Acadl Cas9-CKO Strategy

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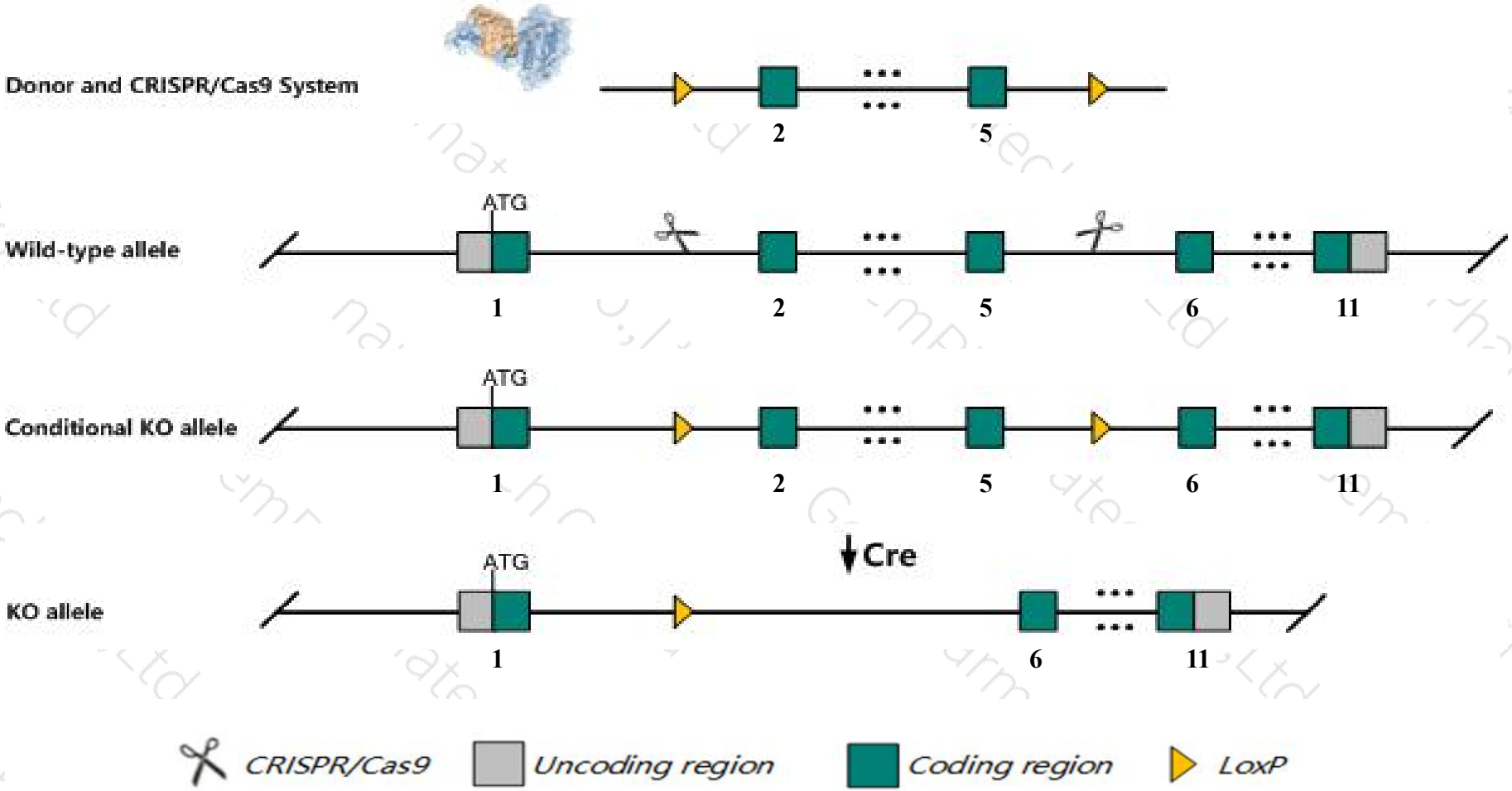
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Acadl* gene has 2 transcripts. According to the structure of *Acadl* gene, exon2-exon5 of *Acadl-201* (ENSMUST00000027153.5) transcript is recommended as the knockout region.The region contains 526bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Acadl* 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,Homozygous mutation of this gene results in reduced litter size, sudden death between 2-14 weeks of age, reduced serum glucose levels, lipid accumulation in the liver and heart, and cardiomyopathy. Heterozygous mutant animals exhibit reduced litter size.

- *Gm27934* and *Gm15793* gene will be deleted.

- The *Acadl* gene is located on the Chr1. 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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Acadl acyl-Coenzyme A dehydrogenase, long-chain [*Mus musculus* (house mouse)]

Gene ID: 11363, updated on 13-Mar-2020

Summary

Official Symbol	Acadl provided by MGI
Official Full Name	acyl-Coenzyme A dehydrogenase, long-chain provided by MGI
Primary source	MGI:MGI:87866
See related	Ensembl:ENSMUSG00000026003
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	LCAD; C79855; AA960361; AU018452
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 C12- and C16-acylCoA. In mice, deficiency of this gene can cause sudden death, cardiomyopathy as well as fasting and cold intolerance. [provided by RefSeq, Nov 2012]
Expression	Broad expression in heart adult (RPKM 89.2), liver E18 (RPKM 50.6) and 22 other tissues See more
Orthologs	human all

Genomic context

Location: 1 C3; 1 33.64 cM

See Acadl in Genome Data Viewer

Exon count: 11

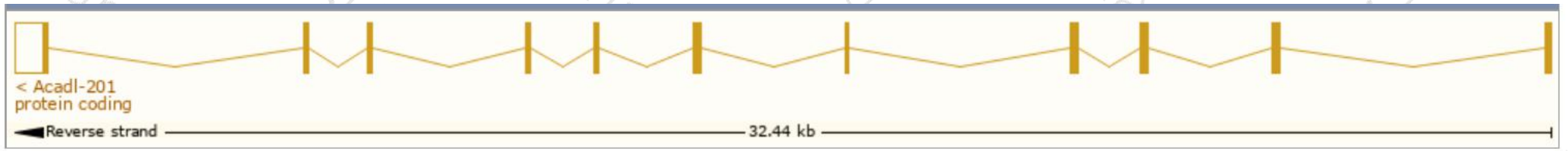
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	1	NC_000067.6 (66830839..66863309, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	1	NC_000067.5 (66877427..66909841, complement)

Transcript information（Ensembl）

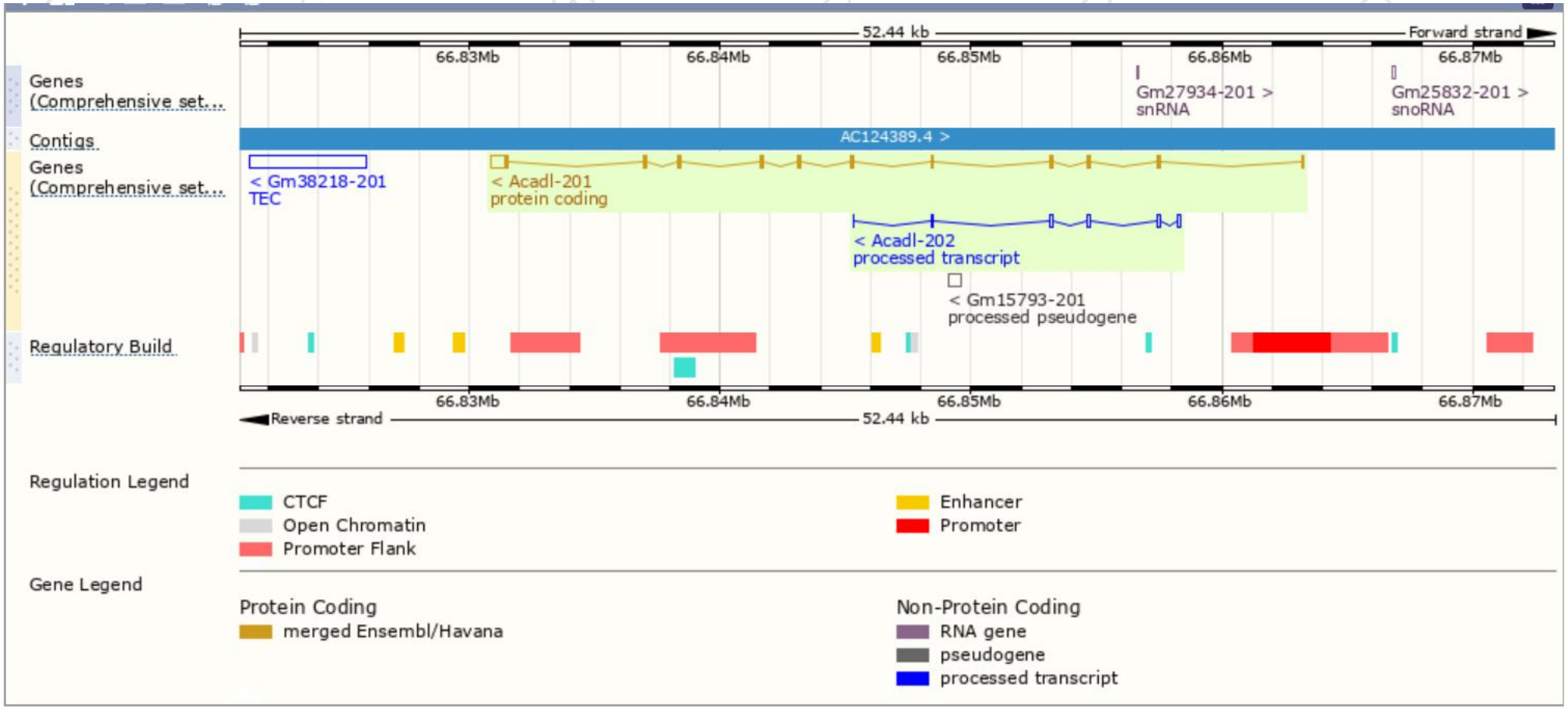
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Acadl-201	ENSMUST00000027153.5	1916	430aa	Protein coding	CCDS15023	A0A0R4J083	TSL:1 GENCODE basic APPRIS P1
Acadl-202	ENSMUST00000139208.1	649	No protein	Processed transcript	-	-	TSL:3

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

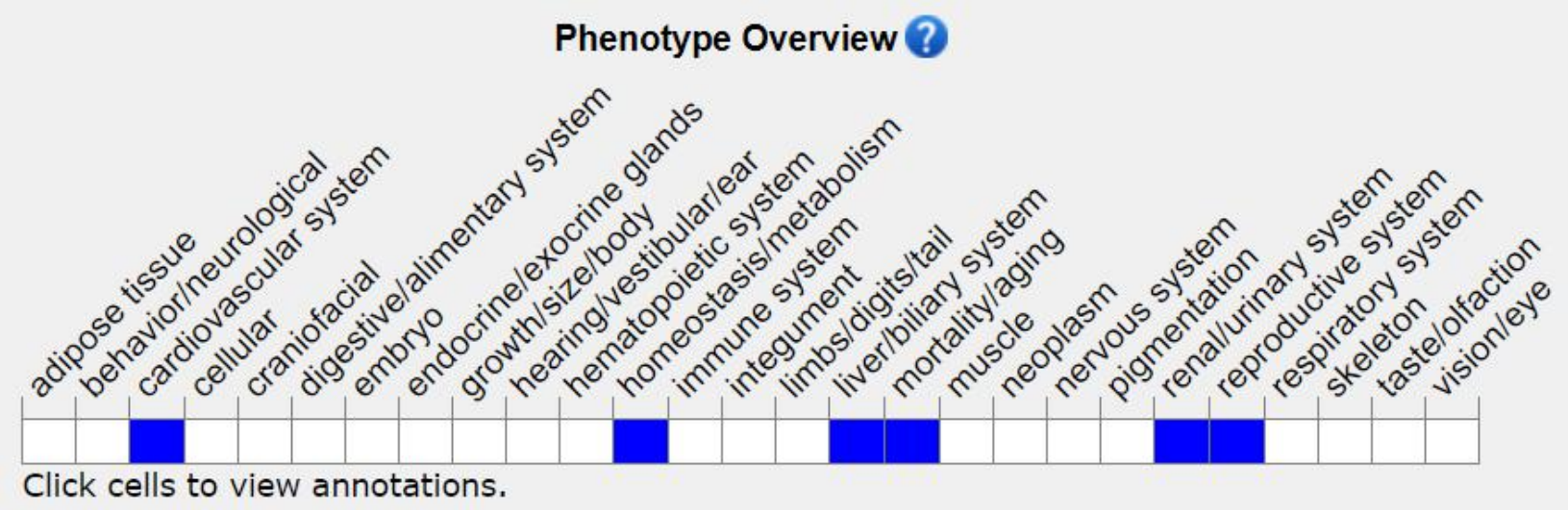


Genomic location distribution



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/).

Homozygous mutation of this gene results in reduced litter size, sudden death between 2-14 weeks of age, reduced serum glucose levels, lipid accumulation in the liver and heart, and cardiomyopathy. Heterozygous mutant animals exhibit reduced litter size.

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

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