

# Alkbh1 Cas9-CKO Strategy

Designer: Jinling Wang

**Design Date:** 2019-7-19

# **Project Overview**



**Project Name** 

Alkbh1

**Project type** 

Cas9-CKO

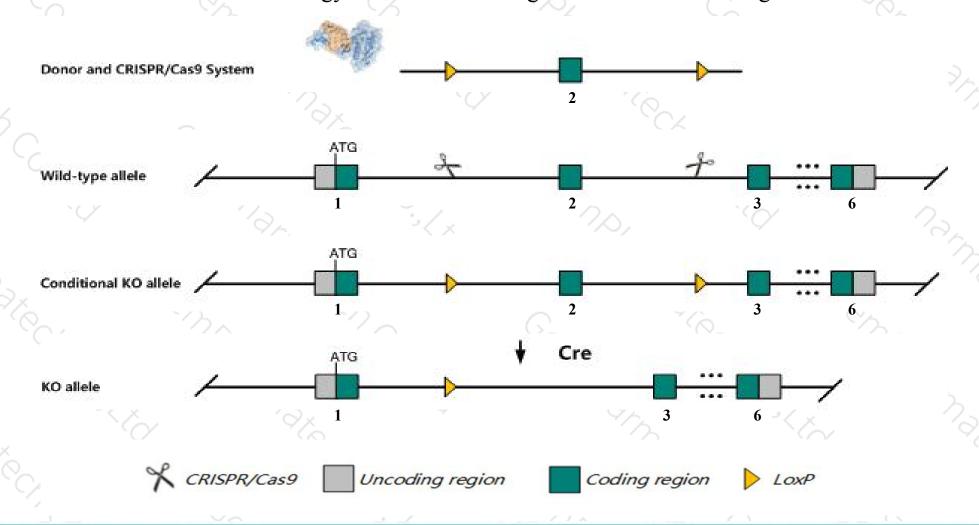
Strain background

C57BL/6JGpt

# Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Alkbh1* gene. The schematic diagram is as follows:



### Technical routes



- ➤ The *Alkbh1* gene has 12 transcripts. According to the structure of *Alkbh1* gene, exon2 of *Alkbh1-209*(ENSMUST00000162961.7) transcript is recommended as the knockout region. The region contains 109bp coding sequence.

  Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Alkbh1* 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, Mice homozygous for a null allele show delayed fetal growth, impaired placental development and low birth weight. Mice homozygous for another null allele show sex-ratio distortion, reduced survival, spermatogenic defects and incompletely penetrant eye, neural tube, skeleton and craniofacial defects.
- The *Alkbh1* gene is located on the Chr12. 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)



#### Alkbh1 alkB homolog 1, histone H2A dioxygenase [Mus musculus (house mouse)]

Gene ID: 211064, updated on 19-Mar-2019

#### Summary

☆ ?

Official Symbol Alkbh1 provided by MGI

Official Full Name alkB homolog 1, histone H2A dioxygenase provided by MGI

Primary source MGI:MGI:2384034

See related Ensembl: ENSMUSG00000079036

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 2700073G19Rik, Abh, Alkbh, alkB, hABH

Expression Ubiquitous expression in limb E14.5 (RPKM 17.2), CNS E11.5 (RPKM 14.3) and 28 other tissuesSee more

Orthologs <u>human</u> all

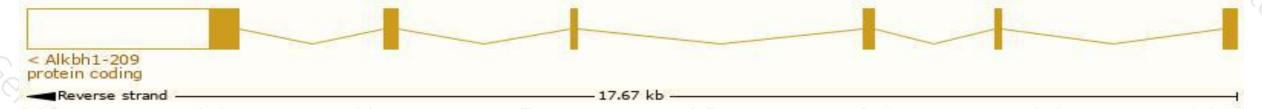
# Transcript information (Ensembl)



The gene has 12 transcripts, all transcripts are shown below:

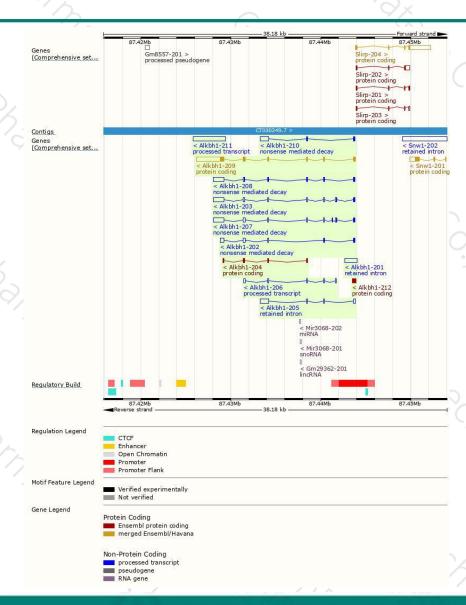
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Alkbh1-209	ENSMUST00000162961.7	3857	389aa	Protein coding	CCDS49120	P0CB42	TSL:1 GENCODE basic APPRIS P1
Alkbh1-204	ENSMUST00000160687.1	541	<u>180aa</u>	Protein coding	-8	F6U5G1	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Alkbh1-212	ENSMUST00000185301.1	408	<u>135aa</u>	Protein coding	2	Q5XLJ1	TSL:NA GENCODE basic
Alkbh1-207	ENSMUST00000161712.7	2154	<u>68aa</u>	Nonsense mediated decay	24	E0CX46	TSL:1
Alkbh1-203	ENSMUST00000160113.7	2019	<u>68aa</u>	Nonsense mediated decay	-	E0CX46	TSL:1
Alkbh1-208	ENSMUST00000162247.7	1883	200aa	Nonsense mediated decay	-8	E0CYA7	TSL:1
Alkbh1-210	ENSMUST00000162986.1	1319	<u>78aa</u>	Nonsense mediated decay	-	E0CZH7	TSL:3
Alkbh1-202	ENSMUST00000159079.1	1042	<u>101aa</u>	Nonsense mediated decay	24	E0CY72	TSL:5
Alkbh1-211	ENSMUST00000176758.2	3598	No protein	Processed transcript	-	-	TSL:NA
Alkbh1-206	ENSMUST00000160919.7	611	No protein	Processed transcript	-8	8-	TSL:3
Alkbh1-205	ENSMUST00000160850.1	1423	No protein	Retained intron	2/	S2	TSL:1
Alkbh1-201	ENSMUST00000091090.4	1335	No protein	Retained intron	2	92	TSL:NA

The strategy is based on the design of Alkbh1-209 transcript, The transcription is shown below



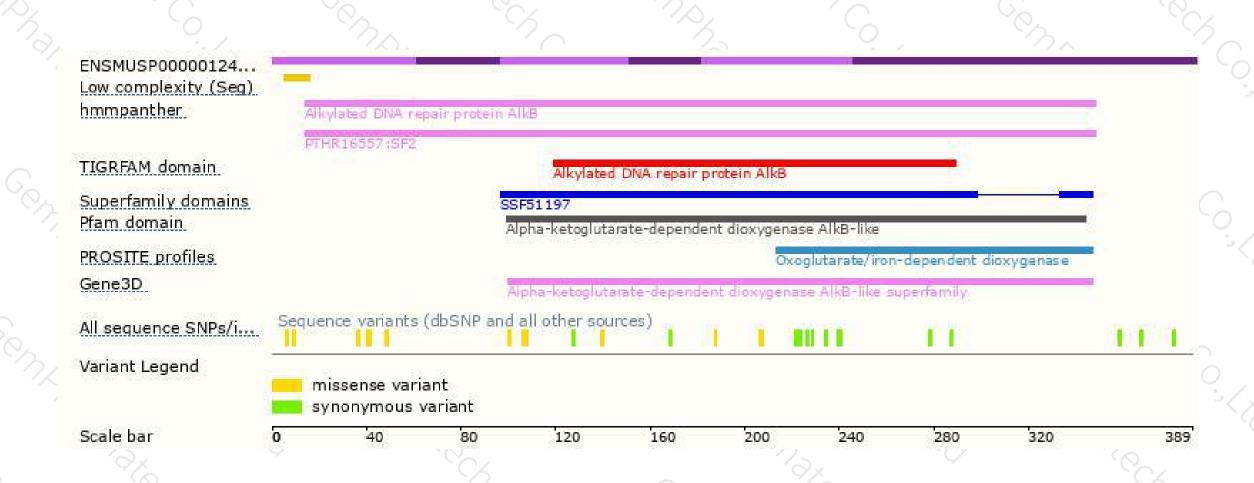
### Genomic location distribution





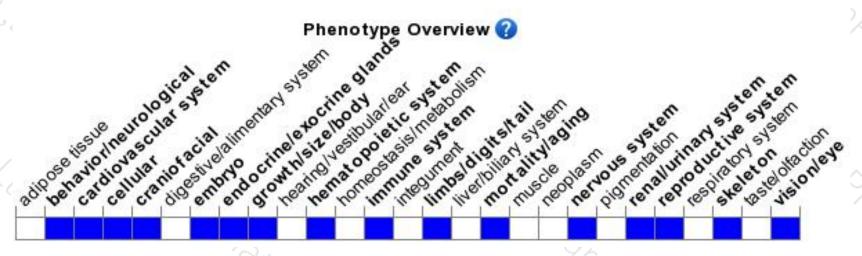
### Protein domain





# 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 null allele show delayed fetal growth, impaired placental development and low birth weight. Mice homozygous for another null allele show sex-ratio distortion, reduced survival, spermatogenic defects and incompletely penetrant eye, neural tube, skeleton and craniofacial defects.



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





