Dtna Cas9-CKO Strategy

Designer: Yang Zeng
Reviewer: Ruirui Zhang

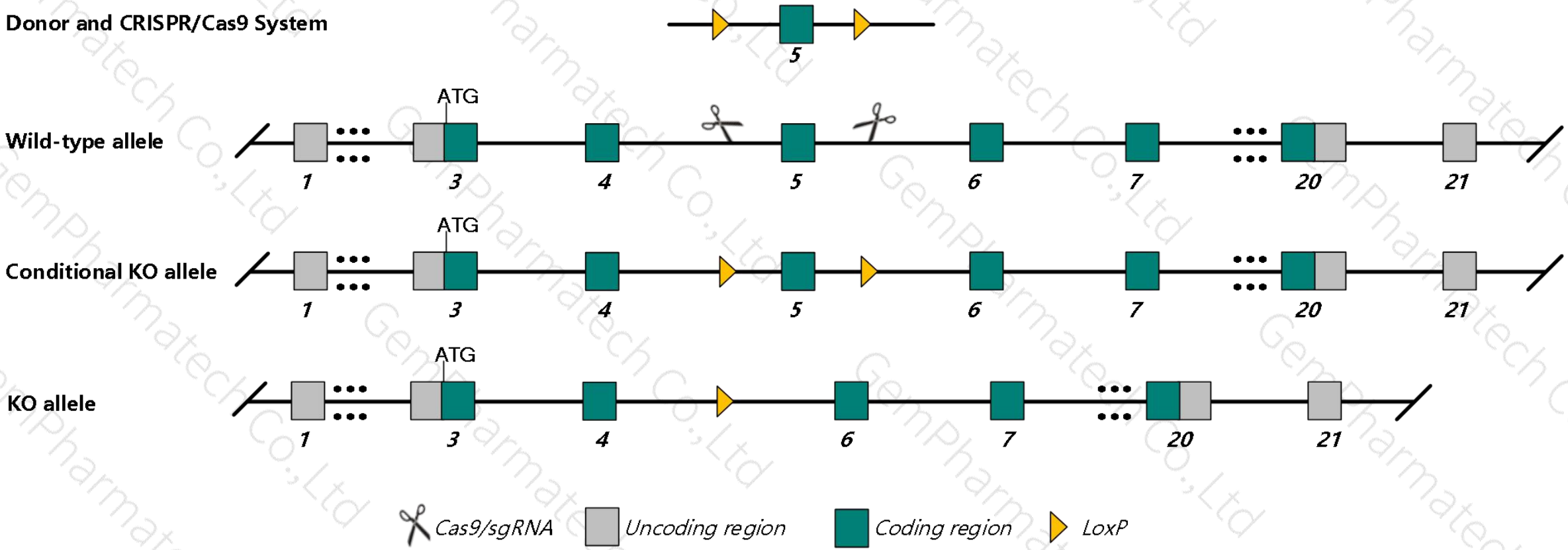
Design Date: 2019-11-25

Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Dtna* gene has 10 transcripts. According to the structure of *Dtna* gene, exon5 of *Dtna-202* (ENSMUST00000115832.3) transcript is recommended as the knockout region.The region contains 214bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Dtna* 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,Homozygous targeted mutants exhibit skeletal and cardiac myopathies. Neuromuscular junctions appear to form normally, but their postnatal maturation is compromised. Dtna mutations do not increase the severity of Dmd or Utrn mutants whose products are also part of the dystrophin-glycoprotein complex.

➢ The CDS of transcript *Dtna*-208,209 are incomplete, whether they will be affected is unknown.

➢ The *Dtna* gene is located on the Chr18. 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

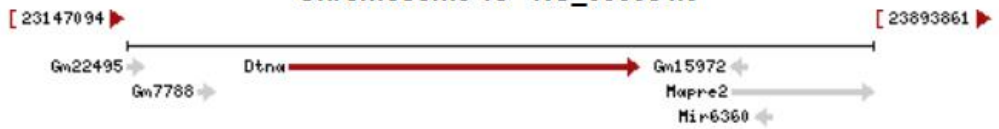
Dtna dystrobrevin alpha [*Mus musculus* (house mouse)]

Gene ID: 13527, updated on 24-Oct-2019

Summary

Official Symbol	Dtna provided by MGI
Official Full Name	dystrobrevin alpha provided by MGI
Primary source	MGI:MGI:106039
See related	Ensembl:ENSMUSG00000024302
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	Dtn; adbn; DTN-A; a-DB-1; Gm19389; 2210407P21Rik
Expression	Broad expression in cerebellum adult (RPKM 9.3), cortex adult (RPKM 8.0) and 16 other tissues See more
Orthologs	human all

Chromosome 18 - NC_000084.6

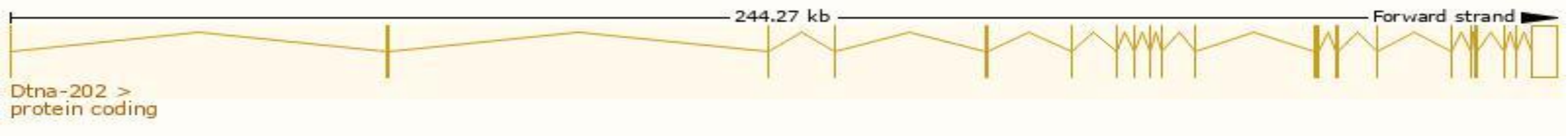


Transcript information（Ensembl）

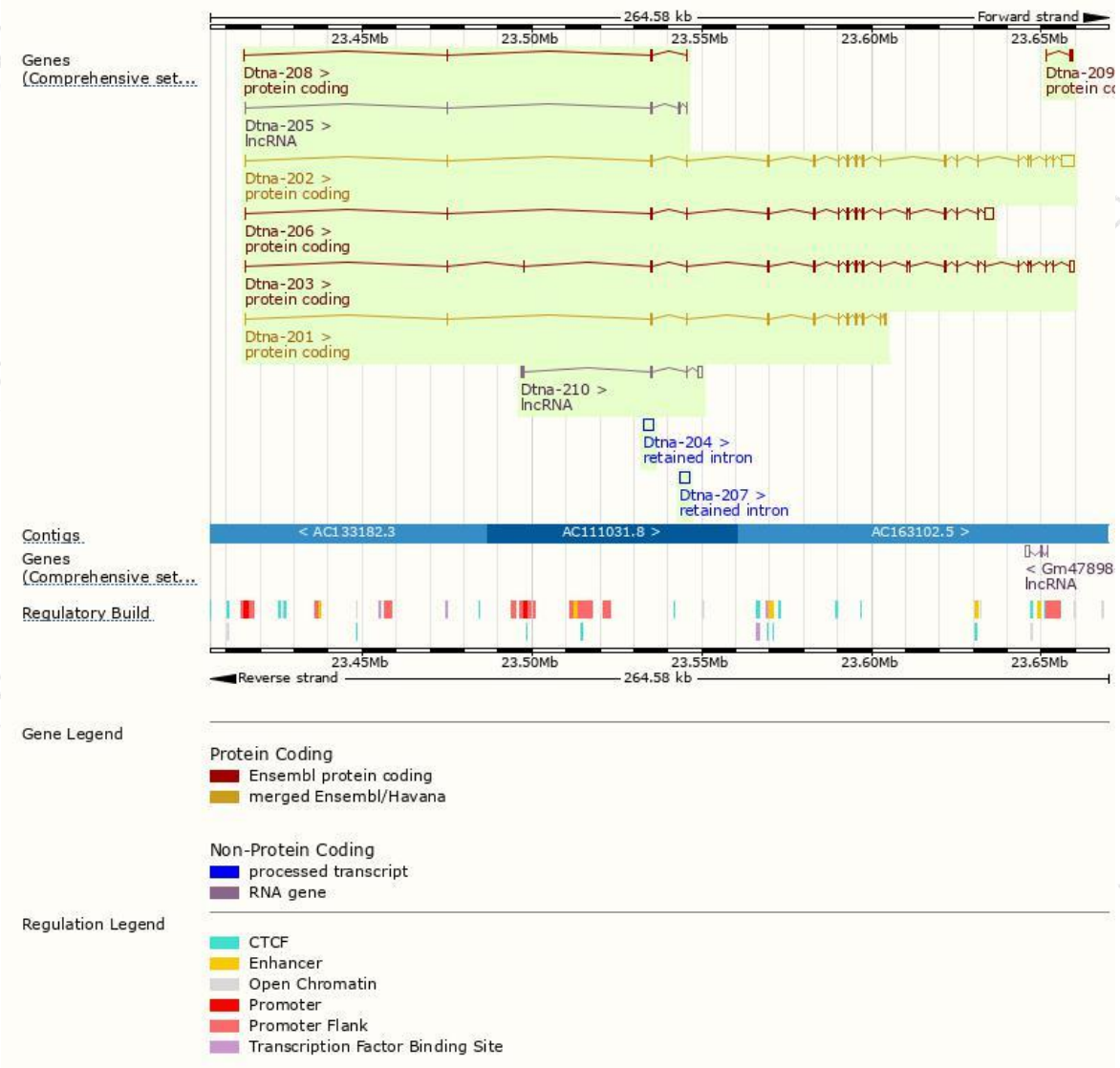
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dtna-202	ENSMUST00000115832.3	6295	682aa	Protein coding	CCDS50236	Q8CFR5	TSL:1 GENCODE basic APPRIS ALT1
Dtna-201	ENSMUST00000047954.14	1596	371aa	Protein coding	CCDS29094	Q8BTD9 Q9D2N4	TSL:1 GENCODE basic APPRIS P3
Dtna-206	ENSMUST00000221880.1	4490	567aa	Protein coding	-	A0A1Y7VJN9	TSL:2 GENCODE basic APPRIS ALT1
Dtna-203	ENSMUST00000220904.1	3860	746aa	Protein coding	-	A0A1Y7VL34	TSL:1 GENCODE basic
Dtna-209	ENSMUST00000222726.1	863	73aa	Protein coding	-	A0A1Y7VLZ6	CDS 5' incomplete TSL:5
Dtna-208	ENSMUST00000222515.1	359	35aa	Protein coding	-	A0A1Y7VK79	CDS 3' incomplete TSL:2
Dtna-207	ENSMUST00000222351.1	3086	No protein	Retained intron	-	-	TSL:NA
Dtna-204	ENSMUST00000220967.1	2941	No protein	Retained intron	-	-	TSL:NA
Dtna-210	ENSMUST00000223441.1	1489	No protein	lncRNA	-	-	TSL:1
Dtna-205	ENSMUST00000221471.1	723	No protein	lncRNA	-	-	TSL:3

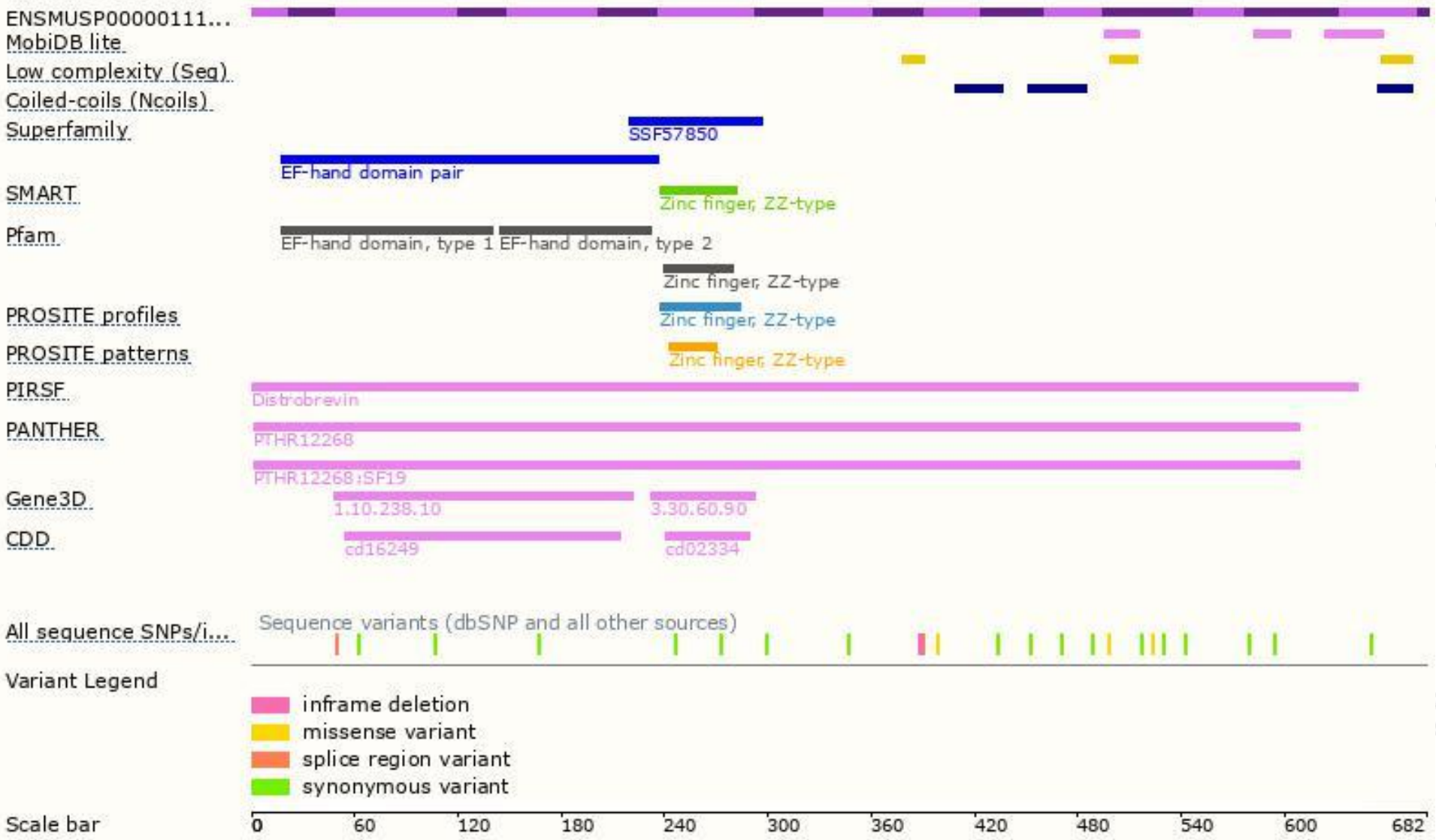
The strategy is based on the design of *Dtna-202* transcript,The transcription is shown below



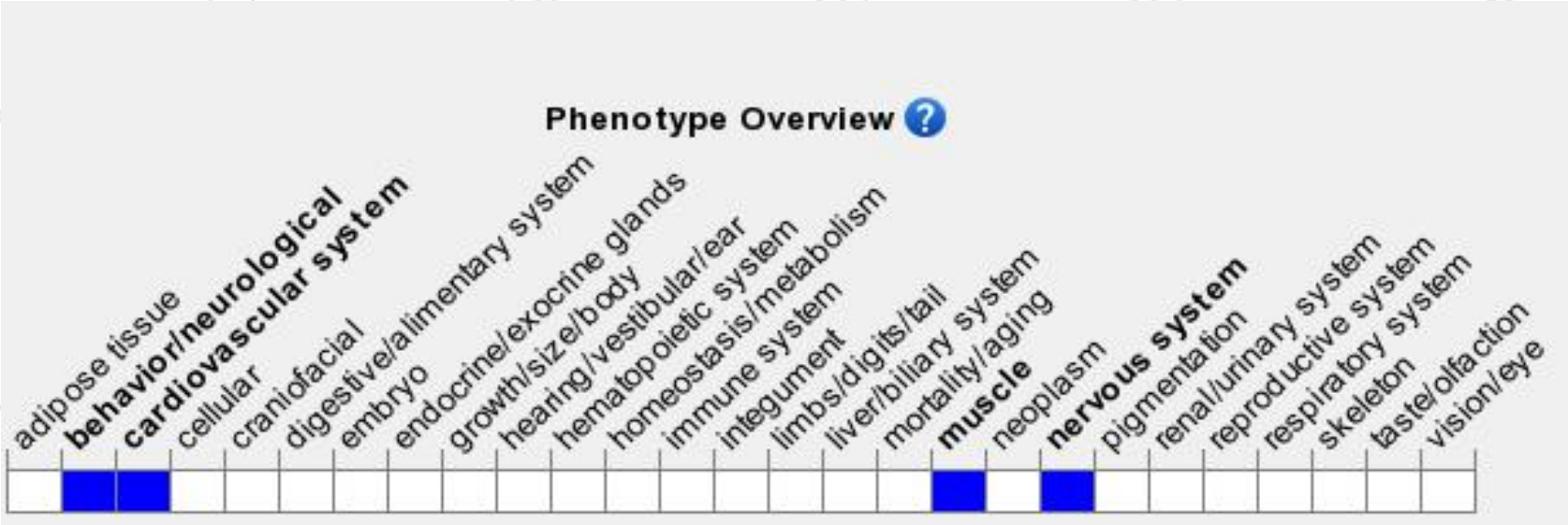
Genomic location distribution



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 targeted mutants exhibit skeletal and cardiac myopathies. Neuromuscular junctions appear to form normally, but their postnatal maturation is compromised. Dtna mutations do not increase the severity of Dmd or Utrn mutants whose products are also part of the dystrophin-glycoprotein complex.

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