

# Hacd2 Cas9-CKO Strategy

Designer:Xueting Zhang Reviewer:Yanhua Shen

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## **Project Overview**



Project Name Hacd2

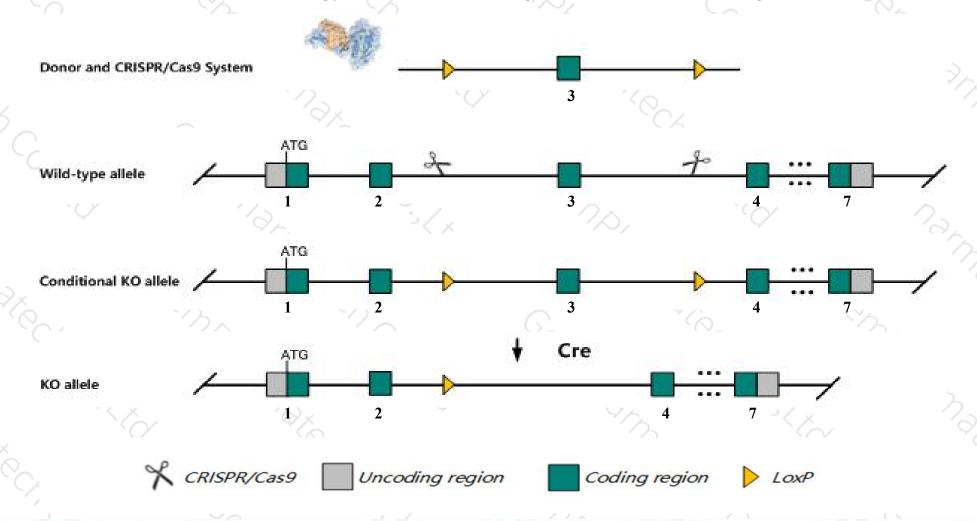
Project type Cas9-CKO

Strain background C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Hacd2* gene. The schematic diagram is as follows:



## Technical routes



- ➤ The *Hacd2* gene has 3 transcripts. According to the structure of *Hacd2* gene, exon3 of *Hacd2-201*(ENSMUST00000061156.9) transcript is recommended as the knockout region. The region contains 19bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Hacd2* 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**



- > The N-terminal of *Hacd2* gene will remain 91aa,it may remain the partial function of *Hacd2* gene.
- ➤ The floxed region is near to the N-terminal of *Gm21691* gene, this strategy may influence the regulatory function of the N-terminal of *Gm21691* gene.
- > The *Hacd2* gene is located on the Chr16. 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)



#### Hacd2 3-hydroxyacyl-CoA dehydratase 2 [ Mus musculus (house mouse) ]

Gene ID: 70757, updated on 12-Aug-2019

#### Summary

△ ?

Official Symbol Hacd2 provided by MGI

Official Full Name 3-hydroxyacyl-CoA dehydratase 2 provided by MGI

Primary source MGI:MGI:1918007

See related Ensembl: ENSMUSG00000035376

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 Hcad2; Ptplb; Al255777; Al481689; 6330408J20Rik

Expression Ubiquitous expression in genital fat pad adult (RPKM 24.6), subcutaneous fat pad adult (RPKM 18.9) and 28 other tissues See more

Orthologs human all

#### Genomic context

2

Location: 16; 16 B3

See Hacd2 in Genome Data Viewer

Exon count: 9

Annotation release	Status	Assembly	Chr	Location	
108	current	GRCm38.p6 (GCF_000001635.26)	16	NC_000082.6 (3502242135109190)	
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	16	NC_000082.5 (3502250735109261)	

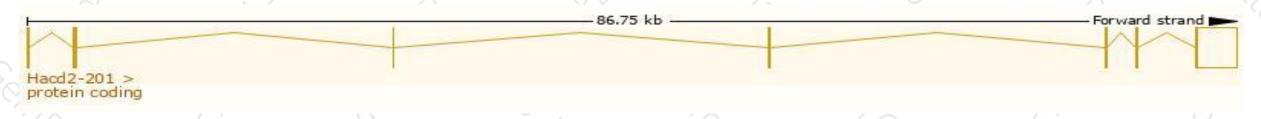
## Transcript information (Ensembl)



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

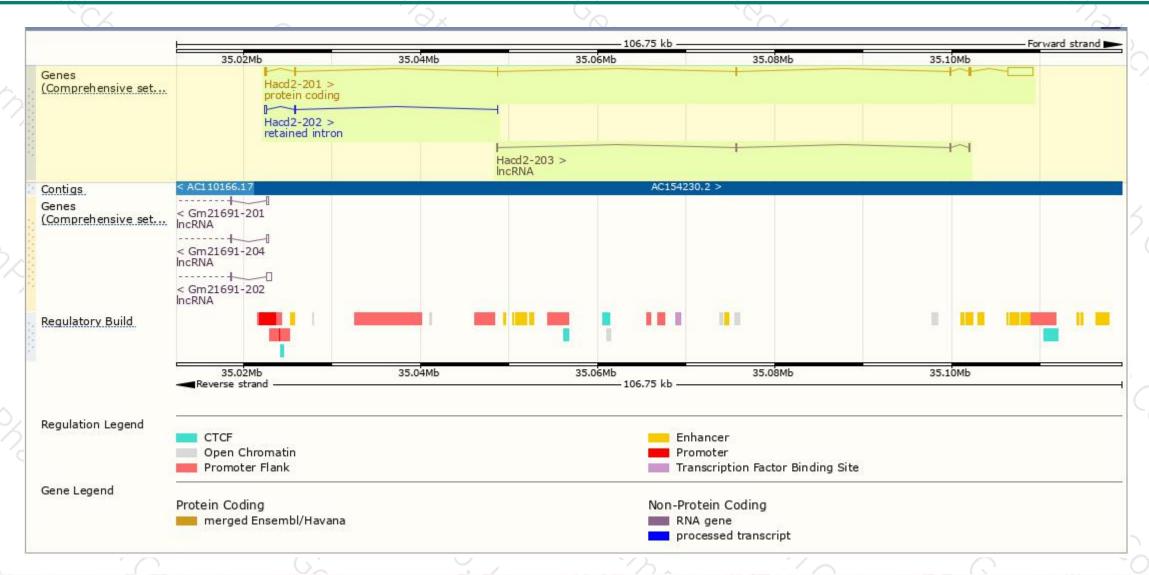
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Hacd2-201	ENSMUST00000061156.9	3598	254aa	Protein coding	CCDS37321	Q9D3B1	TSL:1 GENCODE basic APPRIS P1
Hacd2-203	ENSMUST00000231556.1	421	No protein	Processed transcript	-	-	
Hacd2-202	ENSMUST00000231516.1	389	No protein	Retained intron	ų.	2	

The strategy is based on the design of *Hacd2-201* transcript, The transcription is shown below



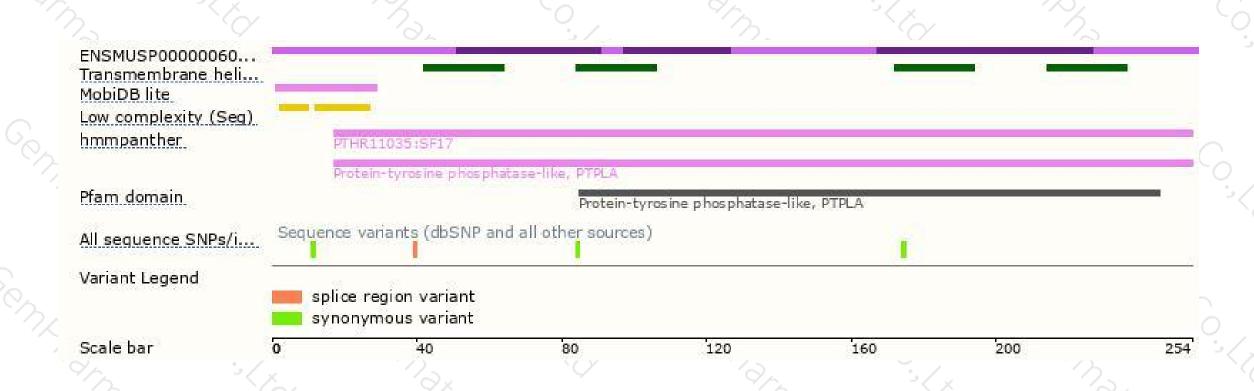
## Genomic location distribution





### Protein domain







If you have any questions, you are welcome to inquire. Tel: 400-9660890





