Casp6 Cas9-KO Strategy

Designer: Jing Jin

Reviewer: Yang Zeng

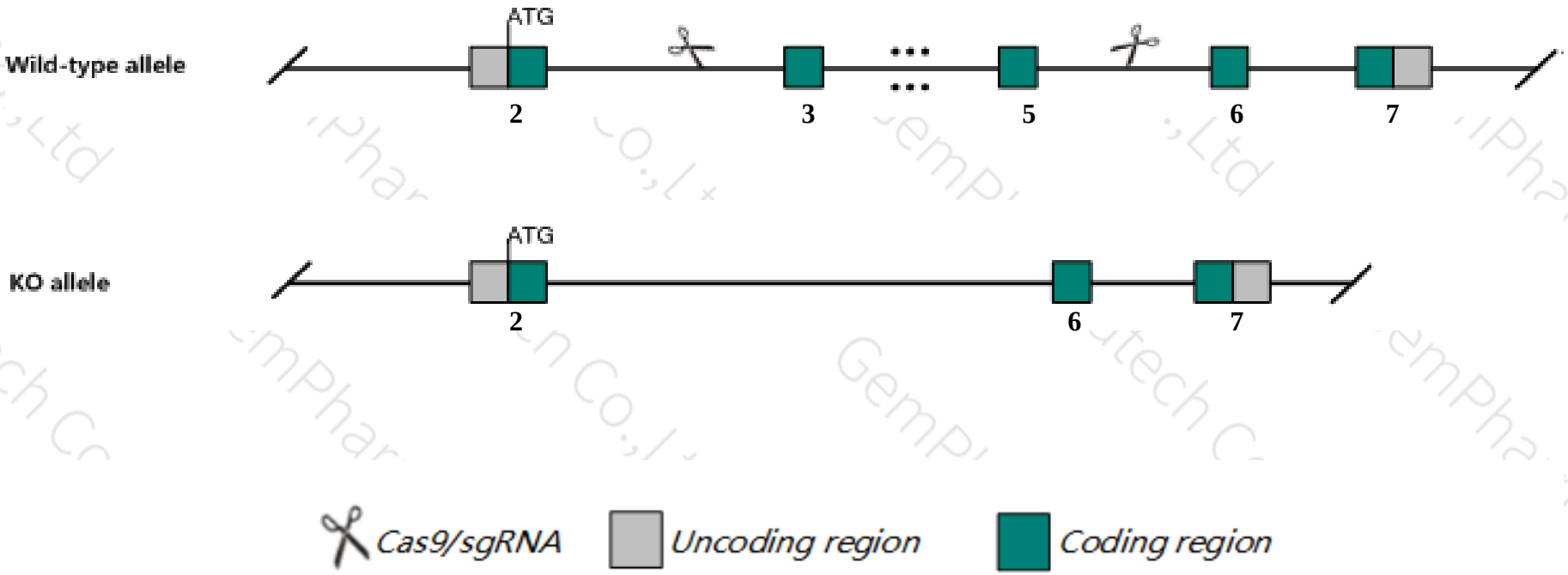
Design Date: 2018-7-6

Project Overview

Project Name	***Casp6***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Casp6* gene. The schematic diagram is as follows:



Technical routes

➢ The *Casp6* gene has 4 transcripts. According to the structure of *Casp6* gene, exon3-exon5 of *Casp6-201* (ENSMUST00000029626.8) transcript is recommended as the knockout region.The region contains 403bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Casp6* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

Notice

➢ According to the existing MGI data,Mice homozygous for a knock-out allele exhibit failure to induce increased lysis of fluorogenic substrate VEID-AMC in staurosporine treated of lenses. Mice homozygous for a different knock-out allele exhibit resistance to excitotoxicity and axonal degeneration.

➢ The *Casp6* gene is located on the Chr3. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

集萃药康 GemPharmatech

Casp6 caspase 6 [Mus musculus (house mouse)]

Gene ID: 12368, updated on 19-Mar-2019

Summary

Official Symbol	Casp6 provided by MGI
Official Full Name	caspase 6 provided byMGI
Primary source	MGI:MGI:1312921
See related	Ensembl:ENSMUSG00000027997
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	CASP-6, Mch2
Summary	This gene encodes a member of the cysteine proteases that plays important roles in regulating apoptosis and neurodegeneration. The encoded protein is involved in the transmission of pain and axonal degeneration. Genetic deletion of this gene in mice results in the delay of axon pruning and protects from axon degeneration. [provided by RefSeq, Apr 2015]
Expression	Ubiquitous expression in duodenum adult (RPKM 36.2), small intestine adult (RPKM 29.7) and 27 other tissuesSee more
Orthologs	human all

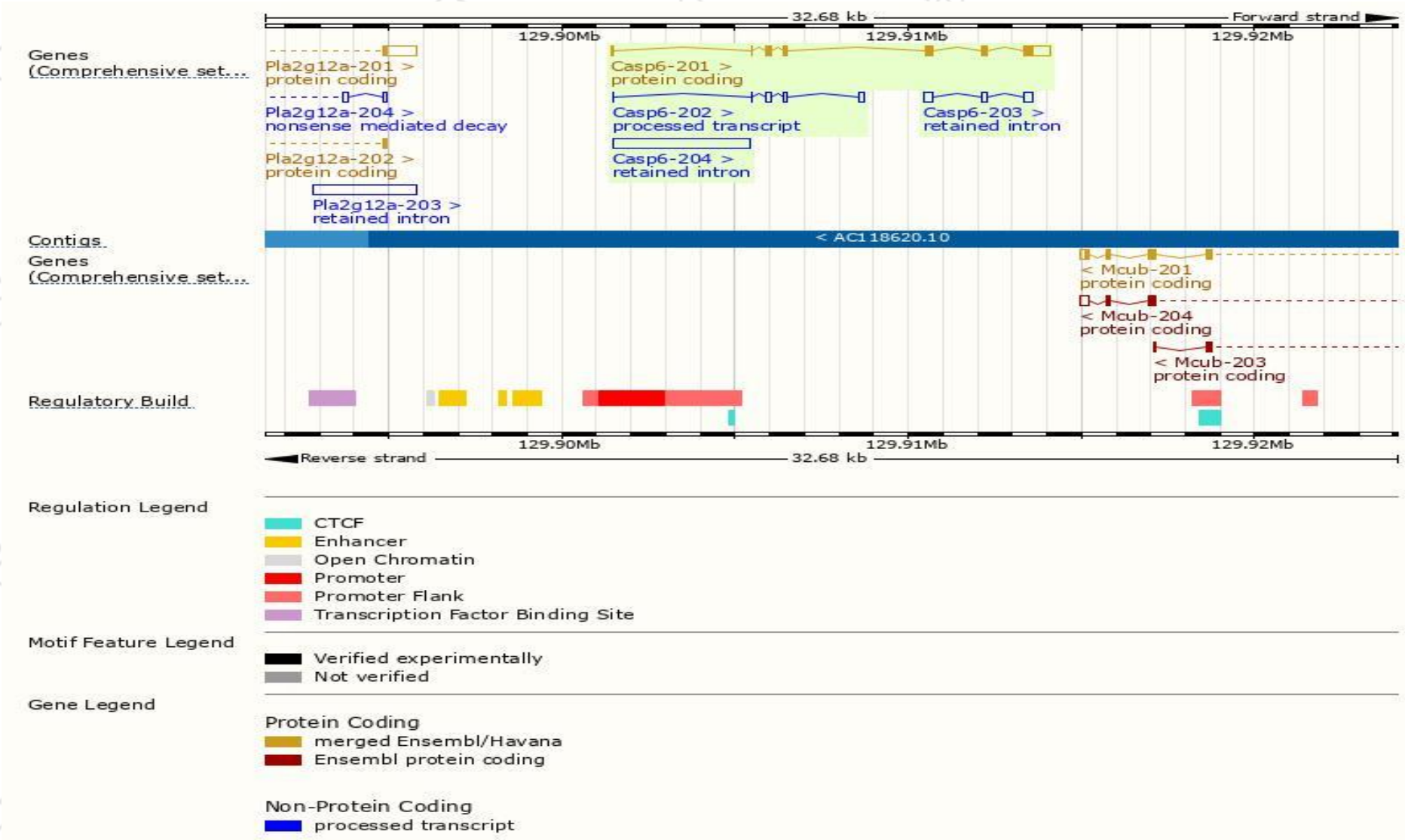
Transcript information（Ensembl）

The gene has 4 transcripts,all transcripts are shown below:

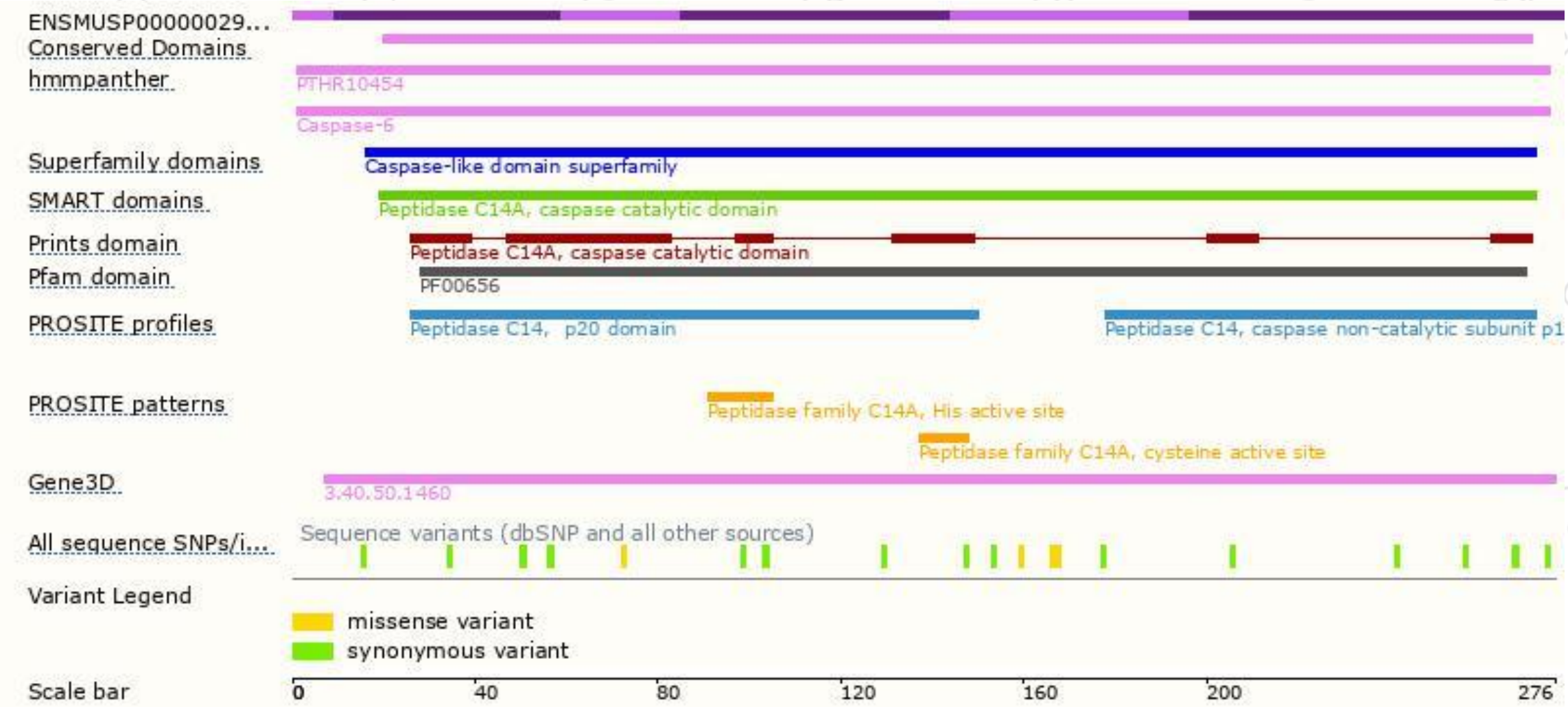
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Casp6-201	ENSMUST00000029626.8	1456	276aa	Protein coding	CCDS17838	O08738 Q3TPJ9	TSL:1 GENCODE basic APPRIS P1
Casp6-202	ENSMUST00000137314.1	477	No protein	Processed transcript	-	-	TSL:2
Casp6-204	ENSMUST00000197175.1	3914	No protein	Retained intron	-	-	TSL:NA
Casp6-203	ENSMUST00000152622.1	664	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Casp6-201* transcript,The transcription is shown below

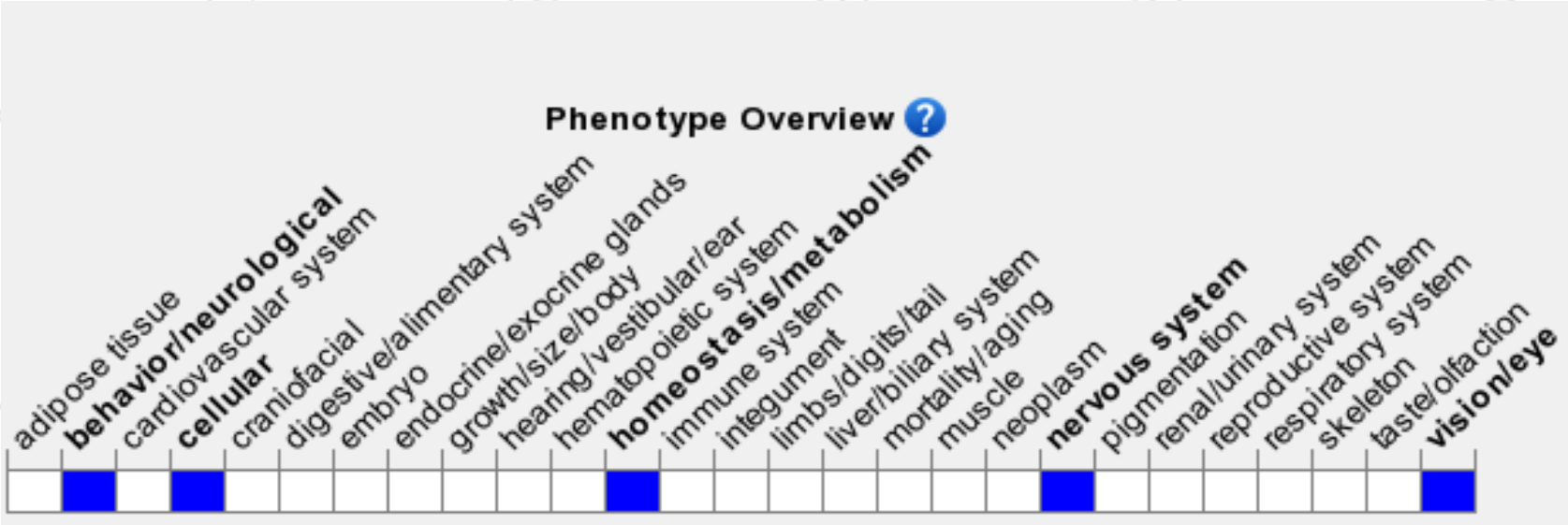
Genomic location distribution



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,Mice homozygous for a knock-out allele exhibit failure to induce increased lysis of fluorogenic substrate VEID-AMC in staurosporine treated of lenses. Mice homozygous for a different knock-out allele exhibit resistance to excitotoxicity and axonal degeneration.

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

Tel: 025-5864 1534

集萃药康生物科技
GemPharmatech Co.,Ltd