Cit Cas9-CKO Strategy

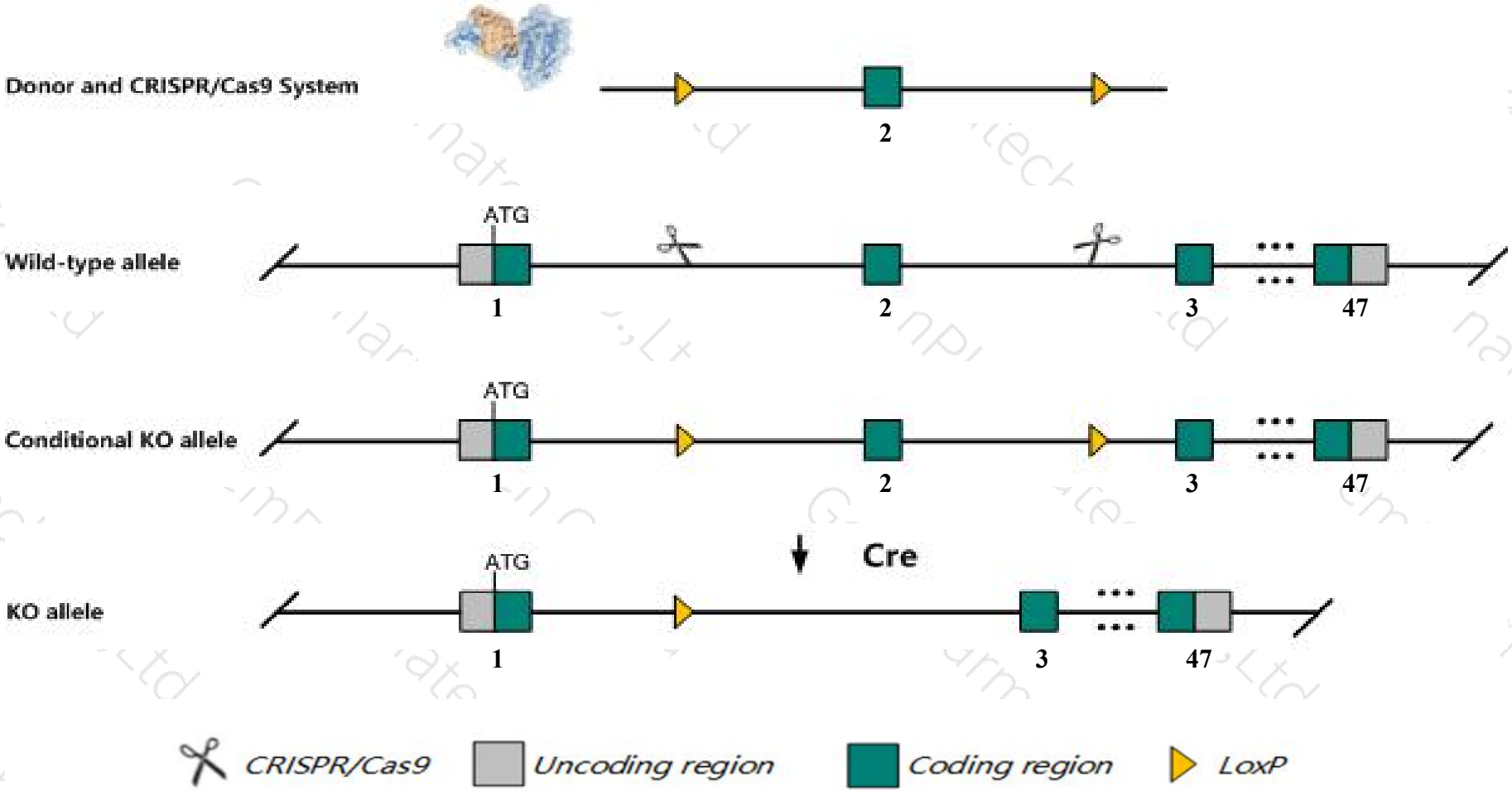
Designer: Daohua Xu
Reviewer: Huimin Su
Design Date: 2020-2-14

Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Cit* gene has 19 transcripts. According to the structure of *Cit* gene, exon2 of *Cit-201* (ENSMUST00000051704.14) transcript is recommended as the knockout region.The region contains 142bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Cit* 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 a null mutation are 20% smaller than wild-type and exhibit tremors, ataxia, and fatal seizures. Brains of mutant mice show a 50% size reduction with abnormalities in the hippocampus, cerebellum, and olfactory lobes. Mutant males show aberrant cytokinesis of spermatogenic precursors.

➢ The *Cit* gene is located on the Chr5. 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

Cit citron [Mus musculus (house mouse)]

Gene ID: 12704, updated on 31-Jan-2019

Summary

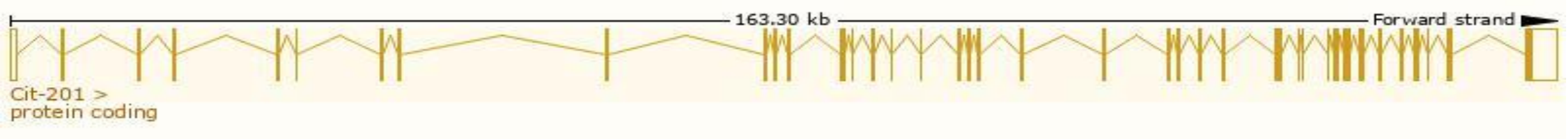
Official Symbol	Cit provided by MGI
Official Full Name	citron provided by MGI
Primary source	MGI:MGI:105313
See related	Ensembl:ENSMUSG00000029516
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	C030025P15Rik, CRIK, CRIK-SK, Cit-k
Expression	Broad expression in cortex adult (RPKM 9.3), frontal lobe adult (RPKM 8.3) and 24 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

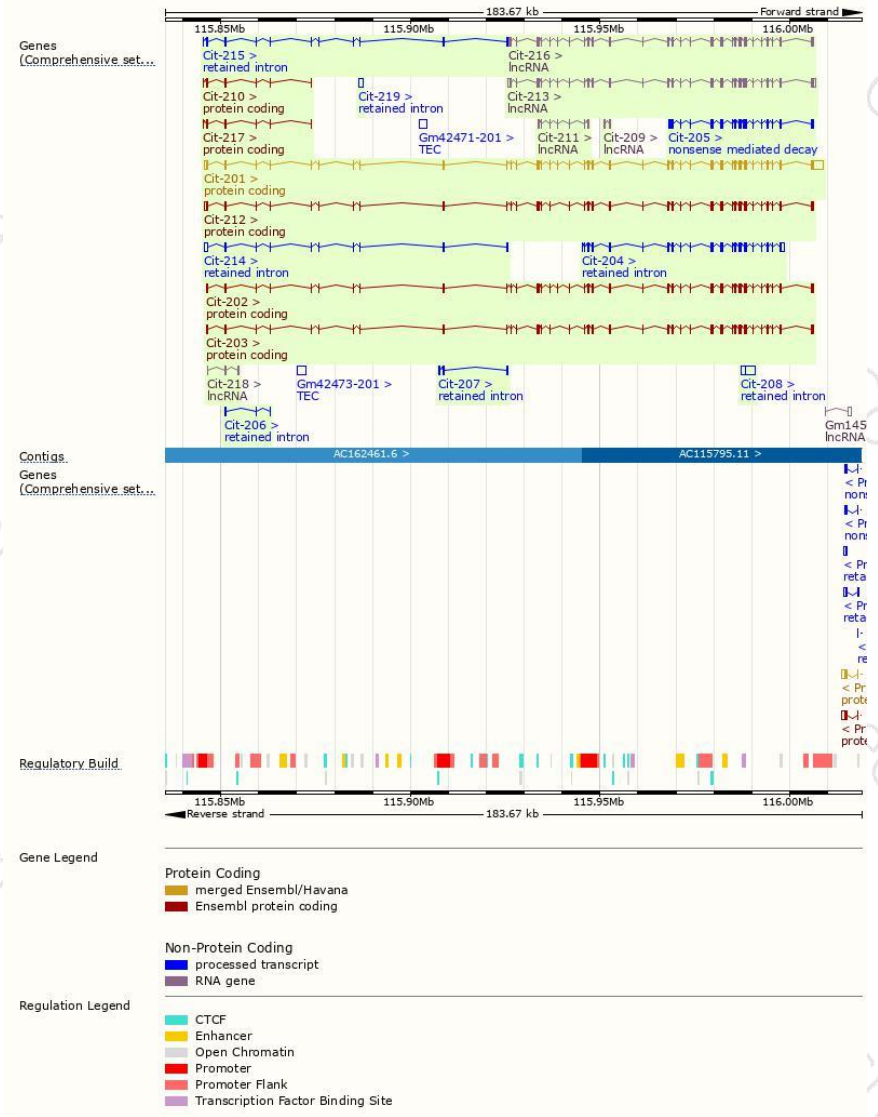
The gene has 19 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cit-201	ENSMUST00000051704.14	9514	2055aa	Protein coding	CCDS19597	E9QL53	TSL:1 GENCODE basic APPRIS P2
Cit-212	ENSMUST00000141101.4	6794	2013aa	Protein coding	-	F6SBR5	TSL:5 GENCODE basic
Cit-202	ENSMUST00000102560.6	6213	2070aa	Protein coding	-	D3YU89	TSL:5 GENCODE basic APPRIS ALT1
Cit-203	ENSMUST00000112008.8	6087	2028aa	Protein coding	-	D3Z1U0	TSL:5 GENCODE basic APPRIS ALT2
Cit-217	ENSMUST00000148245.7	651	181aa	Protein coding	-	D3Z1K6	CDS 3' incomplete TSL:5
Cit-210	ENSMUST00000137952.7	571	175aa	Protein coding	-	D3Z477	CDS 3' incomplete TSL:5
Cit-205	ENSMUST00000123736.1	3487	23aa	Nonsense mediated decay	-	H3BJ74	CDS 5' incomplete TSL:1
Cit-204	ENSMUST00000122877.7	4398	No protein	Retained intron	-	-	TSL:1
Cit-208	ENSMUST00000134609.1	3456	No protein	Retained intron	-	-	TSL:1
Cit-214	ENSMUST00000146387.7	2335	No protein	Retained intron	-	-	TSL:1
Cit-215	ENSMUST00000147330.7	1671	No protein	Retained intron	-	-	TSL:1
Cit-219	ENSMUST00000202734.1	1184	No protein	Retained intron	-	-	TSL:NA
Cit-207	ENSMUST00000128702.1	756	No protein	Retained intron	-	-	TSL:3
Cit-206	ENSMUST00000127976.2	561	No protein	Retained intron	-	-	TSL:3
Cit-213	ENSMUST00000145363.7	5974	No protein	lncRNA	-	-	TSL:5
Cit-216	ENSMUST00000147479.7	5016	No protein	lncRNA	-	-	TSL:1
Cit-211	ENSMUST00000139881.7	699	No protein	lncRNA	-	-	TSL:3
Cit-218	ENSMUST00000153407.1	293	No protein	lncRNA	-	-	TSL:5
Cit-209	ENSMUST00000136780.1	287	No protein	lncRNA	-	-	TSL:5

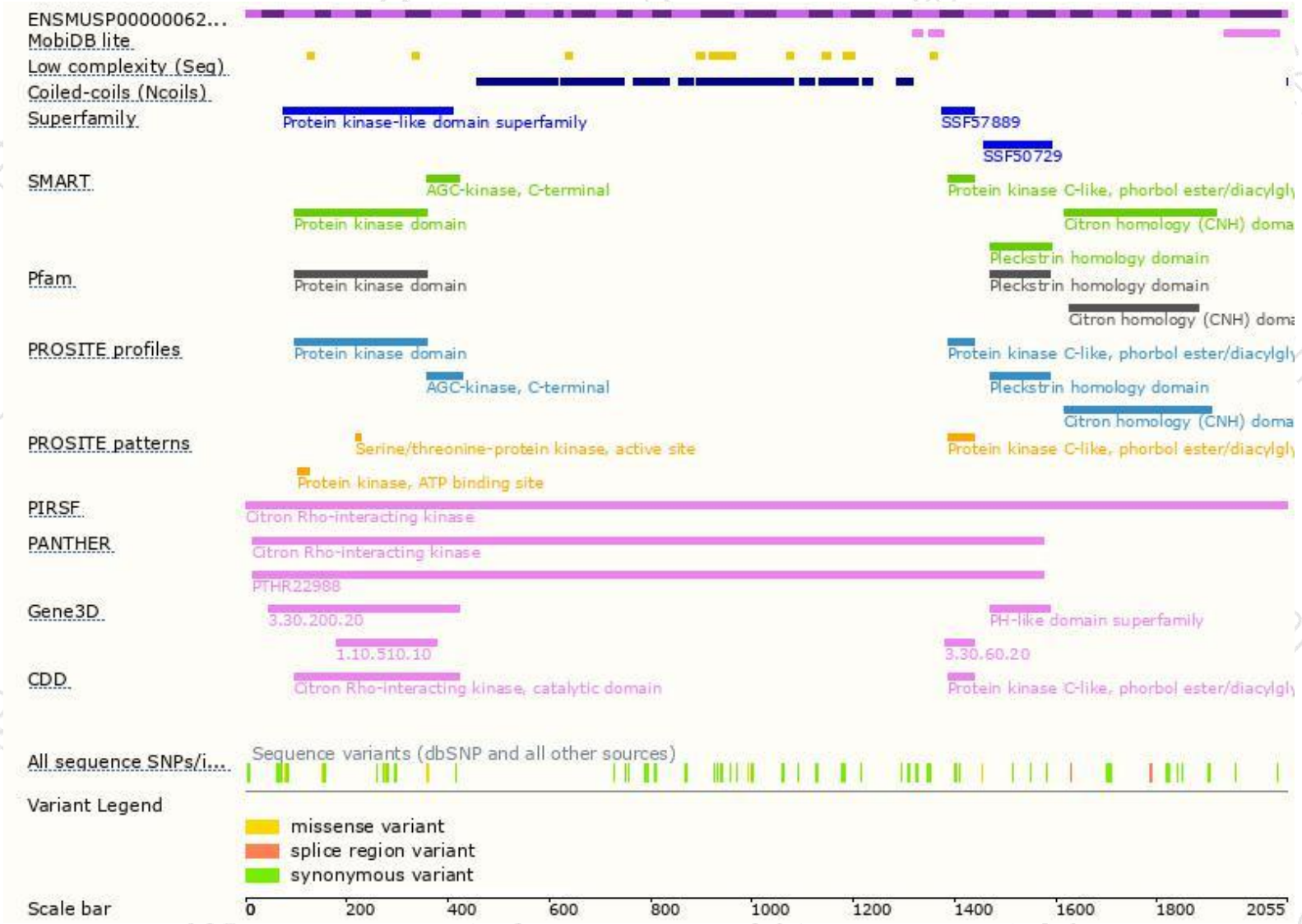
The strategy is based on the design of *Cit-201* transcript,The transcription is shown below



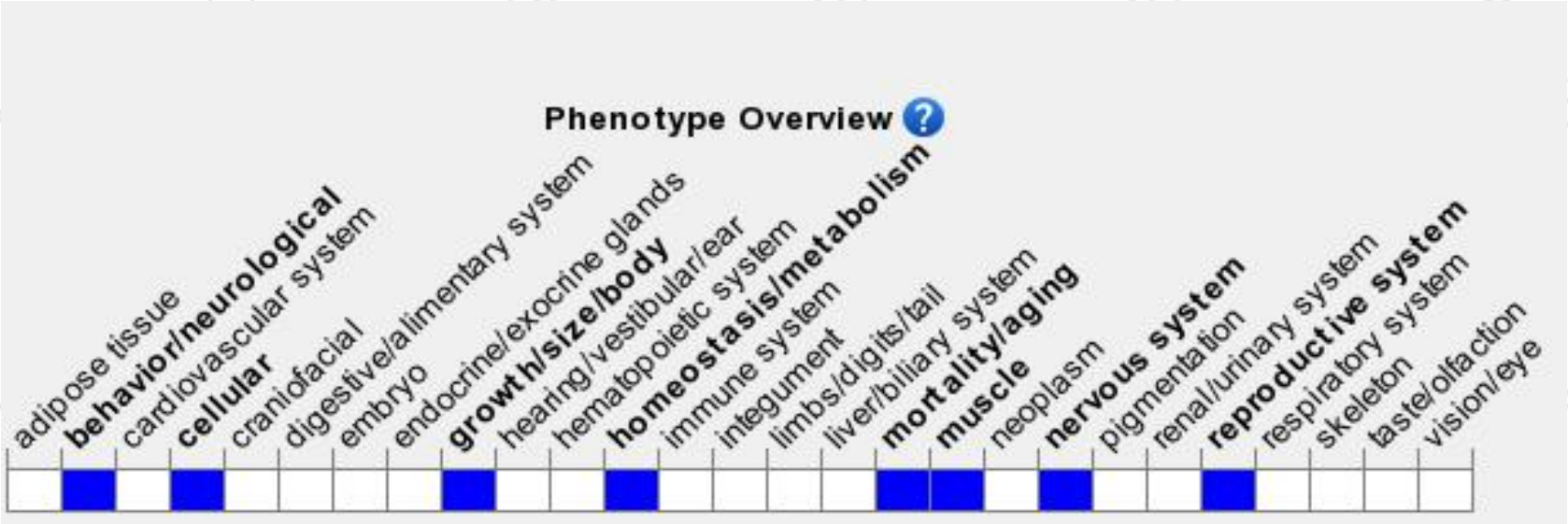
Genomic location distribution



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 null mutation are 20% smaller than wild-type and exhibit tremors, ataxia, and fatal seizures. Brains of mutant mice show a 50% size reduction with abnormalities in the hippocampus, cerebellum, and olfactory lobes. Mutant males show aberrant cytokinesis of spermatogenic precursors.

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