Acads Cas9-CKO Strategy

Designer: Huan Wang

Reviewer: Yumeng Wang

Design Date: 2021-7-14

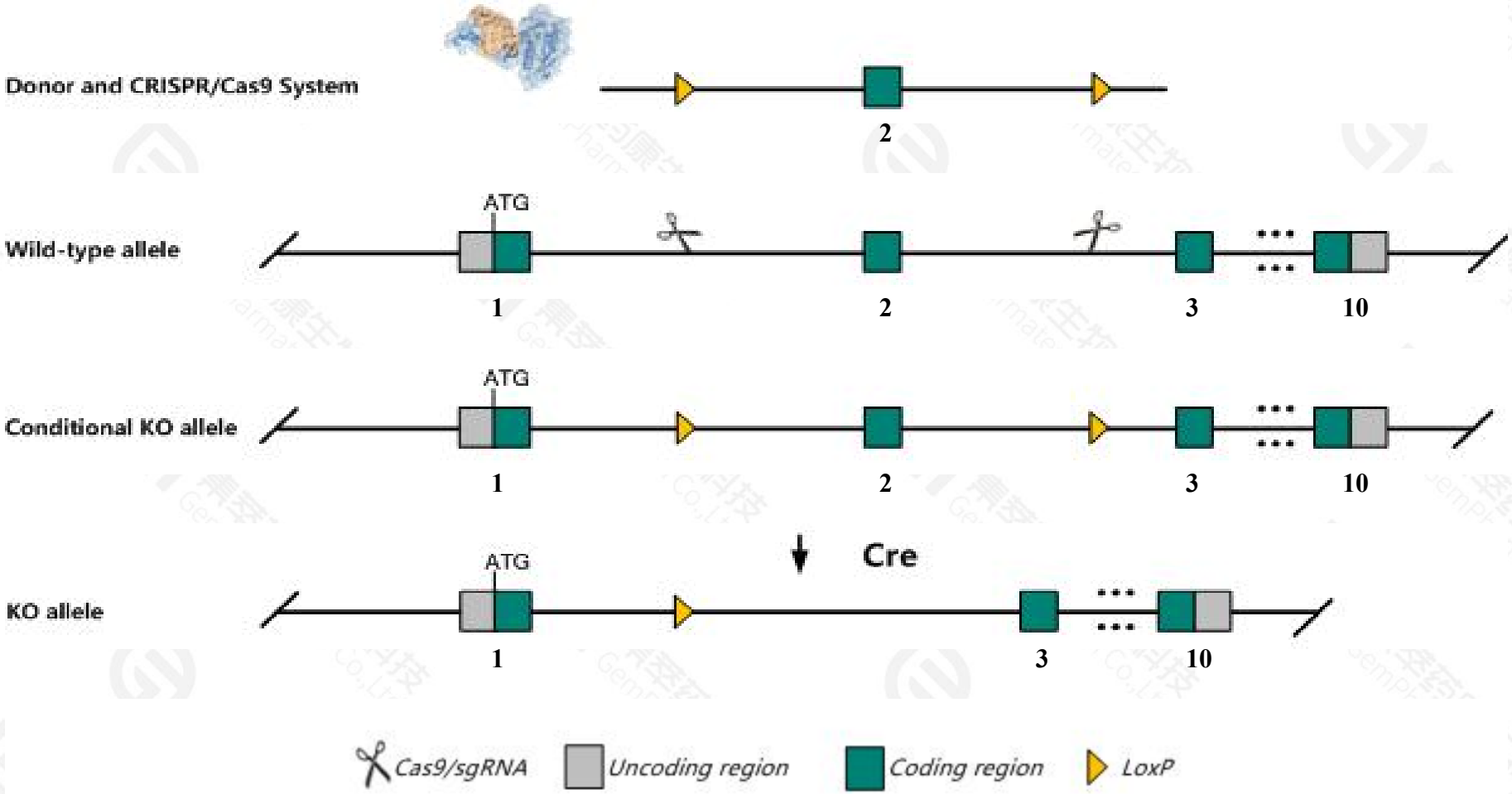
Project Overview

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

Conditional Knockout strategy

集萃药康 GemPharmatech

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



Technical routes

➢ The *Acads* gene has 4 transcripts. According to the structure of *Acads* gene, exon2 of *Acads-201*(ENSMUST00000031524.11) transcript is recommended as the knockout region.The region contains 164bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Acads* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor 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 disruptions in this gene display organic aciduria and develop hypoglycemia and fatty livers after fasting.

➢ The *Acads* 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

Acads acyl-Coenzyme A dehydrogenase, short chain [Mus musculus (house mouse)]

Gene ID: 11409, updated on 13-Mar-2020

Summary

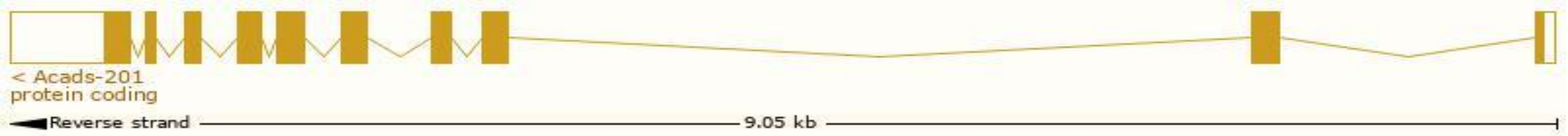
Official Symbol	Acads provided by MGI
Official Full Name	acyl-Coenzyme A dehydrogenase, short chain provided by MGI
Primary source	MGI:MGI:87868
See related	Ensembl:ENSMUSG00000029545
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI196007, Bcd-1, Bcd1, Hdlq8, SCAD
Summary	This gene encodes a homotetrameric mitochondrial flavoprotein and is a member of the acyl-CoA dehydrogenase family. Members of this family catalyze the first step of fatty acid beta-oxidation, forming a C2-C3 trans-double bond in a FAD-dependent reaction. As beta-oxidation cycles through its four steps, each member of the Acyl-CoA dehydrogenase family works at an optimum fatty acid chain-length. This enzyme has its optimum at C(four)-CoA. In mice, deficiency of this gene has been linked to cold sensitivity and increased high-density lipoprotein levels. [provided by RefSeq, Nov 2012]
Expression	Broad expression in colon adult (RPKM 134.5), adrenal adult (RPKM 130.8) and 22 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

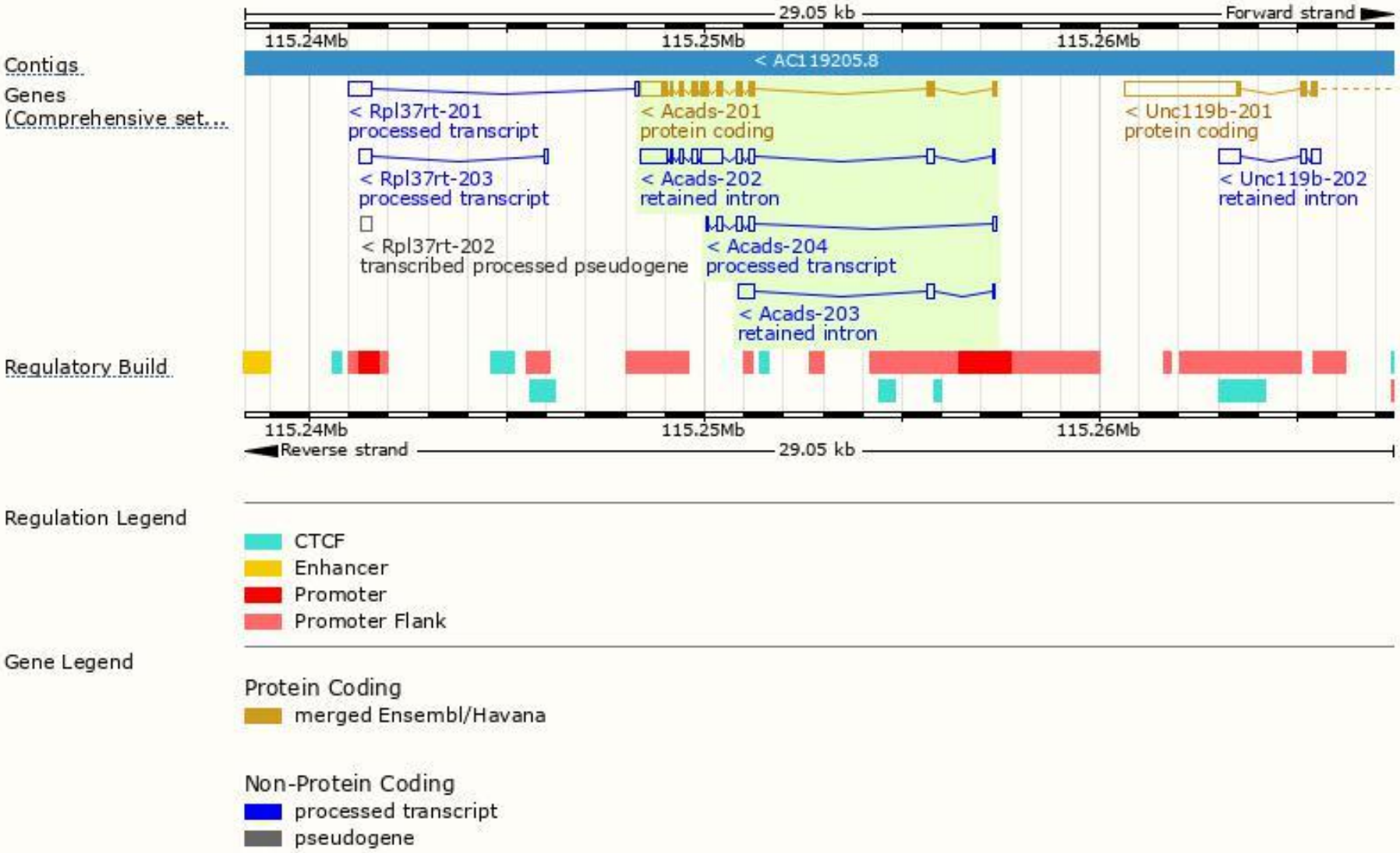
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Acads-201	ENSMUST00000031524.10	1870	412aa	Protein coding	CCDS19579	Q07417	TSL:1 GENCODE basic APPRIS P1
Acads-204	ENSMUST00000153374.1	532	No protein	Processed transcript	-	-	TSL:3
Acads-202	ENSMUST00000131726.7	2023	No protein	Retained intron	-	-	TSL:2
Acads-203	ENSMUST00000141142.1	645	No protein	Retained intron	-	-	TSL:2

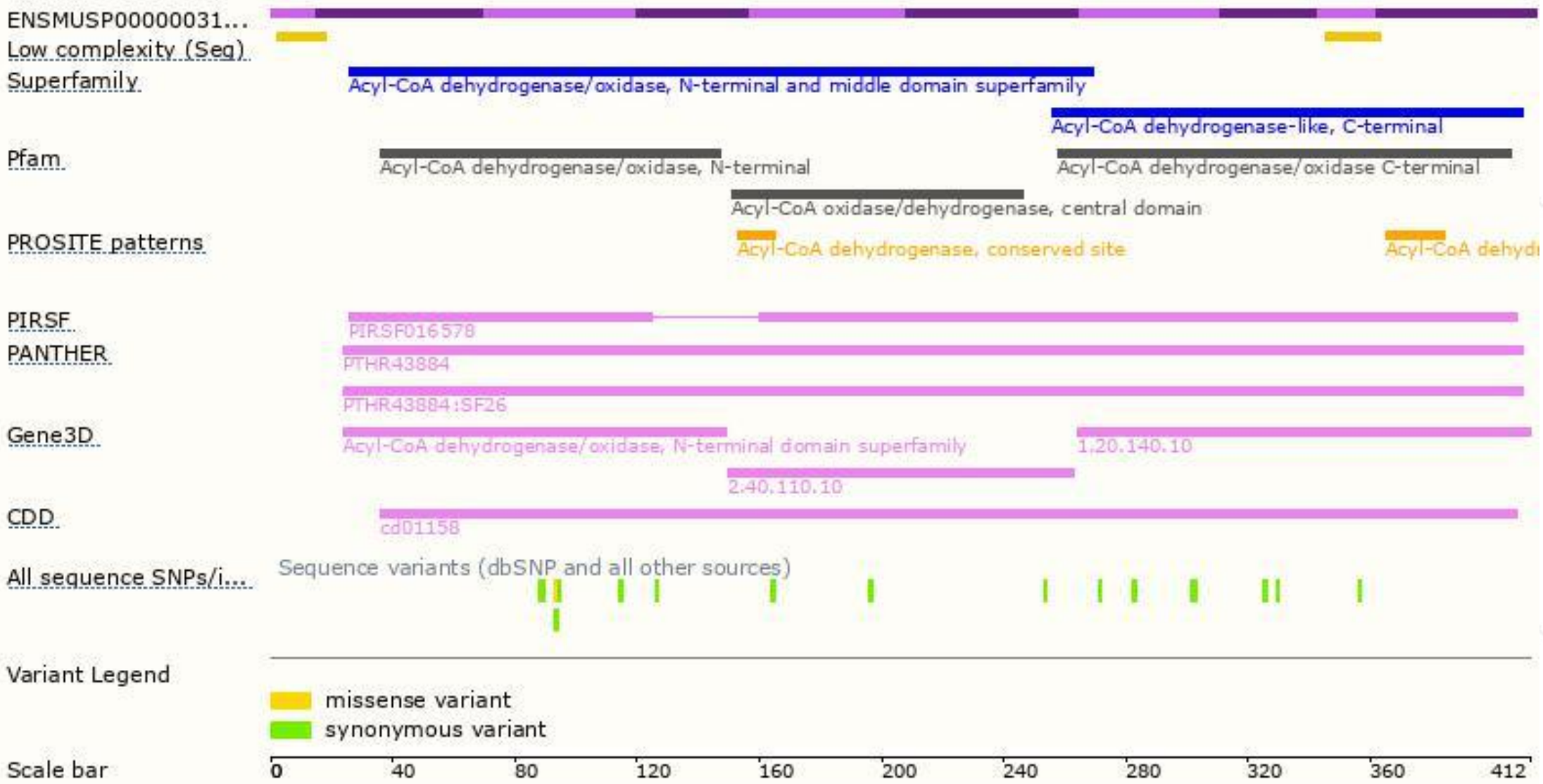
The strategy is based on the design of *Acads-201* transcript,the transcription is shown below:



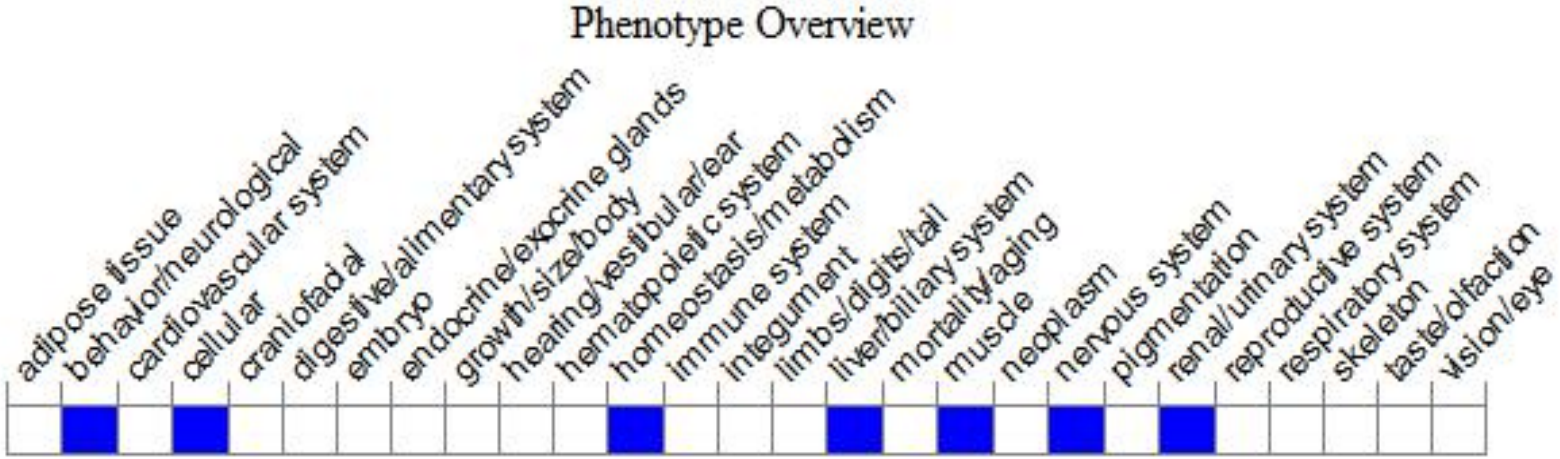
Genomic location distribution



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 disruptions in this gene display organic aciduria and develop hypoglycemia and fatty livers after fasting.

集萃药康
GemPharmatech

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