Acadm Cas9-KO Strategy

Designer: Xueting Zhang
Reviewer:Yanhua Shen
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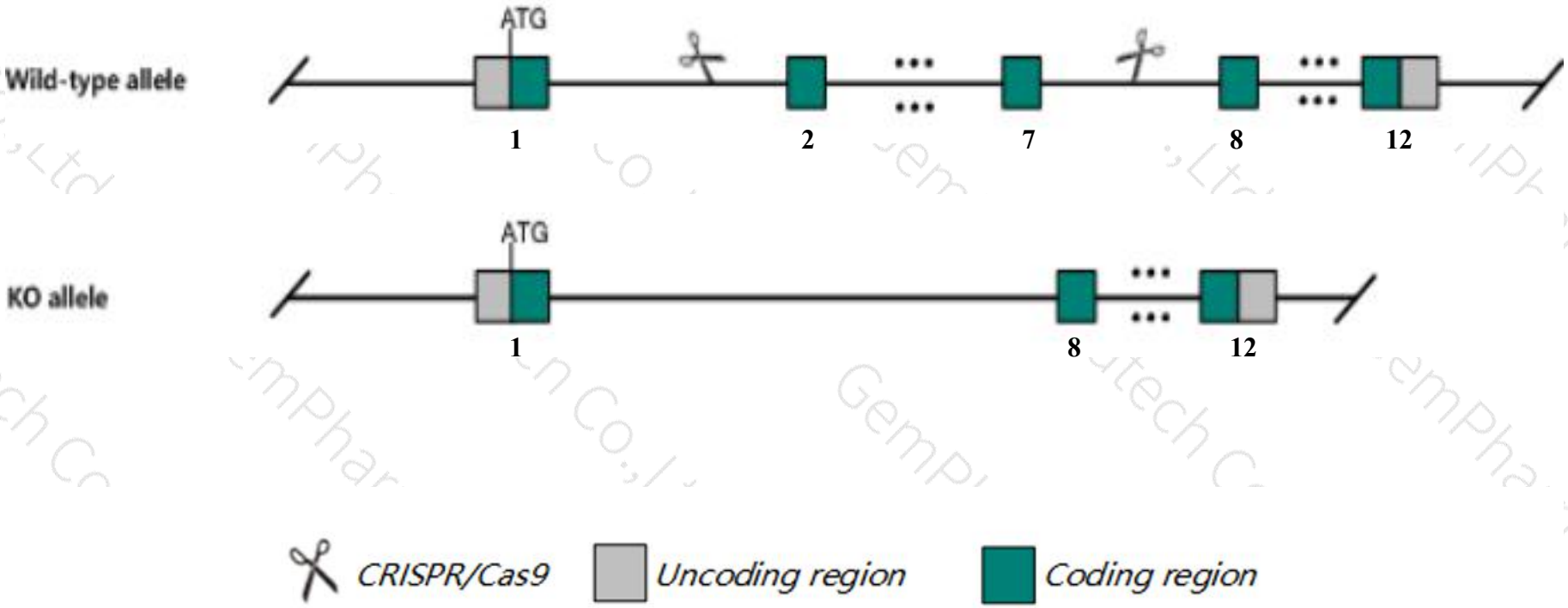
Project Overview

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

Knockout strategy

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



Technical routes

➢ The *Acadm* gene has 10 transcripts. According to the structure of *Acadm* gene, exon2-exon7 of *Acadm-201* (ENSMUST00000072697.12) transcript is recommended as the knockout region.The region contains 569bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Acadm* gene. The brief process is as follows: CRISPR/Cas9 systen

Notice

- According to the existing MGI data,Mice homozygous for a knock-out allele display a high degree of postnatal lethality, develop an organic aciduria, fatty liver and an unexpected diffuse cardiomyopathy with multifocal myocyte degeneration and necrosis, and show severe cold intolerance with prior fasting.

- Transcript *Acadm*-208 may not be affected.

- The *Acadm* 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）

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Acadm acyl-Coenzyme A dehydrogenase, medium chain [*Mus musculus* (house mouse)]

Gene ID: 11364, updated on 13-Mar-2020

Summary

Official Symbol	Acadm provided by MGI
Official Full Name	acyl-Coenzyme A dehydrogenase, medium chain provided by MGI
Primary source	MGI:MGI:87867
See related	Ensembl:ENSMUSG00000062908
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	MCAD; AU018656
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 length between C6- and C12-acylCoA. In mice, deficiency of this gene can cause neonatal mortality as well as fasting and cold intolerance. This gene has multiple, intronless pseudogenes. [provided by RefSeq, Nov 2012]
Expression	Broad expression in heart adult (RPKM 179.5), liver E18 (RPKM 140.4) and 20 other tissues See more
Orthologs	human all

Genomic context

Location: 3 H3; 3 78.77 cM

See Acadm in Genome Data Viewer

Exon count: 12

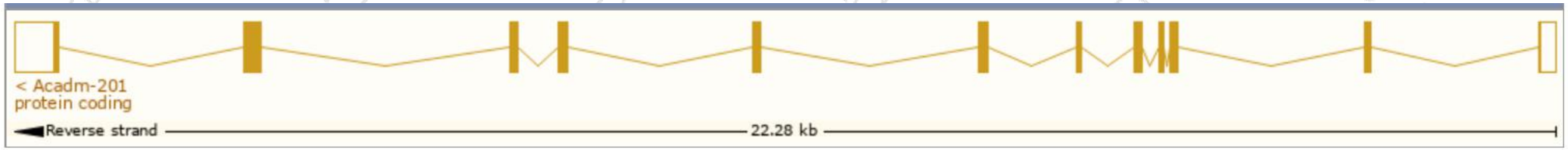
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	3	NC_000069.6 (153922353..153944643, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	3	NC_000069.5 (153585323..153607396, complement)

Transcript information（Ensembl）

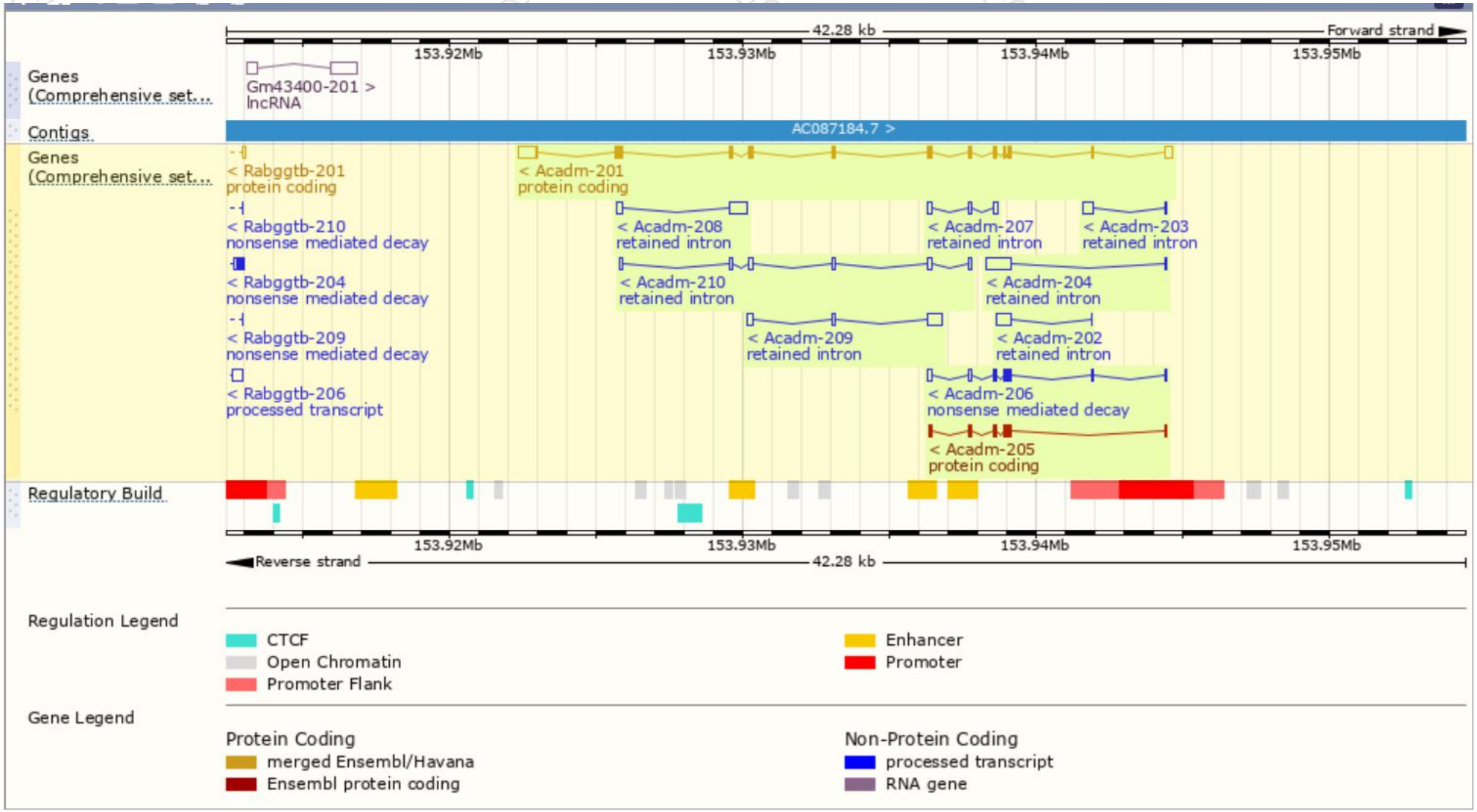
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Acadm-201	ENSMUST00000072697.12	2047	421aa	Protein coding	CCDS17924	P45952	TSL:1 GENCODE basic APPRIS P1
Acadm-205	ENSMUST00000150070.1	565	144aa	Protein coding	-	D3Z2A5	CDS 3' incomplete TSL:5
Acadm-206	ENSMUST00000156310.1	728	157aa	Nonsense mediated decay	-	D6RFD7	TSL:3
Acadm-204	ENSMUST00000137161.1	920	No protein	Retained intron	-	-	TSL:3
Acadm-208	ENSMUST00000196688.1	859	No protein	Retained intron	-	-	TSL:2
Acadm-209	ENSMUST00000199342.1	839	No protein	Retained intron	-	-	TSL:2
Acadm-210	ENSMUST00000200250.4	690	No protein	Retained intron	-	-	TSL:2
Acadm-202	ENSMUST00000130713.1	532	No protein	Retained intron	-	-	TSL:5
Acadm-203	ENSMUST00000135724.1	437	No protein	Retained intron	-	-	TSL:1
Acadm-207	ENSMUST00000196188.4	323	No protein	Retained intron	-	-	TSL:3

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

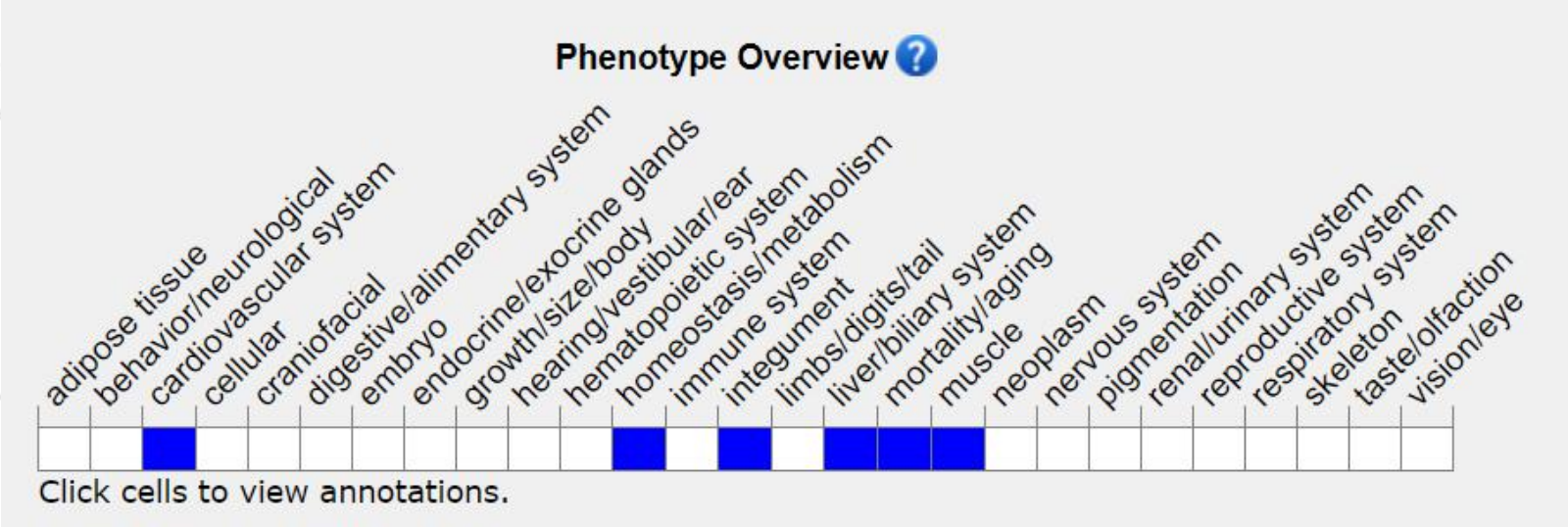


Genomic location distribution



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/).

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

Tel: 400-9660890

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GemPharmatech Co.,Ltd