

# Alkbh5 Cas9-KO Strategy

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# **Project Overview**



Project Name Alkbh5

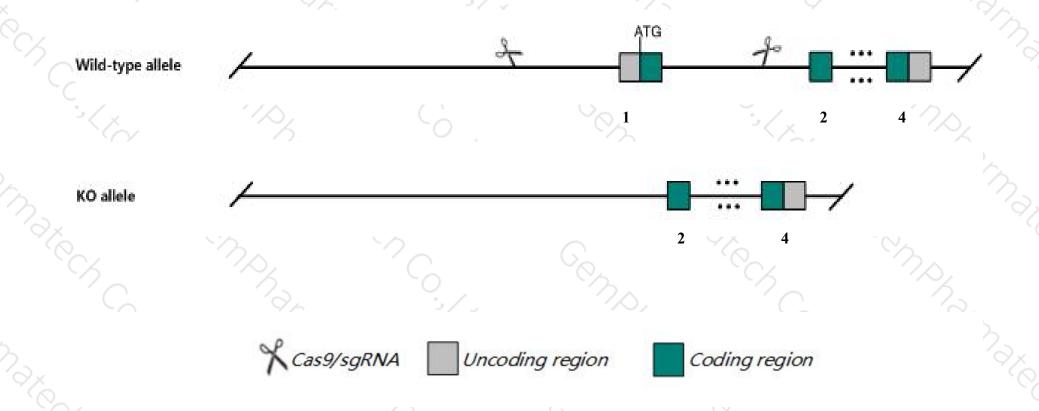
Project type Cas9-KO

Strain background C57BL/6J

# **Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the Alkbh5 gene. The schematic diagram is as follows:



## **Technical routes**



- ➤ The *Alkbh5* gene has 2 transcripts. According to the structure of *Alkbh5* gene, exon1 of *Alkbh5-201* (ENSMUST00000044250.3) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Alkbh5* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.

### **Notice**



- ➤ According to the existing MGI data, Mice homozygous for a knock-out allele exhibit reduced male fertility associated with oligo- and teratozoopermia and male germ cell apoptosis.
- The *Alkbh5* gene is located on the Chr11. 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)



#### Alkbh5 alkB homolog 5, RNA demethylase [Mus musculus (house mouse)]

Gene ID: 268420, updated on 2-Mar-2019

#### Summary

☆ ?

Official Symbol Alkbh5 provided by MGI

Official Full Name alkB homolog 5, RNA demethylase provided by MGI

Primary source MGI:MGI:2144489

See related Ensembl:ENSMUSG00000042650

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 AW050020, AW215868, Abh5, E130207K11, Ofoxd

Expression Ubiquitous expression in adrenal adult (RPKM 20.2), ovary adult (RPKM 17.5) and 28 other tissuesSee more

Orthologs <u>human</u> all

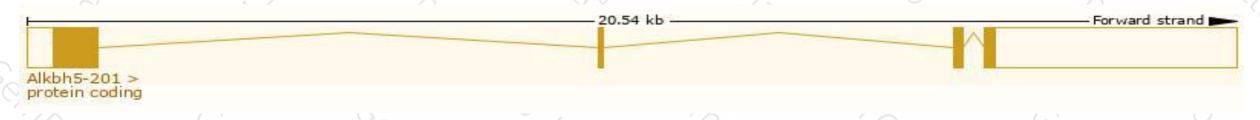
# Transcript information (Ensembl)



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

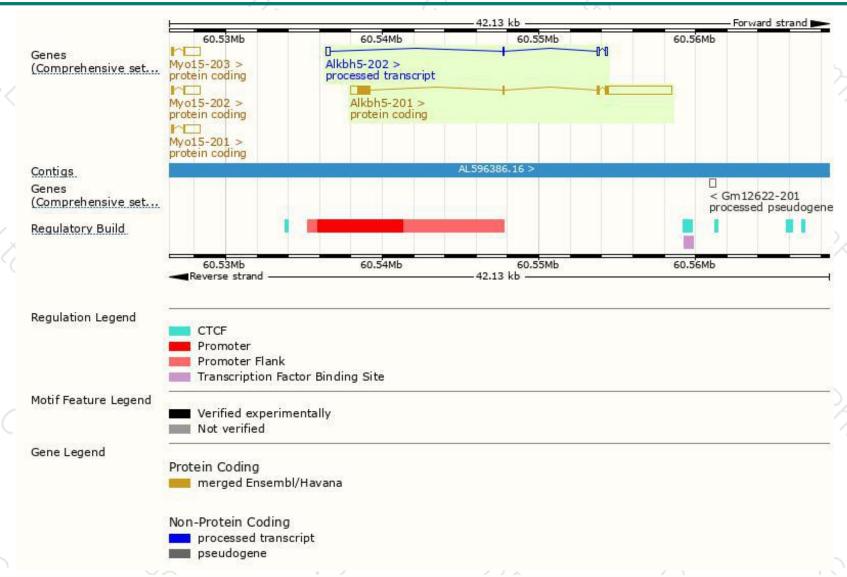
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Alkbh5-201	ENSMUST00000044250.3	5730	395aa	Protein coding	CCDS24794	Q3TSG4	TSL:1 GENCODE basic APPRIS P1
Alkbh5-202	ENSMUST00000134770.1	624	No protein	Processed transcript	<del>-</del> 85	-	TSL:3

The strategy is based on the design of Alkbh5-201 transcript, The transcription is shown below



## Genomic location distribution





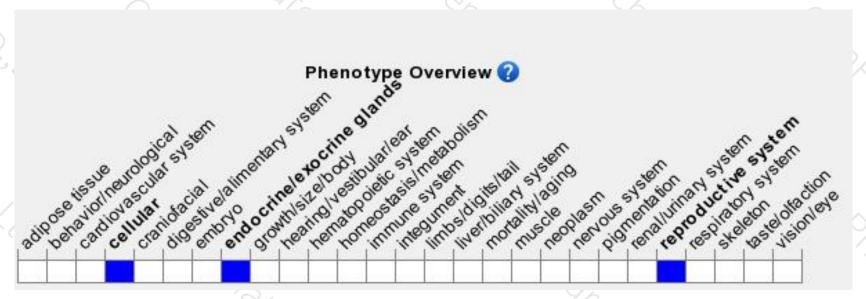
## Protein domain





## 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 a knock-out allele exhibit reduced male fertility associated with oligo- and teratozoopermia and male germ cell apoptosis.



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

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