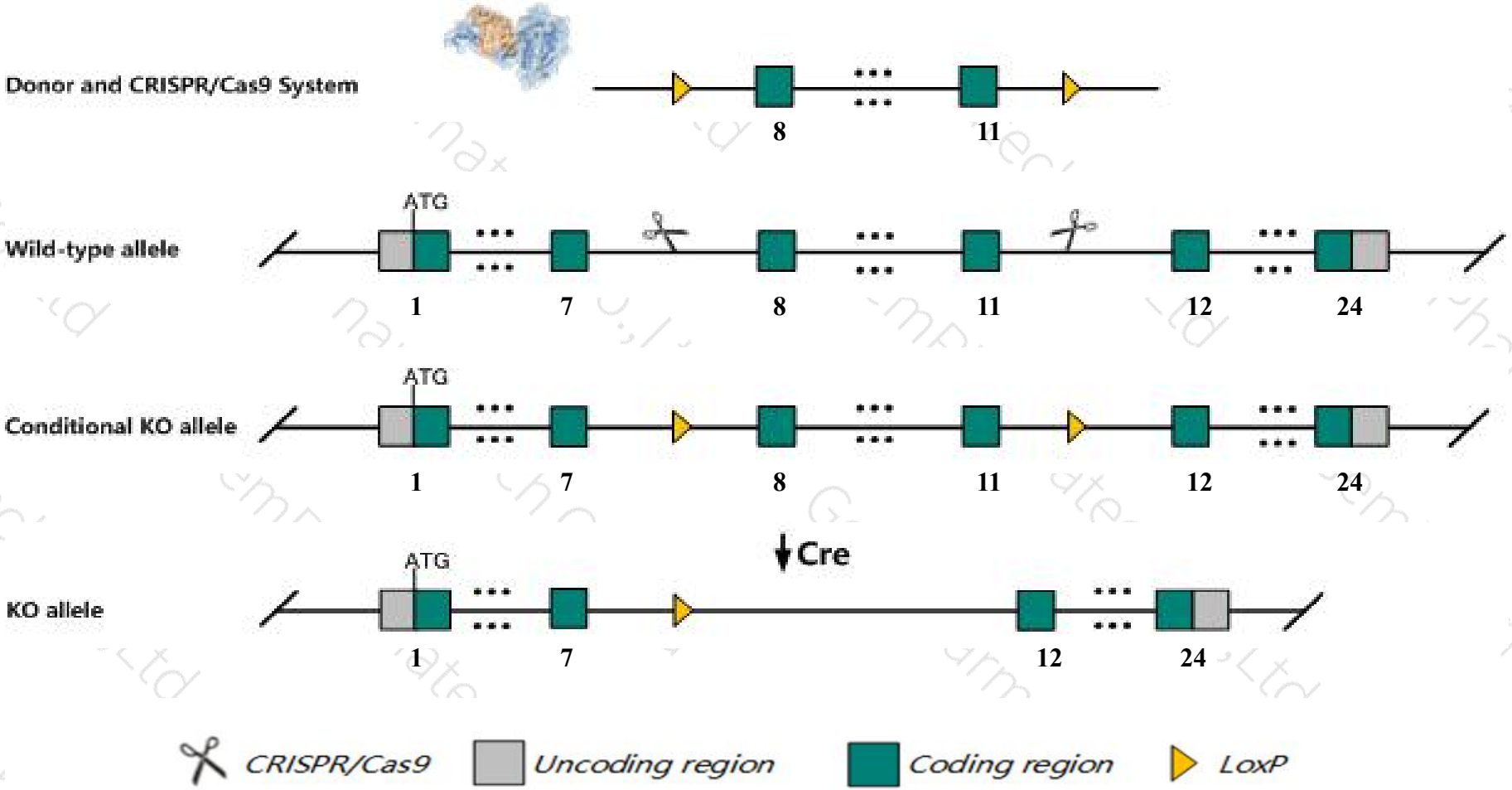
Design Date: 2020-4-20

Project Overview

Project Name	***Msh5***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Msh5* gene. The schematic diagram is as follows:



Technical routes

- The *Msh5* gene has 12 transcripts. According to the structure of *Msh5* gene, exon8-exon11 of *Msh5-201* (ENSMUST00000007250.13) transcript is recommended as the knockout region.The region contains 331bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Msh5* 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 disrupted chromosome pairing in meiosis i resulting in cell death and sterility. in males, testes size is reduced, and in females, there is a total loss of ovarian structure.
- The *Msh5* gene is located on the Chr17. 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

Msh5 mutS homolog 5 [Mus musculus (house mouse)]

Gene ID: 17687, updated on 13-Mar-2020

Summary

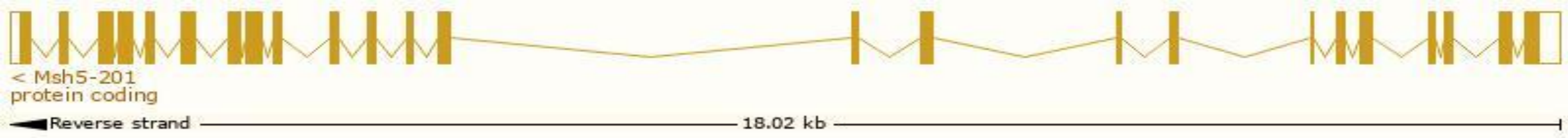
Official Symbol	Msh5 provided by MGI
Official Full Name	mutS homolog 5 provided by MGI
Primary source	MGI:MGI:1329021
See related	Ensembl:ENSMUSG00000007035
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Mut5
Summary	This gene encodes a member of the MutS family of proteins that play critical roles in DNA mismatch repair and meiotic homologous recombination processes. Mice lacking the encoded protein are viable but sterile, with severe defects in spermatogenesis in males and complete loss of ovarian structures in females. Mutations in a similar gene in humans have been shown to cause common variable immune deficiency (CVID) and immunoglobulin A deficiency. Alternative splicing of this gene results in multiple transcript variants. [provided by RefSeq, Jan 2015]
Expression	Ubiquitous expression in testis adult (RPKM 3.5), liver E18 (RPKM 1.6) and 26 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

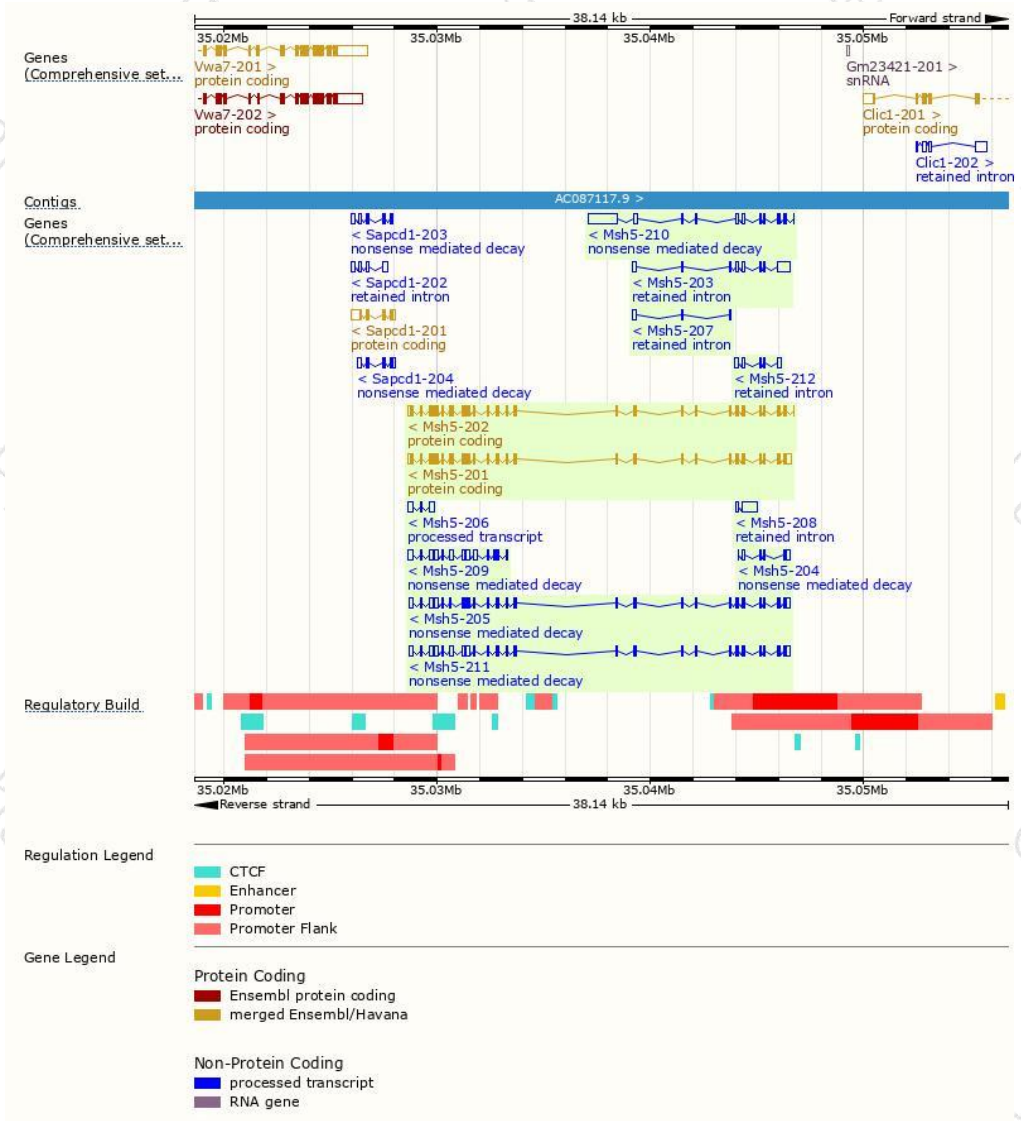
The gene has 12 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Msh5-201	ENSMUST00000007250.13	2886	833aa	Protein coding	CCDS28674	A0A0R4IZY8	TSL:1 GENCODE basic APPRIS P1
Msh5-202	ENSMUST00000097338.10	2678	833aa	Protein coding	CCDS28674	A0A0R4IZY8	TSL:1 GENCODE basic APPRIS P1
Msh5-203	ENSMUST00000165329.8	1248	No protein	Retained intron	-	-	TSL:2
Msh5-204	ENSMUST00000172491.1	591	67aa	Nonsense mediated decay	-	G3UWT5	TSL:3
Msh5-205	ENSMUST00000172536.1	2630	616aa	Nonsense mediated decay	-	G3UZB5	TSL:1
Msh5-206	ENSMUST00000173124.7	538	No protein	Processed transcript	-	-	TSL:2
Msh5-207	ENSMUST00000173685.7	279	No protein	Retained intron	-	-	TSL:3
Msh5-208	ENSMUST00000173928.1	855	No protein	Retained intron	-	-	TSL:2
Msh5-209	ENSMUST00000174026.7	1743	75aa	Nonsense mediated decay	-	G3UZ06	CDS 5' incomplete TSL:1
Msh5-210	ENSMUST00000174556.7	2391	53aa	Nonsense mediated decay	-	G3UYF3	TSL:2
Msh5-211	ENSMUST00000174603.7	2718	499aa	Nonsense mediated decay	-	G3UYF6	TSL:1
Msh5-212	ENSMUST00000174741.7	622	No protein	Retained intron	-	-	TSL:3

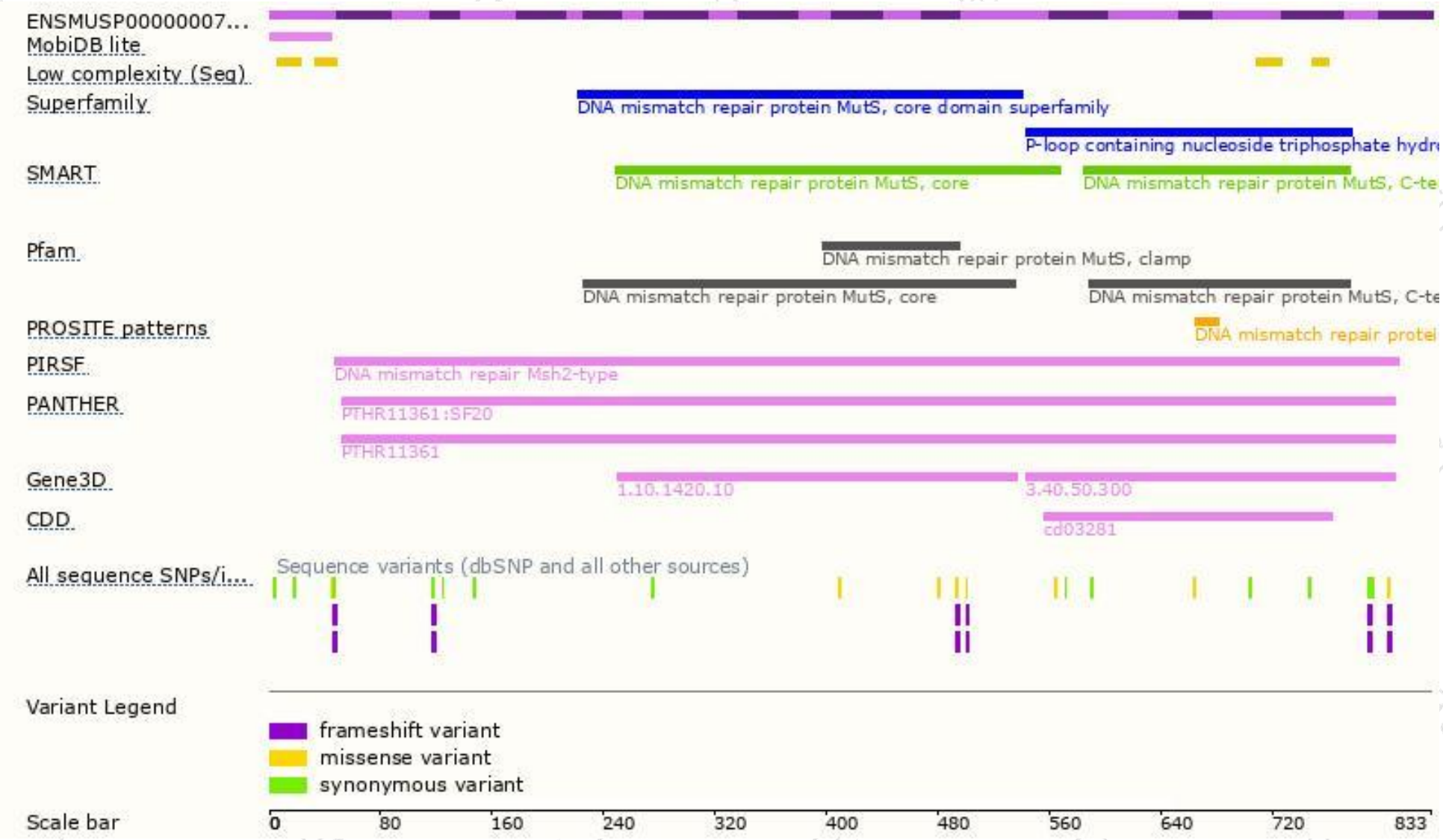
The strategy is based on the design of *Msh5-201* transcript,The transcription is shown below



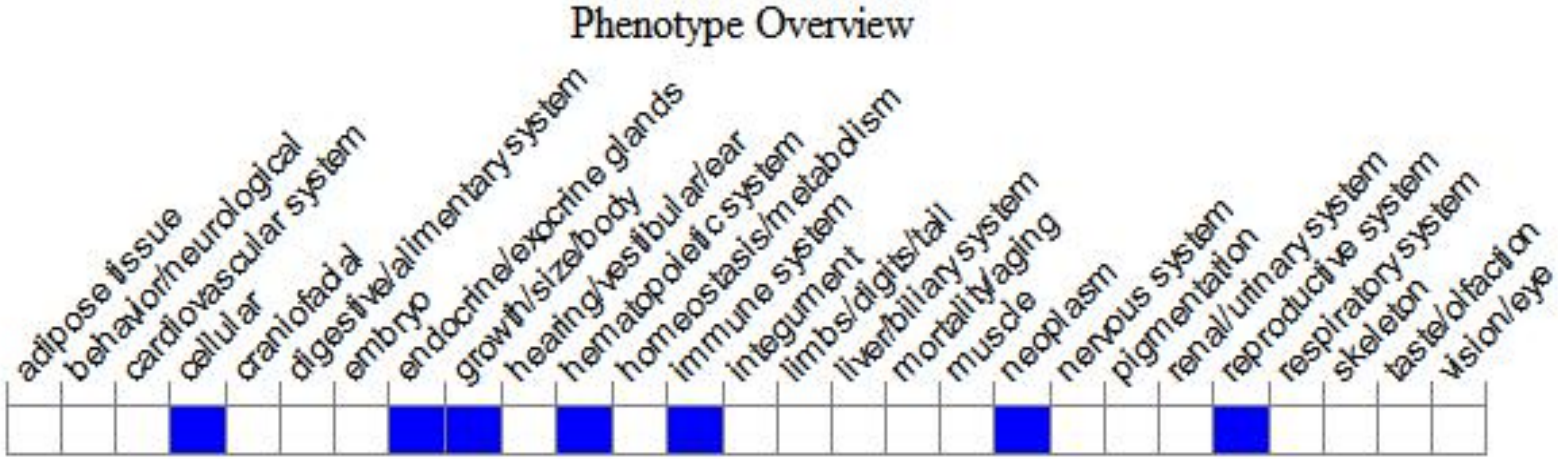
Genomic location distribution



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,Homozygotes for targeted null mutations exhibit disrupted chromosome pairing in meiosis I resulting in cell death and sterility. In males, testes size is reduced, and in females, there is a total loss of ovarian structure.

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