Usp14 Cas9-CKO Strategy

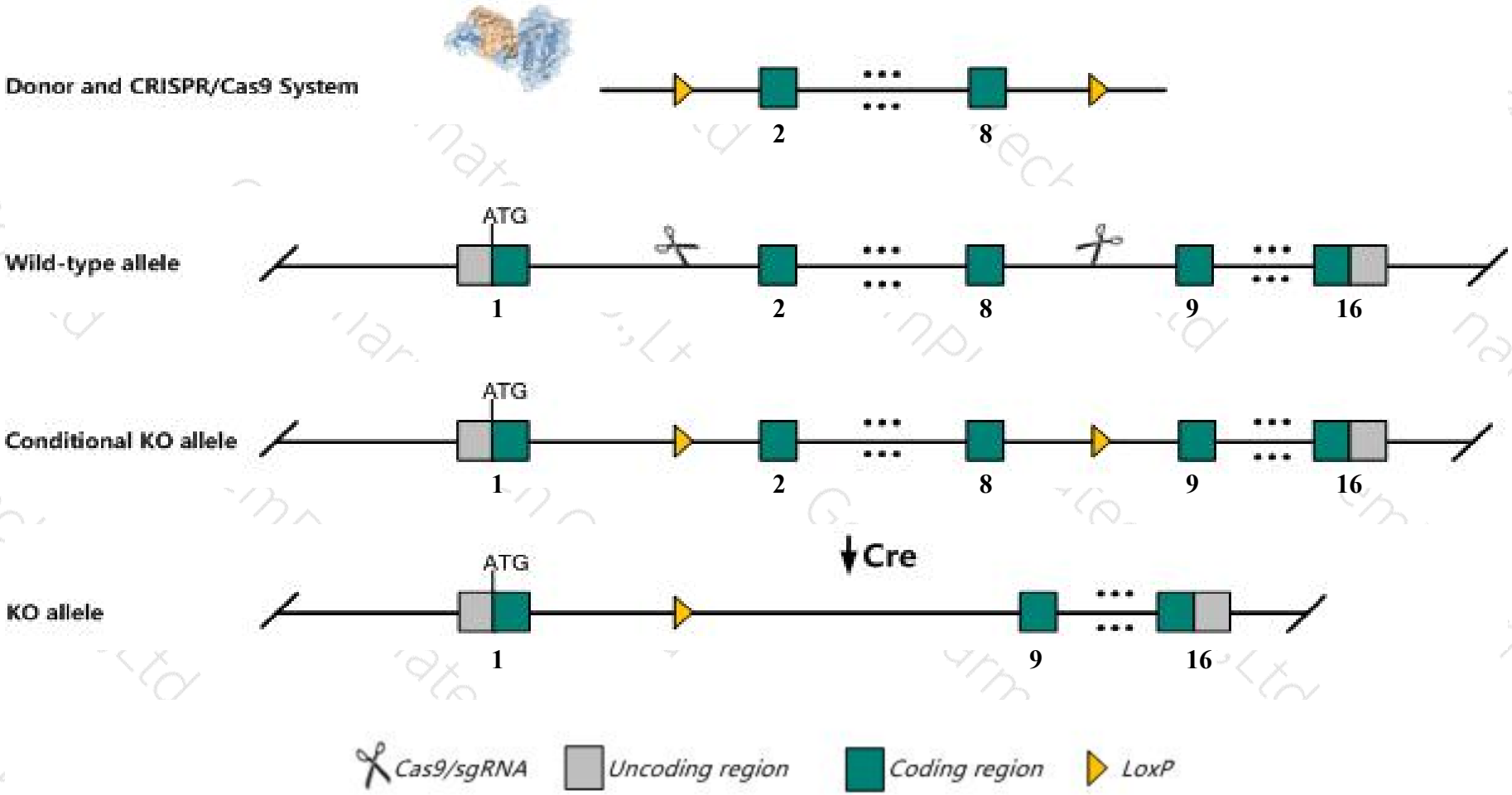
Designer	**Jing Jin**
Reviewer	**Yang Zeng**
Design Date	**2018-6-8**

Project Overview

Project Name	***Usp14***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Usp14* gene. The schematic diagram is as follows:



Technical routes

➢ The *Usp14* gene has 10 transcripts. According to the structure of *Usp14* gene, exon2-exon8 of *Usp14-201* (ENSMUST00000092096.13) transcript is recommended as the knockout region.The region contains 659bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Usp14* 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.

➢ The flox mice was 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 hypomorphic mutation develop severe tremors by 3 weeks of age, followed by hindlimb paralysis and premature death. An underdeveloped corpus callosum, hippocampus, dentate gyrus and forebrain structures, and notable defects in synaptic transmission in both the CNS and PNS are seen.

➢ Transcript *Usp14-204* may not be affected.

➢ The *Usp14* 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）

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Usp14 ubiquitin specific peptidase 14 [*Mus musculus* (house mouse)]

Gene ID: 59025, updated on 5-Mar-2019

Summary

Official Symbol	Usp14 provided by MGI
Official Full Name	ubiquitin specific peptidase 14 provided by MGI
Primary source	MGI:MGI:1928898
See related	Ensembl:ENSMUSG00000047879
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	ax; C78769; nmf375; AW107924; 2610005K12Rik; 2610037B11Rik
Expression	Broad expression in CNS E18 (RPKM 14.6), cortex adult (RPKM 14.0) and 27 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

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

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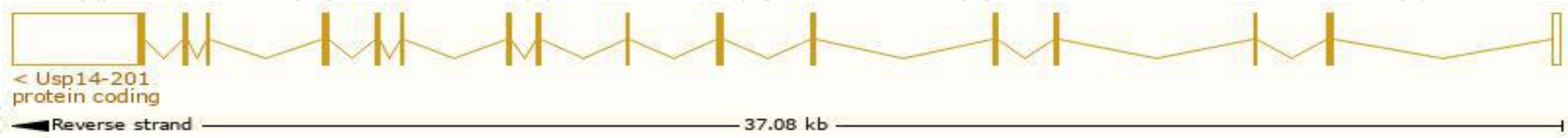
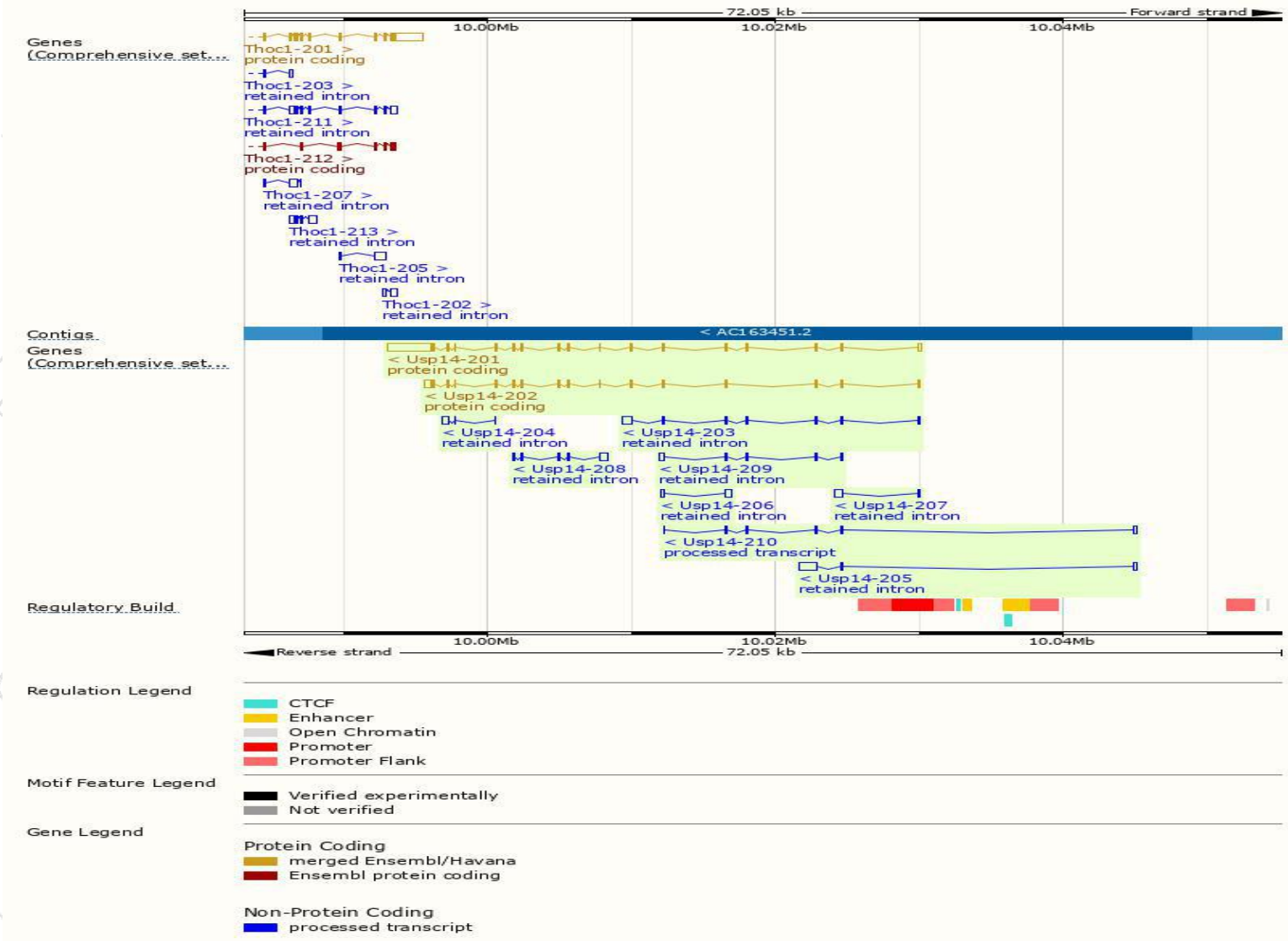
Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Usp14-201	ENSMUST00000092096.13	4726	493aa	ENSMUSP00000089728.6	Protein coding	CCDS37735	Q9JMA1	TSL:1 GENCODE basic APPRIS P1
Usp14-202	ENSMUST00000116669.1	2019	458aa	ENSMUSP00000112368.1	Protein coding	CCDS37734	E9PYI8	TSL:1 GENCODE basic
Usp14-203	ENSMUST00000128334.7	1346	No protein	-	Retained intron	-	-	TSL:1
Usp14-204	ENSMUST00000133594.1	477	No protein	-	Retained intron	-	-	TSL:5
Usp14-205	ENSMUST00000142013.1	1484	No protein	-	Retained intron	-	-	TSL:1
Usp14-206	ENSMUST00000145929.1	656	No protein	-	Retained intron	-	-	TSL:1
Usp14-207	ENSMUST00000150321.1	639	No protein	-	Retained intron	-	-	TSL:2
Usp14-208	ENSMUST00000154088.1	989	No protein	-	Retained intron	-	-	TSL:3
Usp14-209	ENSMUST00000234165.1	664	No protein	-	Retained intron	-	-	-
Usp14-210	ENSMUST00000234243.1	612	No protein	-	lncRNA	-	-	-

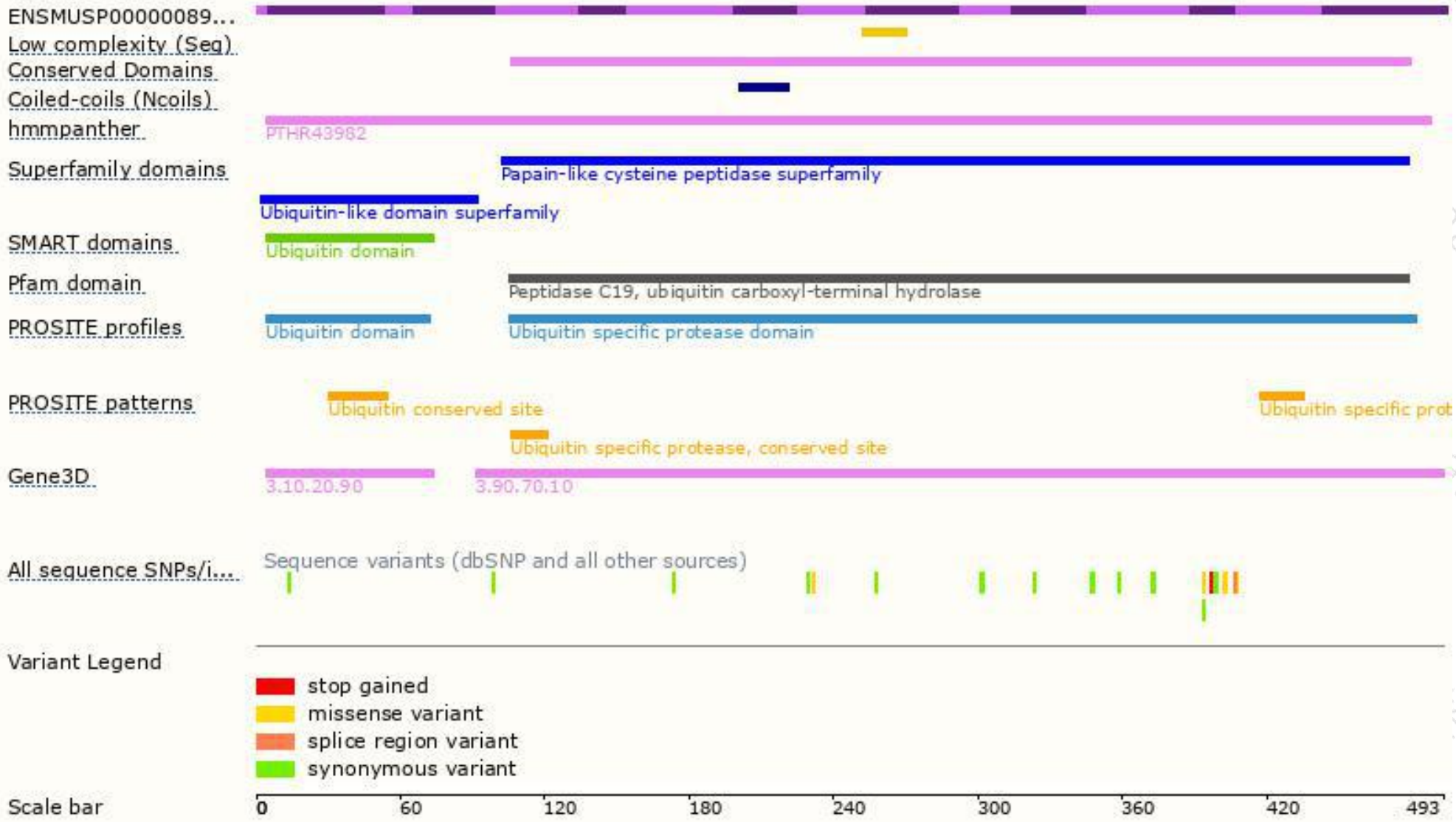
The strategy is based on the design of *Usp14-201* transcript,The transcription is shown below



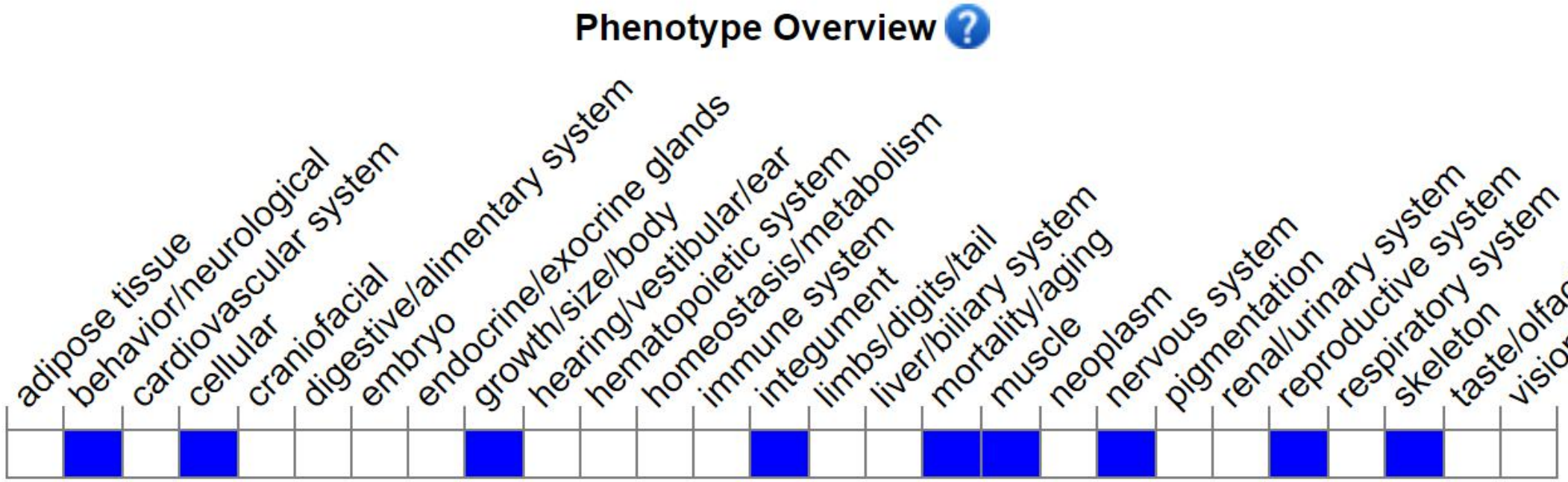
Genomic location distribution



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 a hypomorphic mutation develop severe tremors by 3 weeks of age, followed by hindlimb paralysis and premature death. An underdeveloped corpus callosum, hippocampus, dentate gyrus and forebrain structures, and notable defects in synaptic transmission in both the CNS and PNS are seen.

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GemPharmatech

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

Tel: 025-5864 1534

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GemPharmatech Co.,Ltd