GemPharmatech™

Kdm6b Cas9-CKO Strategy

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Reviewer: Jia Yu

Design Date: 2023-07-25

Overview

Target Gene Name

- Kdm6b

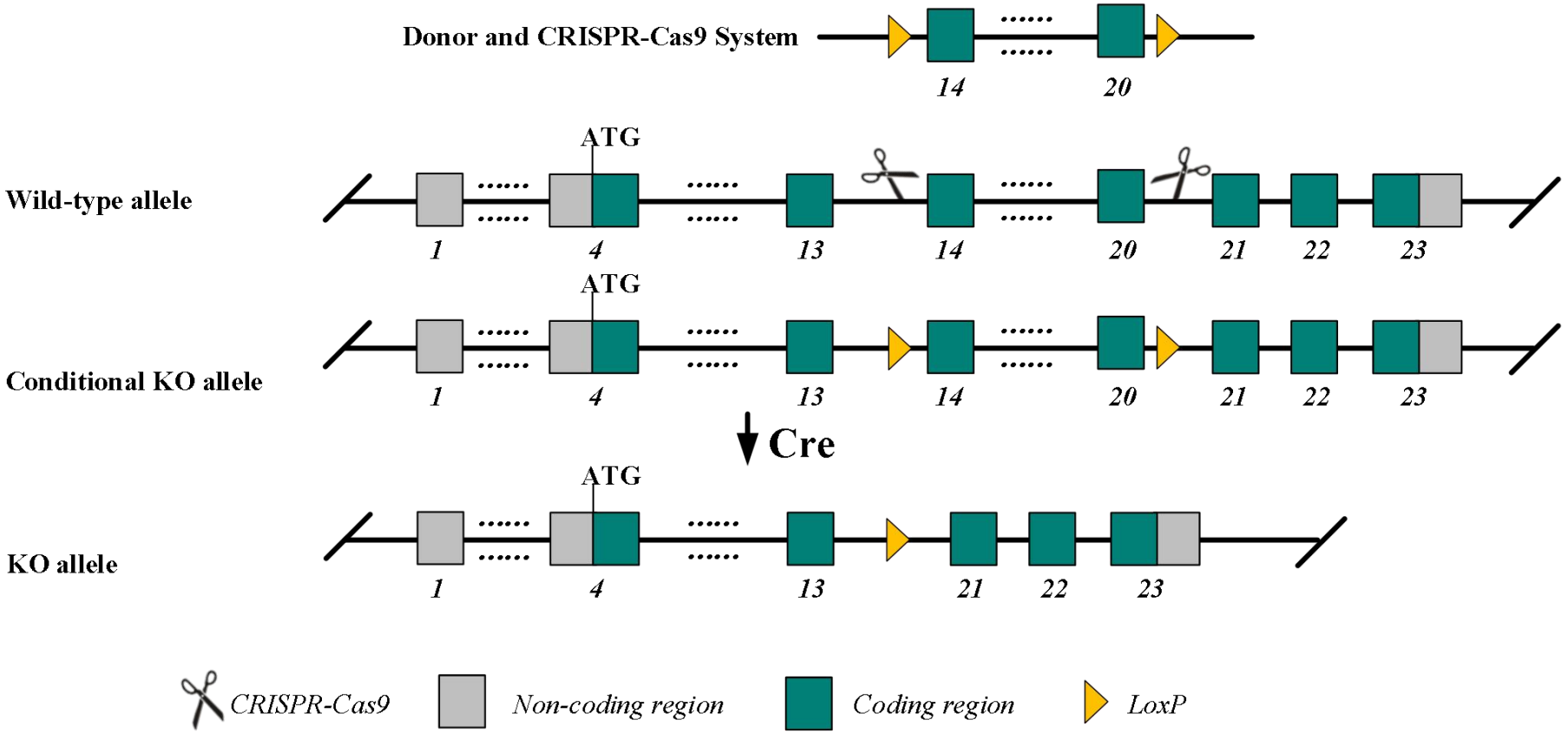
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Kdm6b* gene.

Technical Information

- The *Kdm6b* gene has 2 transcripts. According to the structure of *Kdm6b* gene, exon14-20 of *Kdm6b*-201 (ENSMUST00000094077.5) transcript is recommended as the knockout region. The region contains 937bp of coding sequences. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Kdm6b* 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- 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.

Gene Information

Kdm6b KDM1 lysine (K)-specific demethylase 6B [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 216850, updated on 21-Jun-2023

Summary

Official Symbol	Kdm6b provided by MGI
Official Full Name	KDM1 lysine (K)-specific demethylase 6B provided by MGI
Primary source	MGI:MGI:2448492
See related	Ensembl:ENSMUSG00000018476 AllianceGenome:MGI:2448492
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	Jmjd3; 1700064E03Rik
Summary	Enables beta-catenin binding activity; histone H3-tri/di-methyl-lysine-27 demethylase activity; and sequence-specific DNA binding activity. Involved in several processes, including histone H3-K27 demethylation; mesodermal cell differentiation; and positive regulation of cold-induced thermogenesis. Acts upstream of or within several processes, including cellular response to hydrogen peroxide; histone demethylation; and positive regulation of transcription by RNA polymerase II. Located in nucleus. Is expressed in several structures, including brain; gut; liver; metanephros; and olfactory epithelium. Orthologous to human KDM6B (lysine demethylase 6B). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Ubiquitous expression in thymus adult (RPKM 16.9), duodenum adult (RPKM 10.2) and 25 other tissues See more
Orthologs	human all

NEW **Try the new Gene table**
Try the new Transcript table

Genomic context

Location: 11; 11 B3 — See Kdm6b in Genome Data Viewer

Exon count: 32

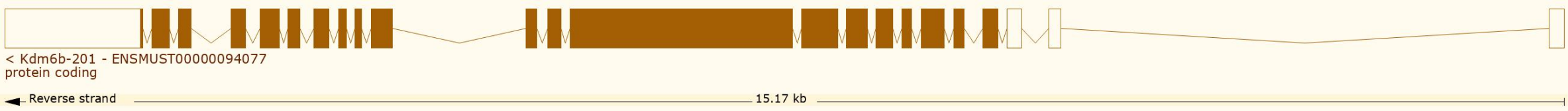
ılm.nih.gov/

Transcript Information

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

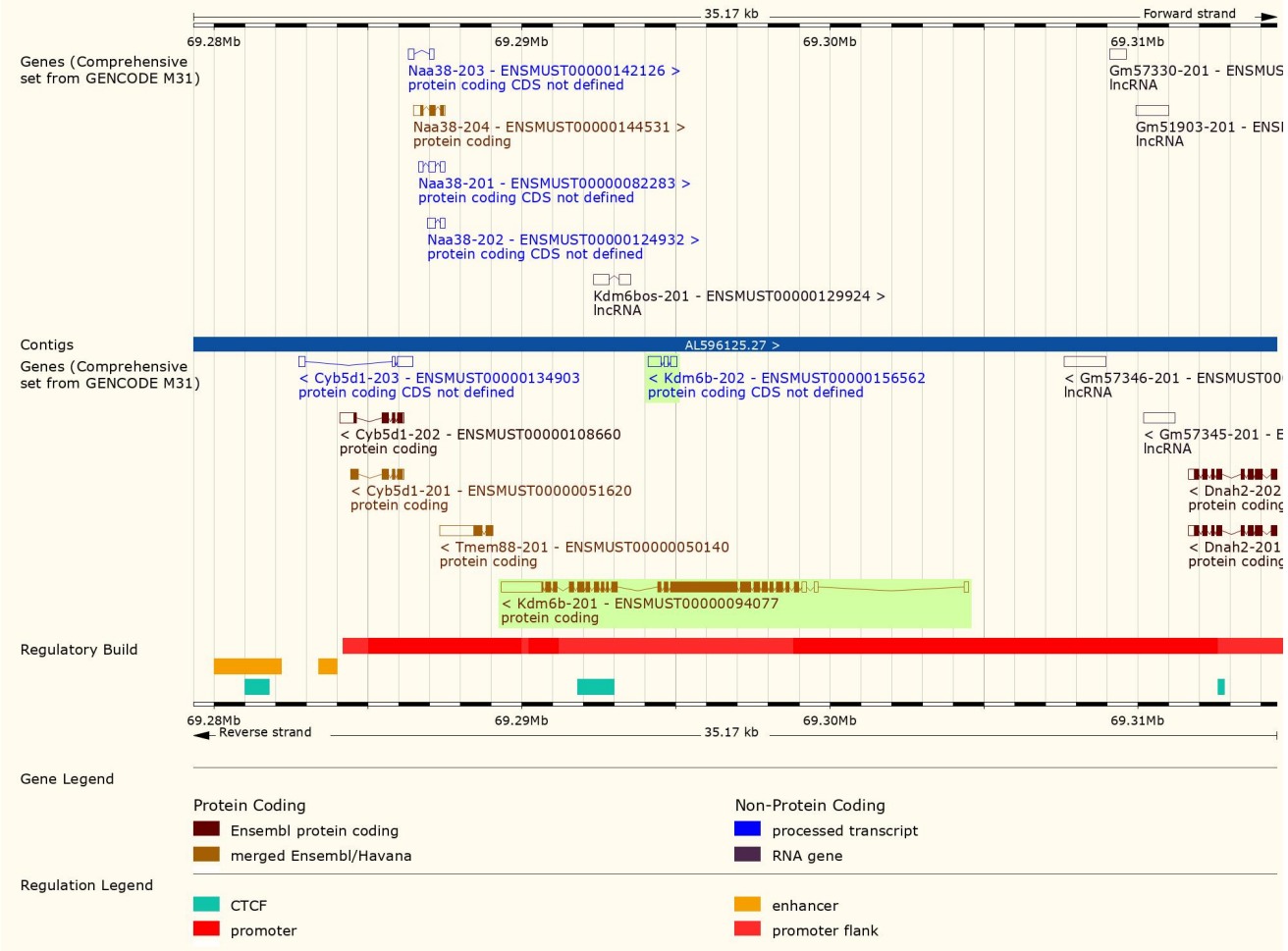
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000094077.5	Kdm6b-201	6654	1641aa	Protein coding	CCDS24895	Q5NCY0	Ensembl Canonical, GENCODE basic, APPRIS P1, TSL:5
ENSMUST00000156562.2	Kdm6b-202	743	No protein	Protein coding CDS not defined		-	TSL:2

The strategy is based on the design of *Kdm6b*-201 transcript, the transcription is shown below:



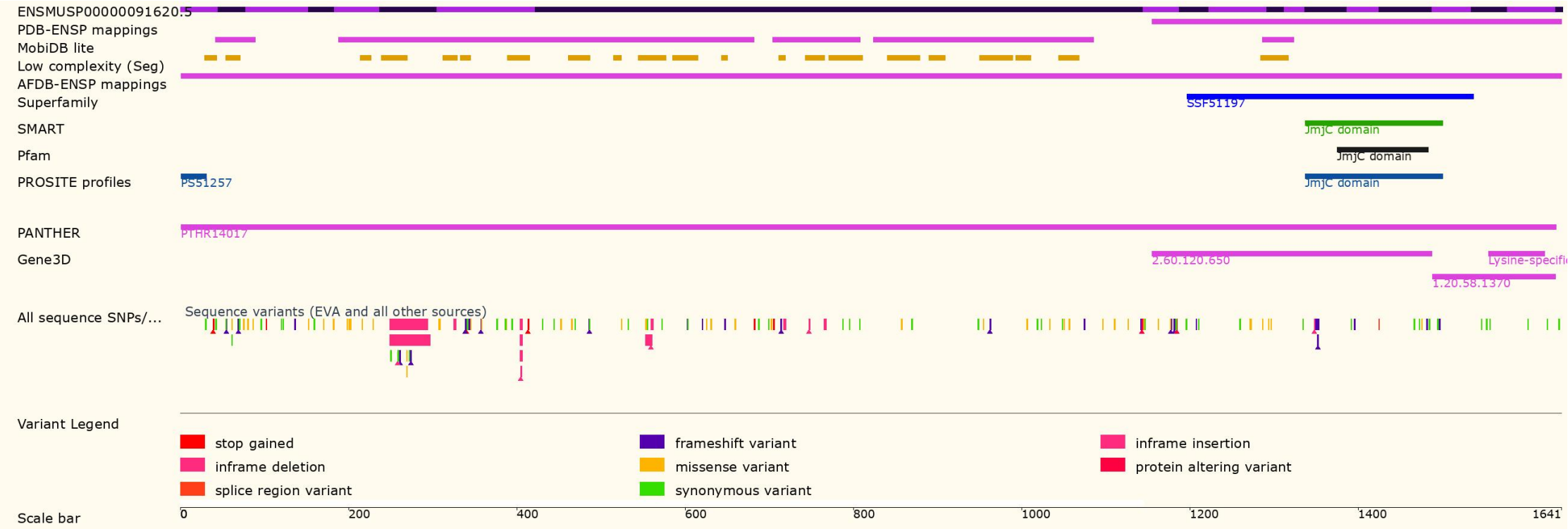
Source: https://www.ensembl.org

Genomic Information



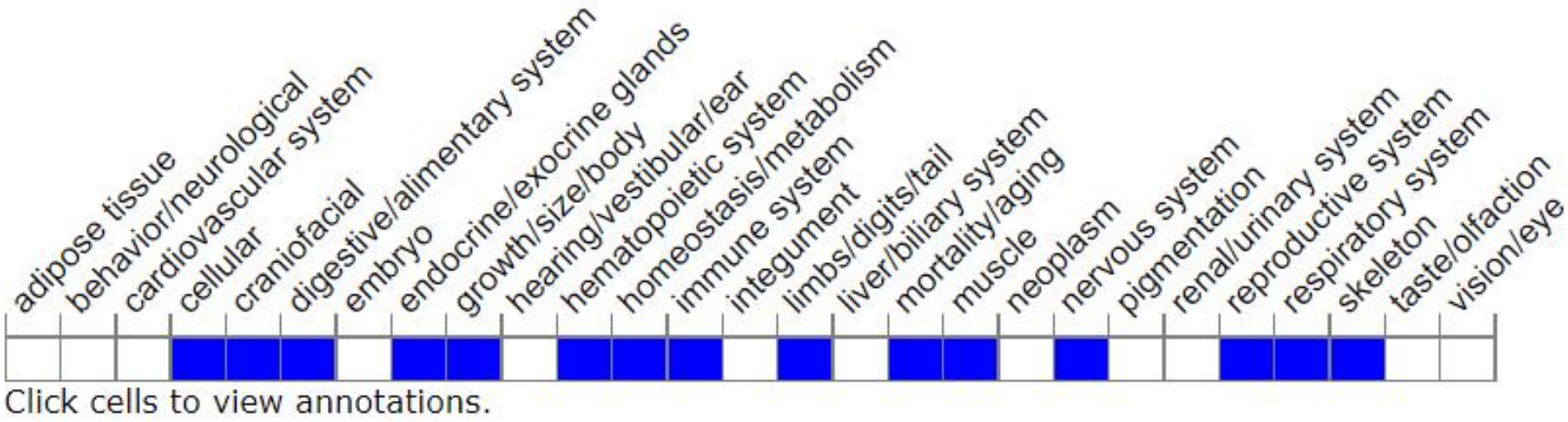
Source: : https://www.ensembl.org

Protein Information



Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



- Mice homozygous for a null allele show perinatal death, thick alveolar septum, and absence of air space in the lungs. Mice homozygous for a different null allele die neonatally displaying abnormal lung development, dwarfism, kyphosis, short limbs, and a severe delay in endochondral ossification.

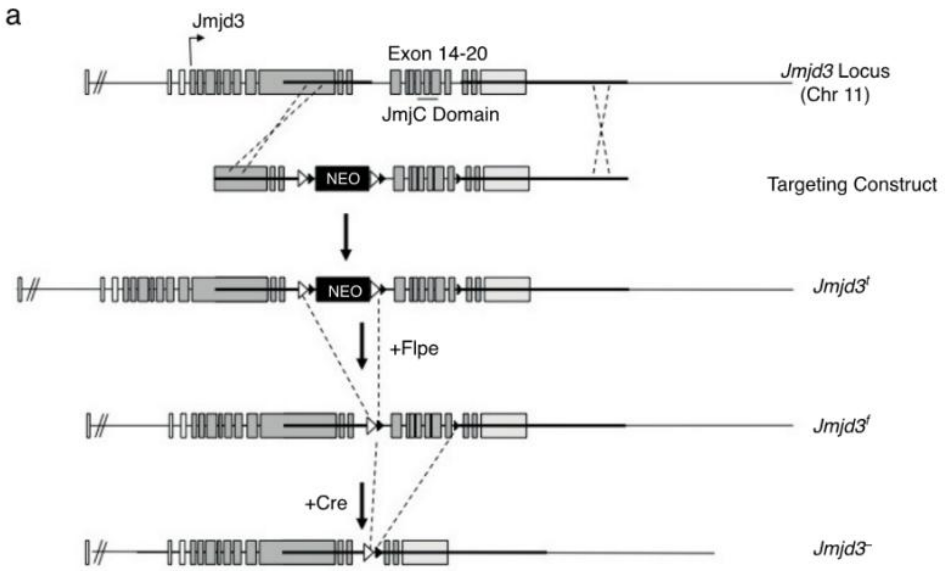
Source: https://www.informatics.jax.org

Important Information

- According theMGI data, Mice homozygous for a null allele show perinatal death, thick alveolar septum, and absence of air space in the lungs. Mice homozygous for a different null allele die neonatally displaying abnormal lung development, dwarfism, kyphosis, short limbs, and a severe delay in endochondral ossification.
- The effect of *Tmem88*-201, *Cyb5d1* and *Kdm6bos*-201 is unknown.
- The most protein will be remained.
- Intron 20-21(384 bp), the effect is unknown.
- *Kdm6b* is located on Chr11. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is 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 the existing technology level.

Reference

Supplementary Figures



Targeting strategy for the generation of conditional Jmjd3- or Utx conditional mice.

(**a, b**) Structure of wild-type *Jmjd3* (**a**) or *Utx* (**b**) loci and targeting vectors. The targeting vector for each gene contains an inserted neomycin (Neo) cassette flanked by Frt (white arrowheads), and three LoxP sites (black arrowheads) flanking the Neo cassette and exon(s) encoding the catalytic JmjC domain for each enzyme. Homologous recombination in embryonic stem cells results in the targeted allele (*Jmjd3*^{t} or *Utx*^{t}).

reference:Histone H3 Lysine 27 demethylases Jmjd3 and Utx are required for T-cell differentiation.