Atp11a Cas9-CKO Strategy

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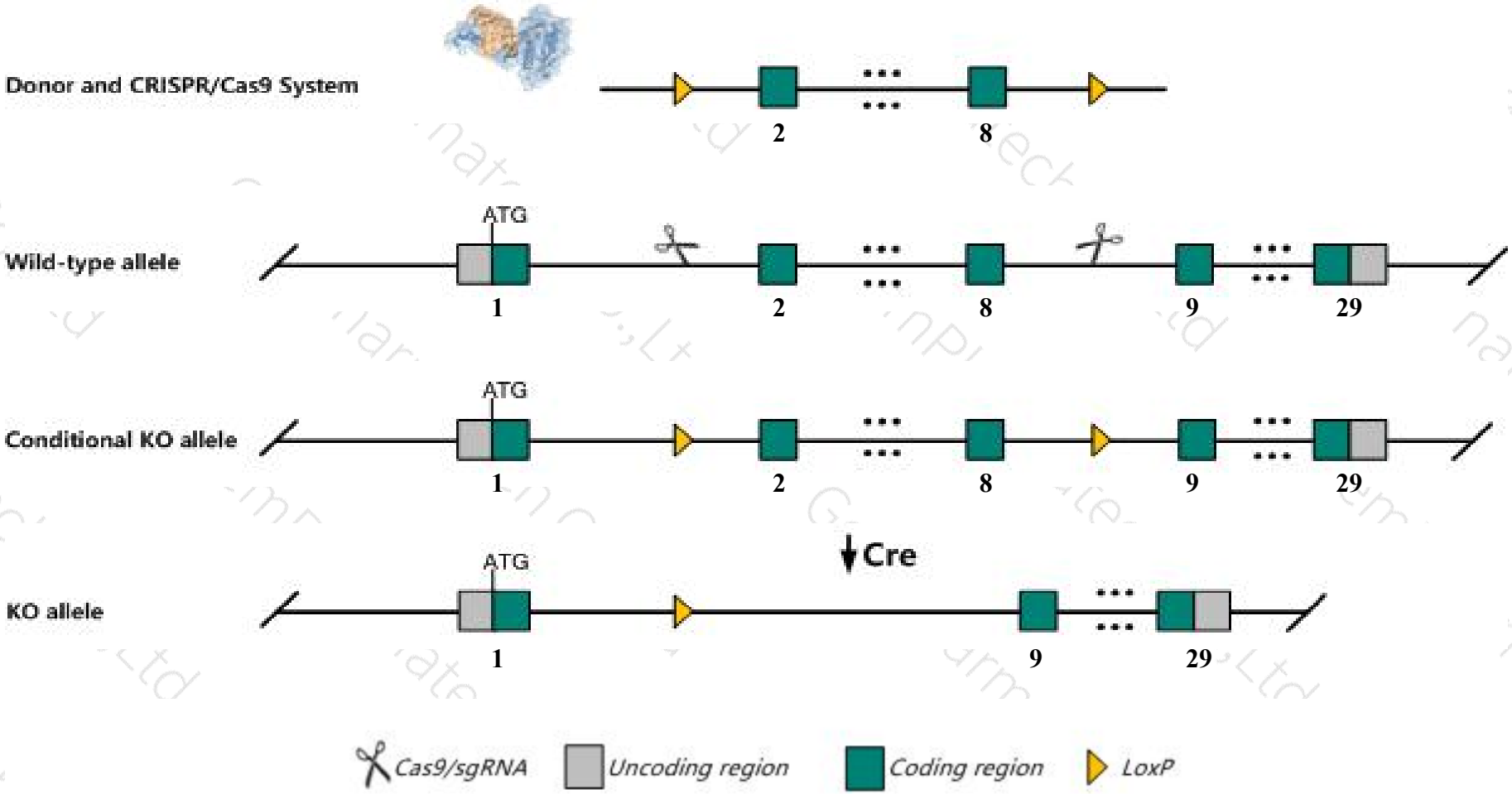
Project Overview

Project Name	***Atp11a***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Atp11a* gene. The schematic diagram is as follows:



Technical routes

- The *Atp11a* gene has 11 transcripts. According to the structure of *Atp11a* gene, exon2-exon8 of *Atp11a-202* (ENSMUST00000107444.7) transcript is recommended as the knockout region.The region contains 686bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Atp11a* 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,Mice homozygous for a conditional allele activated in muscle cells exhibit abnormal myoblast function in culture and abnormal skeletal muscle regeneration.

➢ The *Atp11a* gene is located on the Chr8. 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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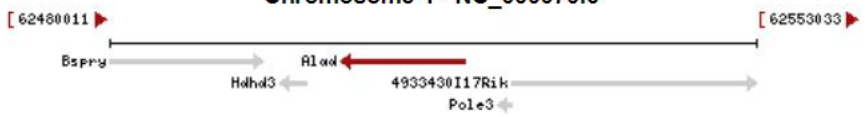
Alad aminolevulinate, delta-, dehydratase [*Mus musculus* (house mouse)]

Gene ID: 17025, updated on 12-Aug-2019

Summary

Official Symbol	Alad provided by MGI
Official Full Name	aminolevulinate, delta-, dehydratase provided by MGI
Primary source	MGI:MGI:96853
See related	Ensembl:ENSMUSG00000028393
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	Lv; ALADH
Expression	Ubiquitous expression in liver E14.5 (RPKM 66.3), liver E14 (RPKM 56.6) and 27 other tissues See more
Orthologs	human all

Chromosome 4 - NC_000070.6

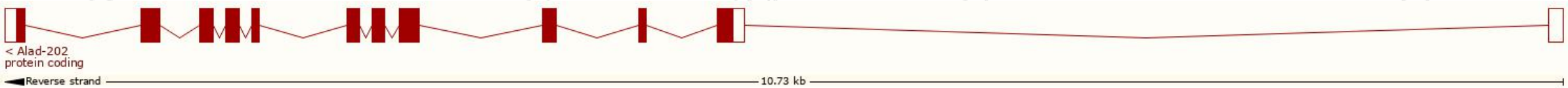


Transcript information（Ensembl）

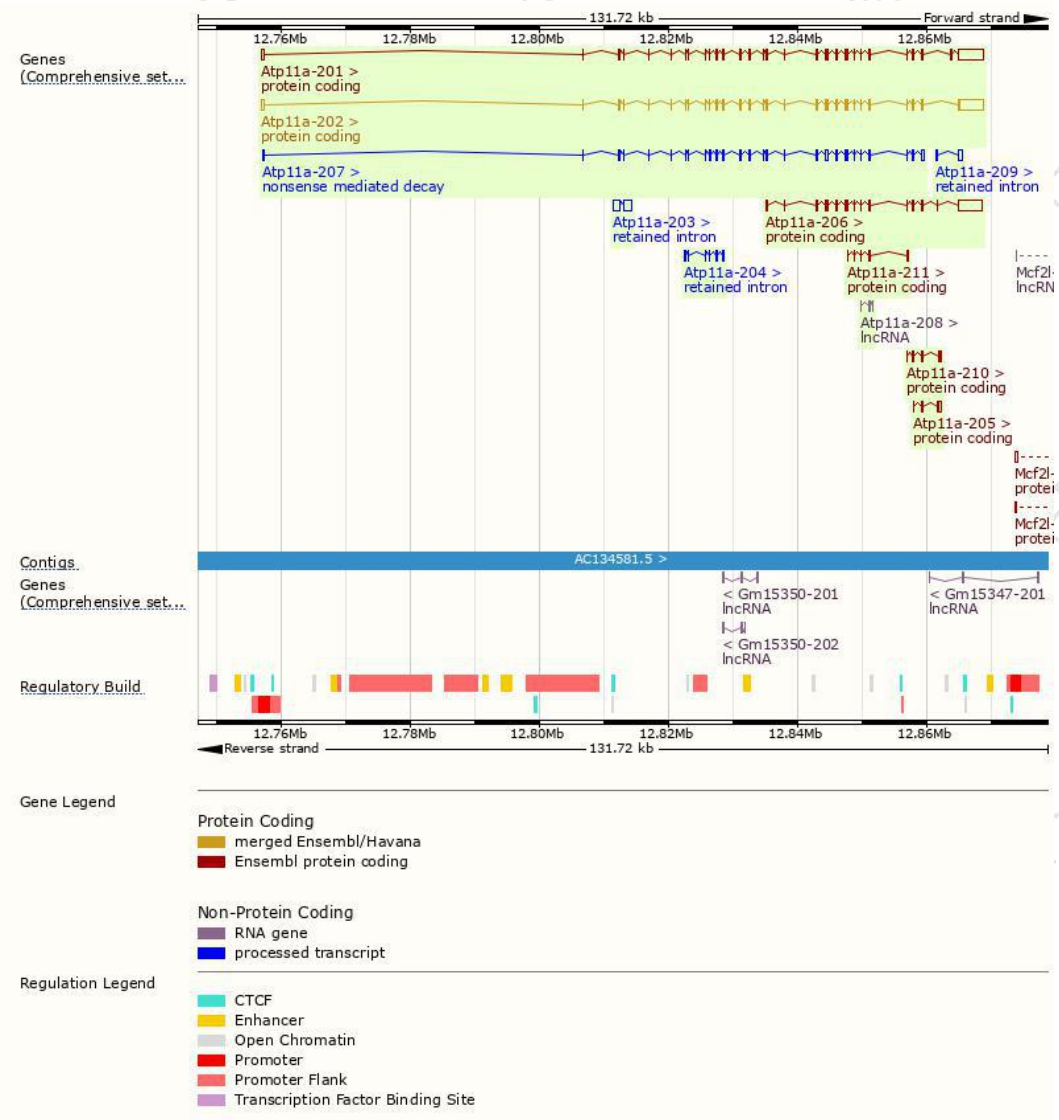
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Alad-202	ENSMUST00000107444.7	1247	330aa	ENSMUSP00000103068.1	Protein coding	CCDS18243	P10518	TSL:1 GENCODE basic APPRIS P1
Alad-201	ENSMUST00000030090.3	1206	330aa	ENSMUSP00000030090.3	Protein coding	CCDS18243	P10518	TSL:1 GENCODE basic APPRIS P1
Alad-203	ENSMUST00000137448.1	1769	No protein	-	lncRNA	-	-	TSL:2

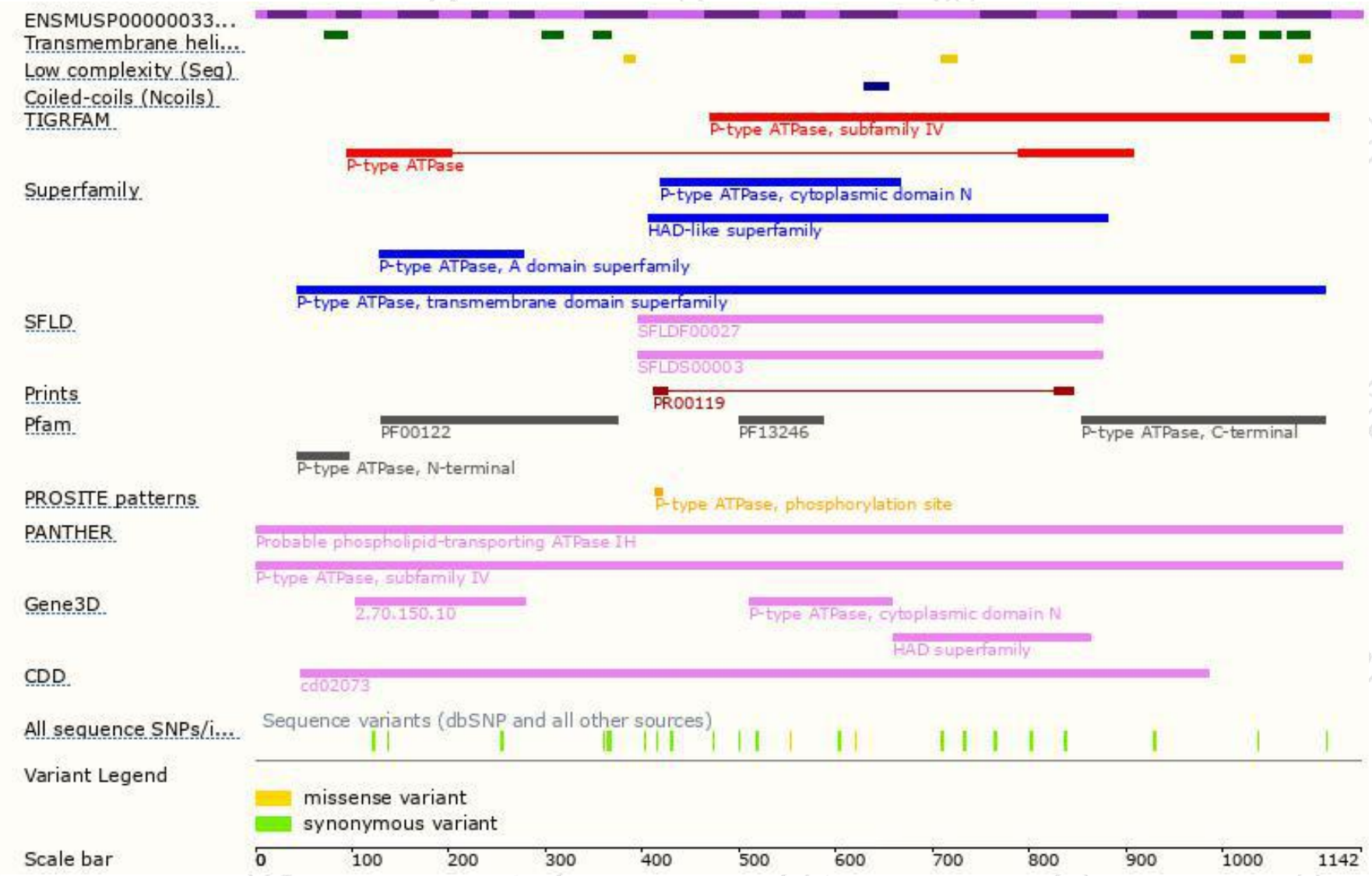
The strategy is based on the design of *Atp11a-202* transcript,The transcription is shown below



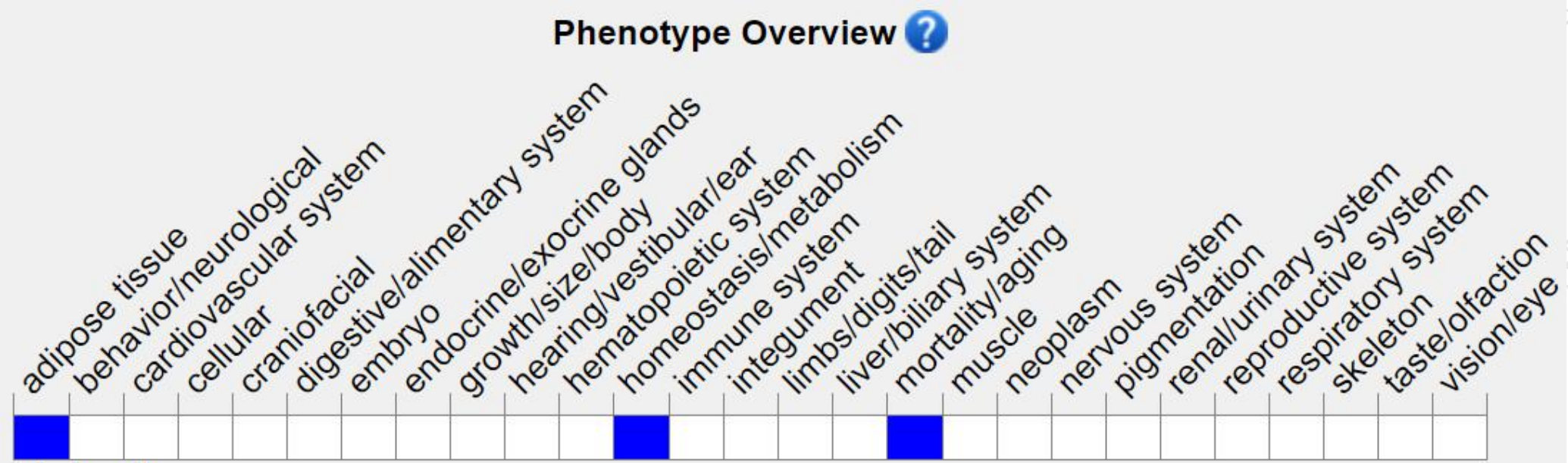
Genomic location distribution



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,Mice homozygous for a conditional allele activated in muscle cells exhibit abnormal myoblast function in culture and abnormal skeletal muscle regeneration.

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

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