

# Alkbh7 Cas9-CKO Strategy

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Reviewer: Ruirui Zhang

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## **Project Overview**



Project Name

Alkbh7

Project type

Cas9-CKO

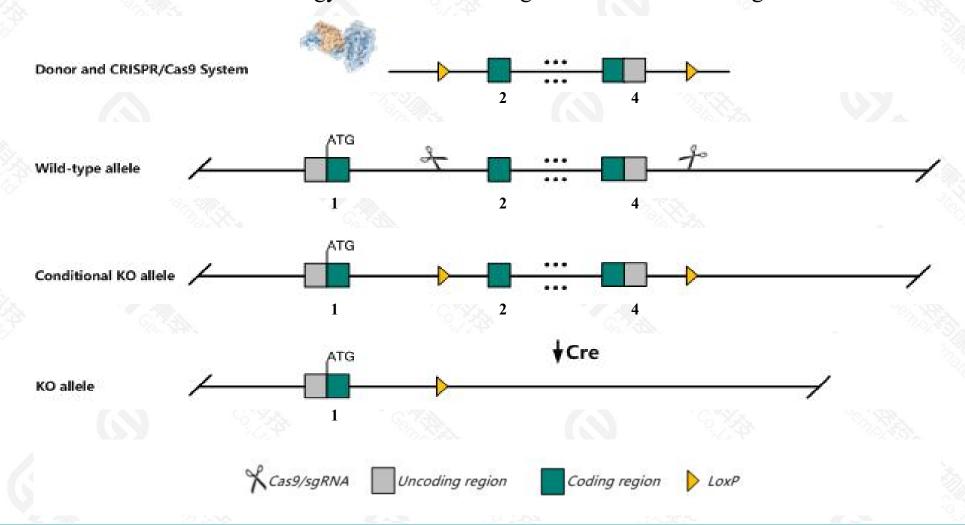
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Alkbh7* gene. The schematic diagram is as follows:



### **Technical routes**



- The *Alkbh7* gene has 3 transcripts. According to the structure of *Alkbh7* gene, exon2-exon4 of *Alkbh7*201(ENSMUST00000002737.7) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Alkbh7* 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 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, homozygous knockout affects lipid and carbohydrate metabolism which leads to obesity and increased sensitivity to diet-induced obesity. It also increases resistance to alkylation-induced cell death and mortality in males (and females for some tissues) and protects the heart from ischemia-reperfusion (IR) injury in males.
- > The KO region is close to *Clpp* and *Pspn* gene. Knockout the region may affect the function of *Clpp* and *Pspn* gene.
- > The *Alkbh7* gene is located on the Chr17. 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)



#### Alkbh7 alkB homolog 7 [ Mus musculus (house mouse) ]

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Gene ID: 66400, updated on 23-Jun-2021

Summary

Official Symbol Alkbh7 provided by MGI

Official Full Name alkB homolog 7 provided by MGI

Primary source MGI:MGI:1913650

See related Ensembl: ENSMUSG000000002661

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 Abh; Spa; Abh7; Spata11; 2310045B01Rik; 2510008E23Rik

Expression Ubiquitous expression in adrenal adult (RPKM 71.0), ovary adult (RPKM 30.9) and 28 other tissues See more

Orthologs <u>human</u> all

Try the new Gene table

Try the new <u>Transcript table</u>

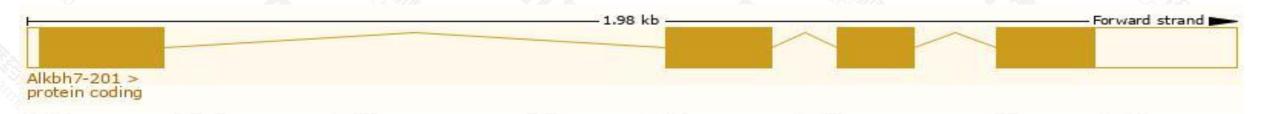
## Transcript information (Ensembl)



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

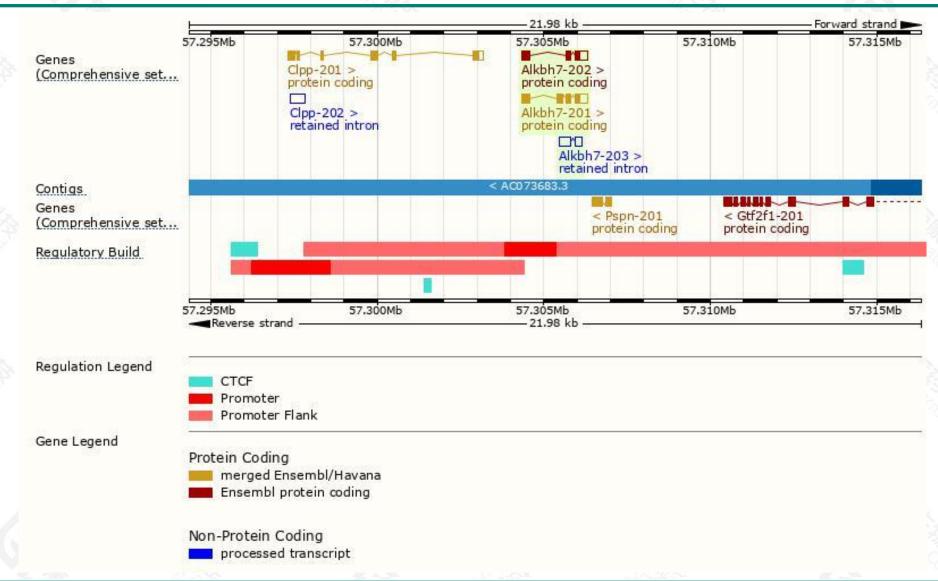
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Alkbh7-201	ENSMUST00000002737.7	917	221aa	Protein coding	CCD528921		TSL:1 , GENCODE basic , APPRIS P1 ,
Alkbh7-202	ENSMUST00000074141.14	736	<u>163aa</u>	Protein coding	CCDS57105		TSL:1 , GENCODE basic ,
Alkbh7-203	ENSMUST00000149632.2	524	No protein	Retained intron	-		TSL:2,

The strategy is based on the design of *Alkbh7-201* transcript, the transcription is shown below:



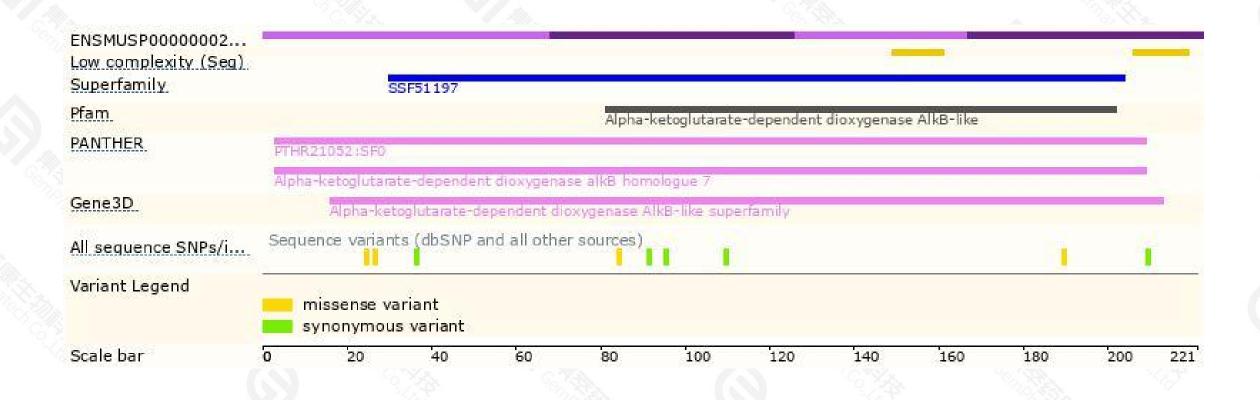
### Genomic location distribution





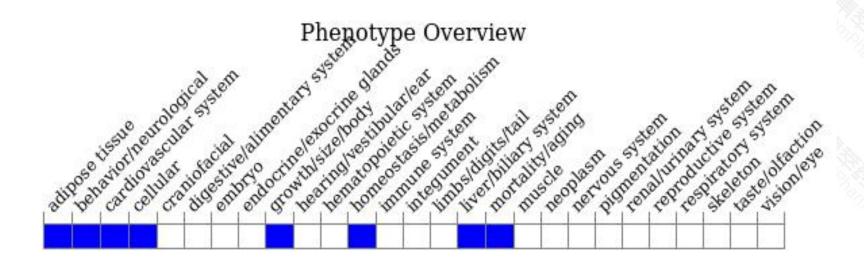
### Protein domain





## 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, homozygous knockout affects lipid and carbohydrate metabolism which leads to obesity and increased sensitivity to diet-induced obesity. It also increases resistance to alkylation-induced cell death and mortality in males (and females for some tissues) and protects the heart from ischemia-reperfusion (IR) injury in males.



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

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