Abcd2 Cas9-CKO Strategy

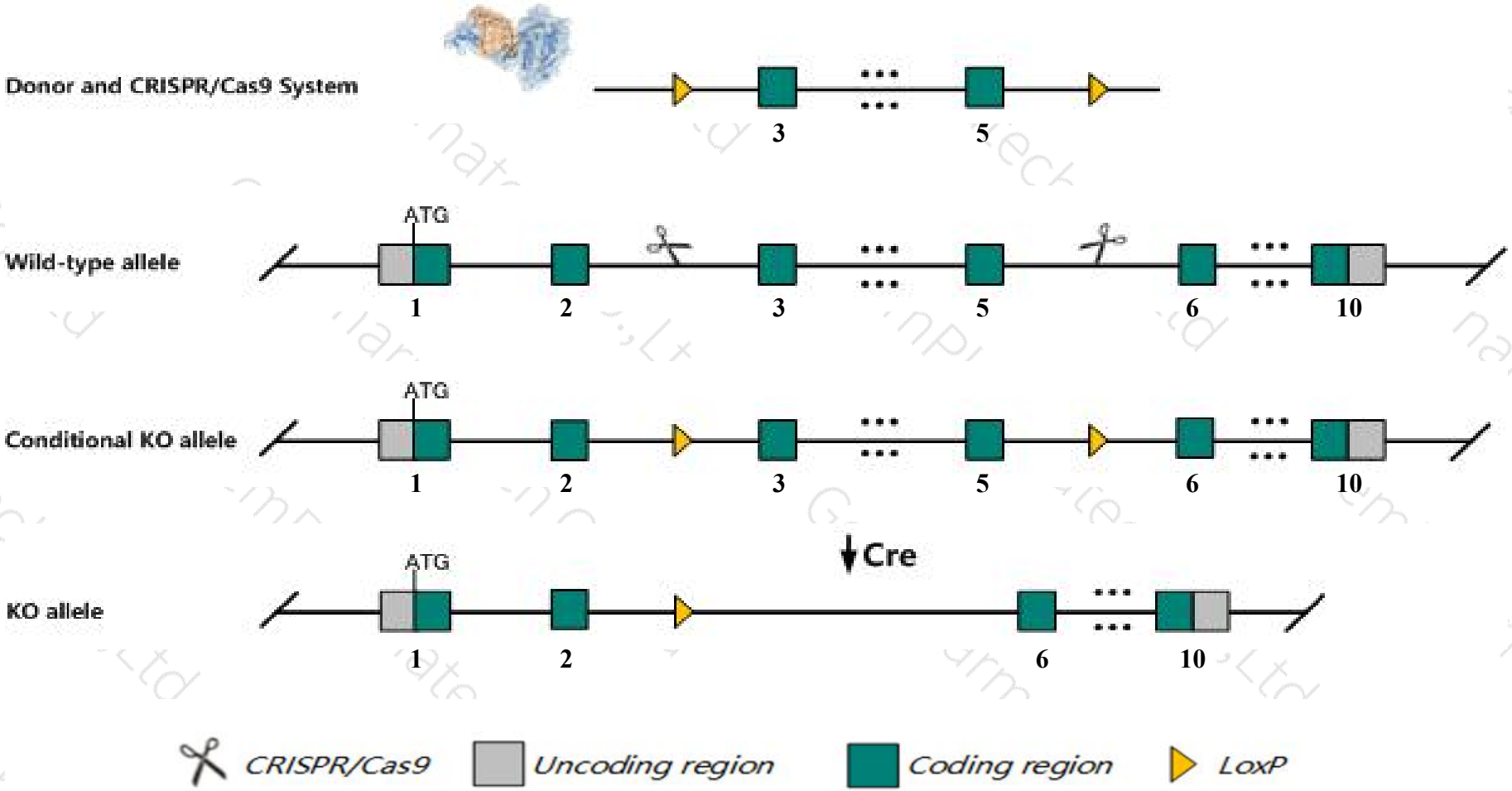
Designer: Bingxuan Li
Reviewer: Ruirui Zhang
Design Date: 2019-10-31

Project Overview

Project Name	***Abcd2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Abcd2* gene. The schematic diagram is as follows:



Technical routes

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- The *Abcd2* gene has 3 transcripts. According to the structure of *Abcd2* gene, exon3-exon5 of *Abcd2-201* (ENSMUST00000069511.7) transcript is recommended as the knockout region.The region contains 383bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Abcd2* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA and Donor were microinjected into the fertilized eggs of C57BL/6J 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/6J 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,Mice homozygous for a disruption in this gene exhibit a late-onset cerebellar and sensory ataxia, loss of Purkinje cells, dorsal root ganglia cell degeneration, axonal degeneration in the spinal cord, and an accumulation of very long chain fatty acids.

- The *Abcd2* gene is located on the Chr15. 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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Abcd2 ATP-binding cassette, sub-family D (ALD), member 2 [*Mus musculus* (house mouse)]

Gene ID: 26874, updated on 12-Aug-2019

Summary

Official Symbol	Abcd2 provided by MGI
Official Full Name	ATP-binding cassette, sub-family D (ALD), member 2 provided by MGI
Primary source	MGI:MGI:1349467
See related	Ensembl:ENSMUSG00000055782
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	ALDR; ABC39; ALDL1; ALDRP
Summary	The membrane-associated protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intra-cellular membranes. ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White). This protein is a member of the ALD subfamily, which is involved in peroxisomal import of fatty acids and/or fatty acyl-CoAs in the organelle. All known peroxisomal ABC transporters are half transporters which require a partner half transporter molecule to form a functional homodimeric or heterodimeric transporter. The function of this peroxisomal membrane protein is unknown; however this protein is speculated to function as a dimerization partner of Abcd1 and/or other peroxisomal ABC transporters. Mutations in the human gene have been observed in patients with adrenoleukodystrophy, a severe demyelinating disease. This gene has been identified as a candidate for a modifier gene, accounting for the extreme variation among adrenoleukodystrophy phenotypes. This gene is also a candidate for a complement group of Zellweger syndrome, a genetically heterogeneous disorder of peroxisomal biogenesis. [provided by RefSeq, Jul 2008]
Expression	Biased expression in subcutaneous fat pad adult (RPKM 15.5), genital fat pad adult (RPKM 12.8) and 12 other tissues See more
Orthologs	human all

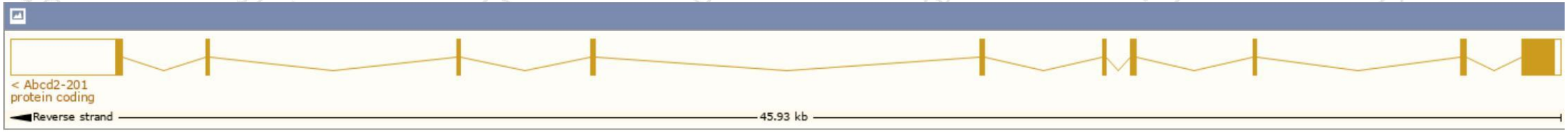
Transcript information（Ensembl）

The gene has 3 transcripts,all transcripts are shown below:

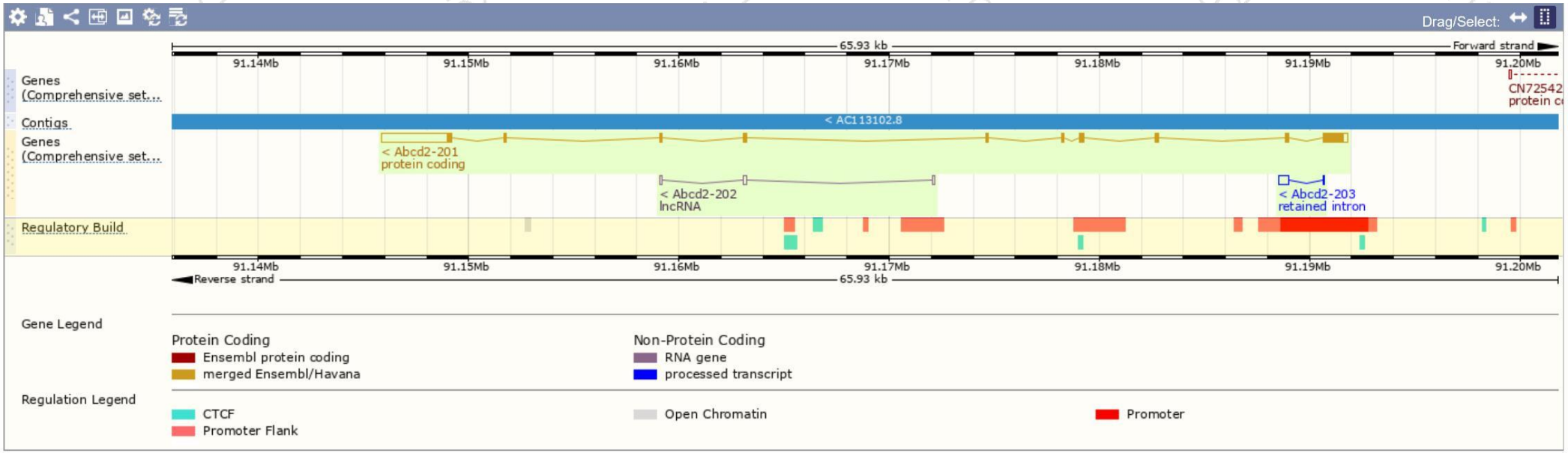
Show/hide columns (1 hidden) Filter

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Abcd2-203	ENSMUST00000230461.1	528	No protein	Retained intron	-	-	-
Abcd2-202	ENSMUST00000229909.1	320	No protein	lncRNA	-	-	-
Abcd2-201	ENSMUST00000069511.7	5532	741aa	Protein coding	CCDS27760	A0A0R4J0U5	TSL:1 GENCODE basic APPRIS P1

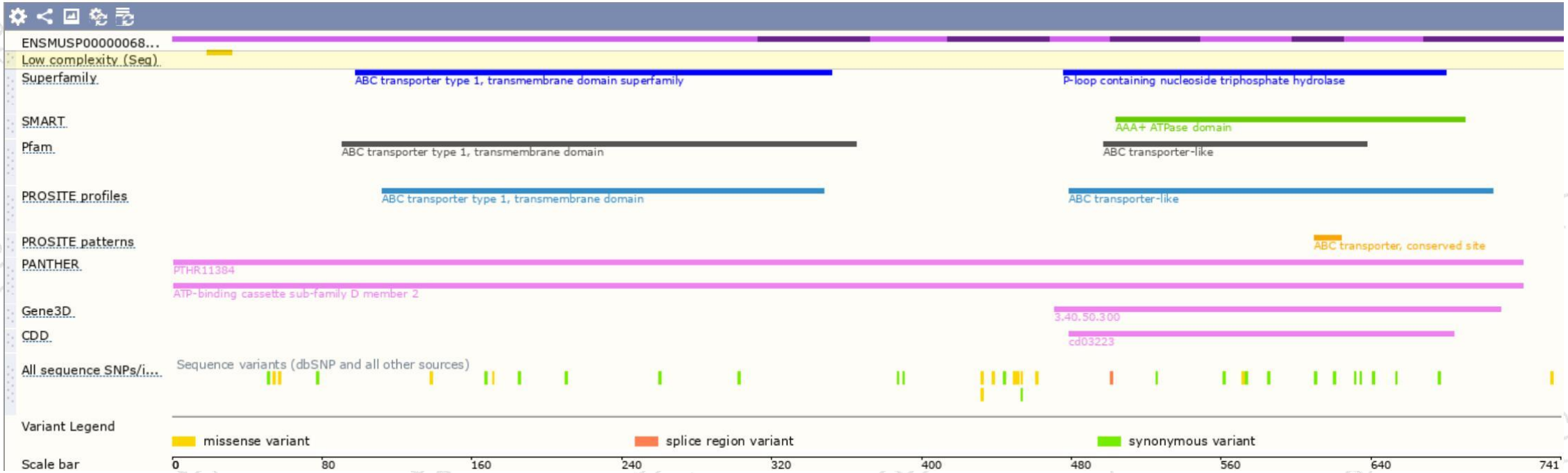
The strategy is based on the design of *Abcd2-201* transcript,The transcription is shown below



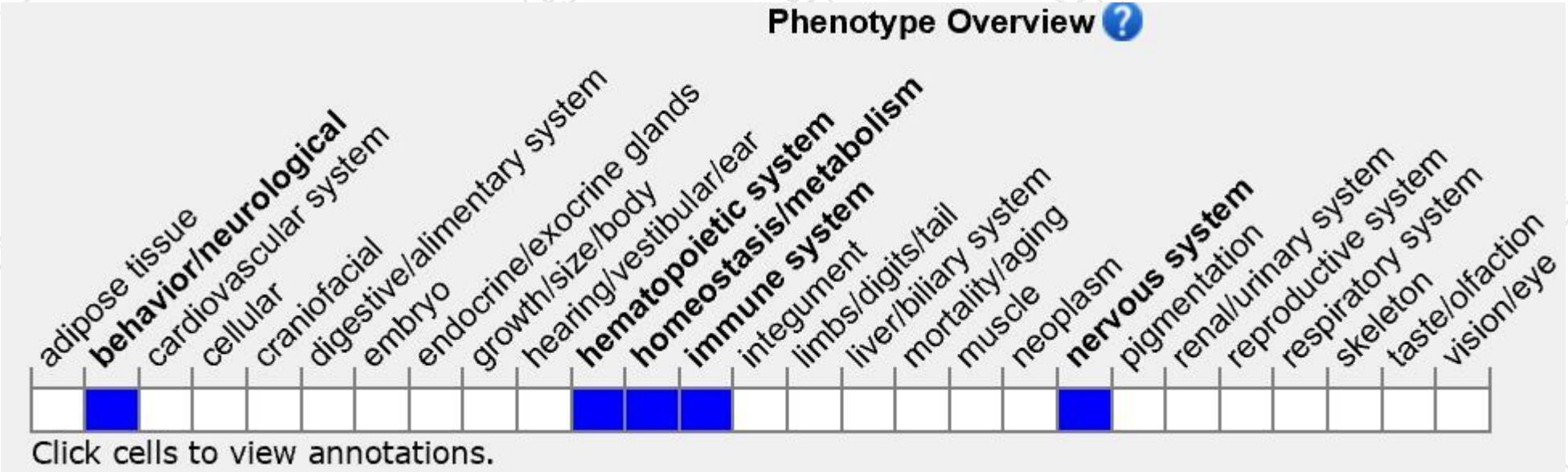
Genomic location distribution



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 disruption in this gene exhibit a late-onset cerebellar and sensory ataxia, loss of Purkinje cells, dorsal root ganglia cell degeneration, axonal degeneration in the spinal cord, and an accumulation of very long chain fatty acids.

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

Tel: 400-9660890

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