

# ***Kdm4b Cas9-CKO Strategy***

|                     |                   |
|---------------------|-------------------|
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| <b>Reviewer</b>     | <b>Huan Fan</b>   |
| <b>Design Date:</b> | <b>2019-11-12</b> |

# Project Overview

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**Project Name**

***Kdm4b***

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**Project type**

**Cas9-CKO**

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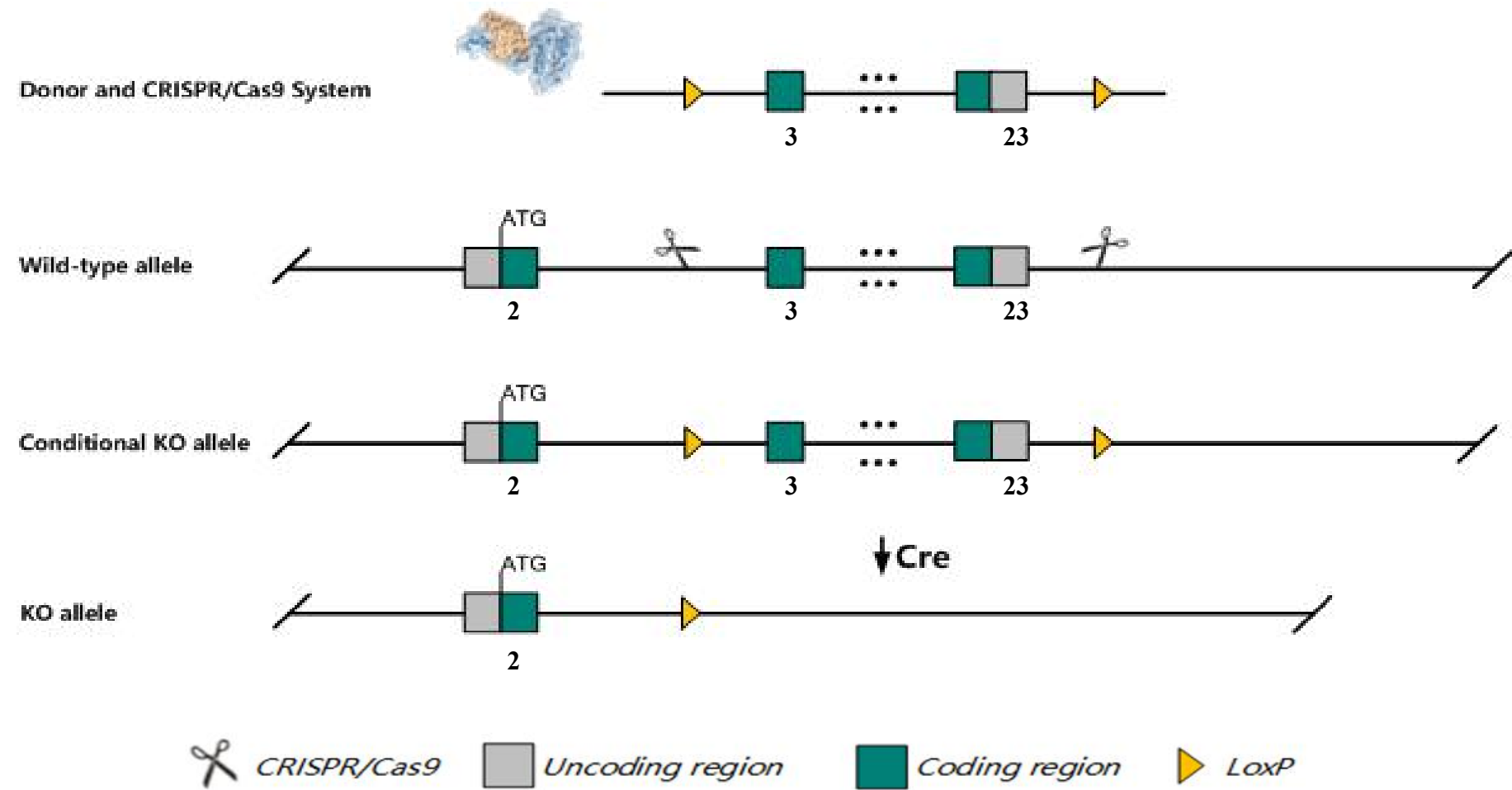
**Strain background**

**C57BL/6JGpt**

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# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Kdm4b* gene. The schematic diagram is as follows:



The *Kdm4b* gene has 6 transcripts. According to the structure of *Kdm4b* gene, exon3-exon23 of *Kdm4b-201* (ENSMUST00000025036.10) 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 *Kdm4b* 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.

According to the existing MGI data, Mice homozygous for a targeted allele lacking demethylase activity exhibit no gross abnormalities. Mice homozygous for a conditional allele activated in mammary gland epithelial cells exhibit delayed mammary gland development with reduced branching.

The *Kdm4b* 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.

**Kdm4b lysine (K)-specific demethylase 4B [Mus musculus (house mouse)]**

Gene ID: 193796, updated on 24-Feb-2019

**Summary**

|                           |   |
|---------------------------|---|
| <b>Official Symbol</b>    | Kdm4b provided by <a href="#">MGI</a>   |
| <b>Official Full Name</b> | lysine (K)-specific demethylase 4B provided by <a href="#">MGI</a>  |
| <b>Primary source</b>     | <a href="#">MGI:MGI:2442355</a>   |
| <b>See related</b>        | <a href="#">Ensembl:ENSMUSG00000024201</a>  |
| <b>Gene type</b>          | protein coding  |
| <b>RefSeq status</b>      | VALIDATED   |
| <b>Organism</b>           | <a href="#">Mus musculus</a>  |
| <b>Lineage</b>            | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| <b>Also known as</b>      | 4732474L06Rik, Jmjd2b   |
| <b>Expression</b>         | Ubiquitous expression in thymus adult (RPKM 19.4), ovary adult (RPKM 19.2) and 28 other tissues <a href="#">See more</a>  |
| <b>Orthologs</b>          | <a href="#">human</a> <a href="#">all</a>   |

# Transcript information      Ensembl

The gene has 6 transcripts,all transcripts are shown below:

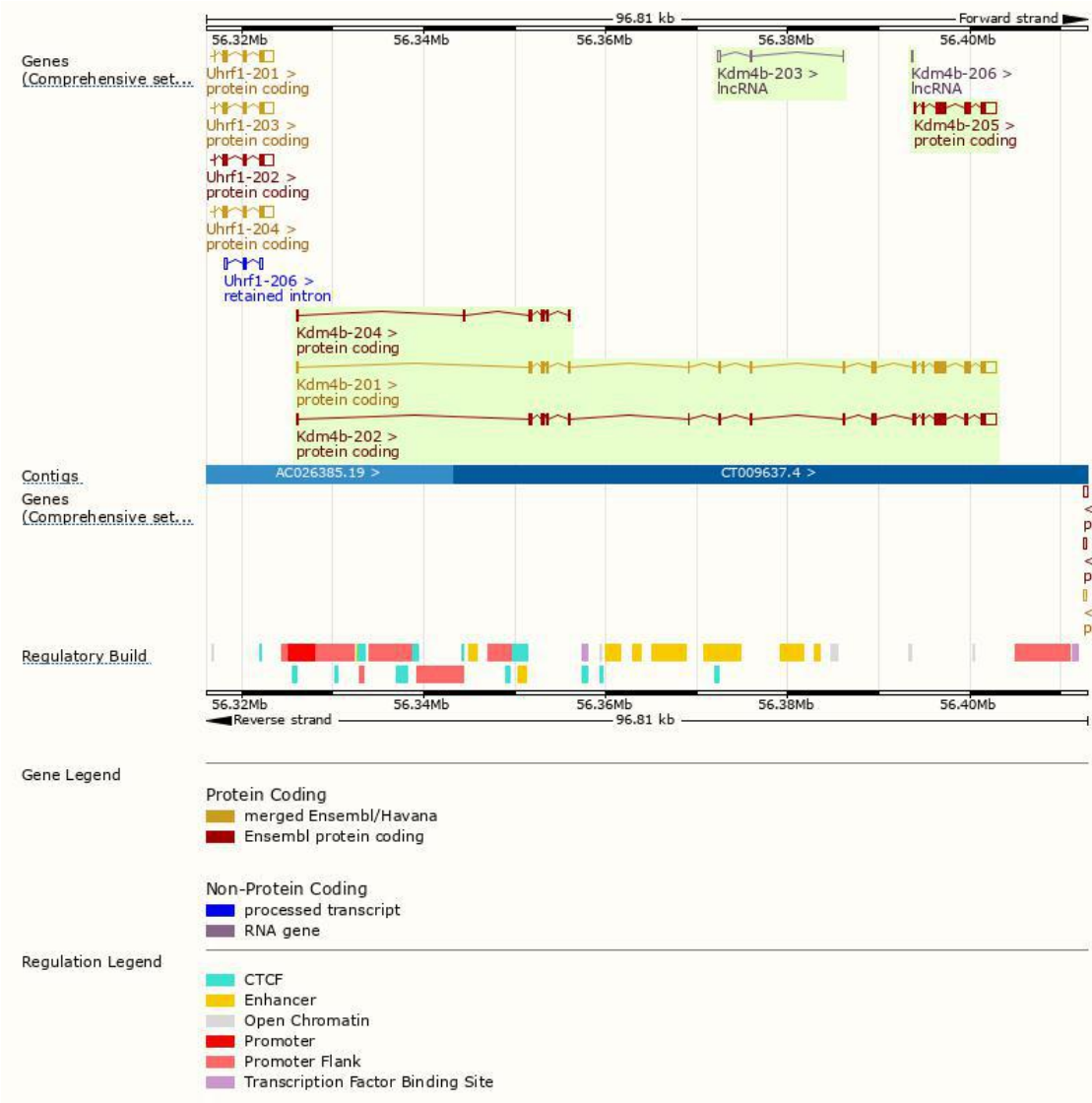
| Name      | Transcript ID                         | bp   | Protein                | Biotype        | CCDS                      | UniProt                | Flags                           |
|-----------|---------------------------------------|------|------------------------|----------------|---------------------------|------------------------|---------------------------------|
| Kdm4b-201 | <a href="#">ENSMUST00000025036.10</a> | 4577 | <a href="#">1086aa</a> | Protein coding | <a href="#">CCDS28904</a> | <a href="#">Q91VY5</a> | TSL:1 GENCODE basic APPRIS P2   |
| Kdm4b-202 | <a href="#">ENSMUST00000086835.11</a> | 4360 | <a href="#">1021aa</a> | Protein coding | -                         | <a href="#">B0V2M9</a> | TSL:5 GENCODE basic APPRIS ALT2 |
| Kdm4b-205 | <a href="#">ENSMUST00000141507.1</a>  | 2775 | <a href="#">544aa</a>  | Protein coding | -                         | <a href="#">F7BTY2</a> | CDS 5' incomplete TSL:1         |
| Kdm4b-204 | <a href="#">ENSMUST00000139679.7</a>  | 927  | <a href="#">208aa</a>  | Protein coding | -                         | <a href="#">B0V2M7</a> | CDS 3' incomplete TSL:3         |
| Kdm4b-203 | <a href="#">ENSMUST00000132363.1</a>  | 408  | No protein             | lncRNA         | -                         | -                      | TSL:3                           |
| Kdm4b-206 | <a href="#">ENSMUST00000147489.1</a>  | 130  | No protein             | lncRNA         | -                         | -                      | TSL:3                           |

The strategy is based on the design of *Kdm4b-201* transcript,The transcription is shown below



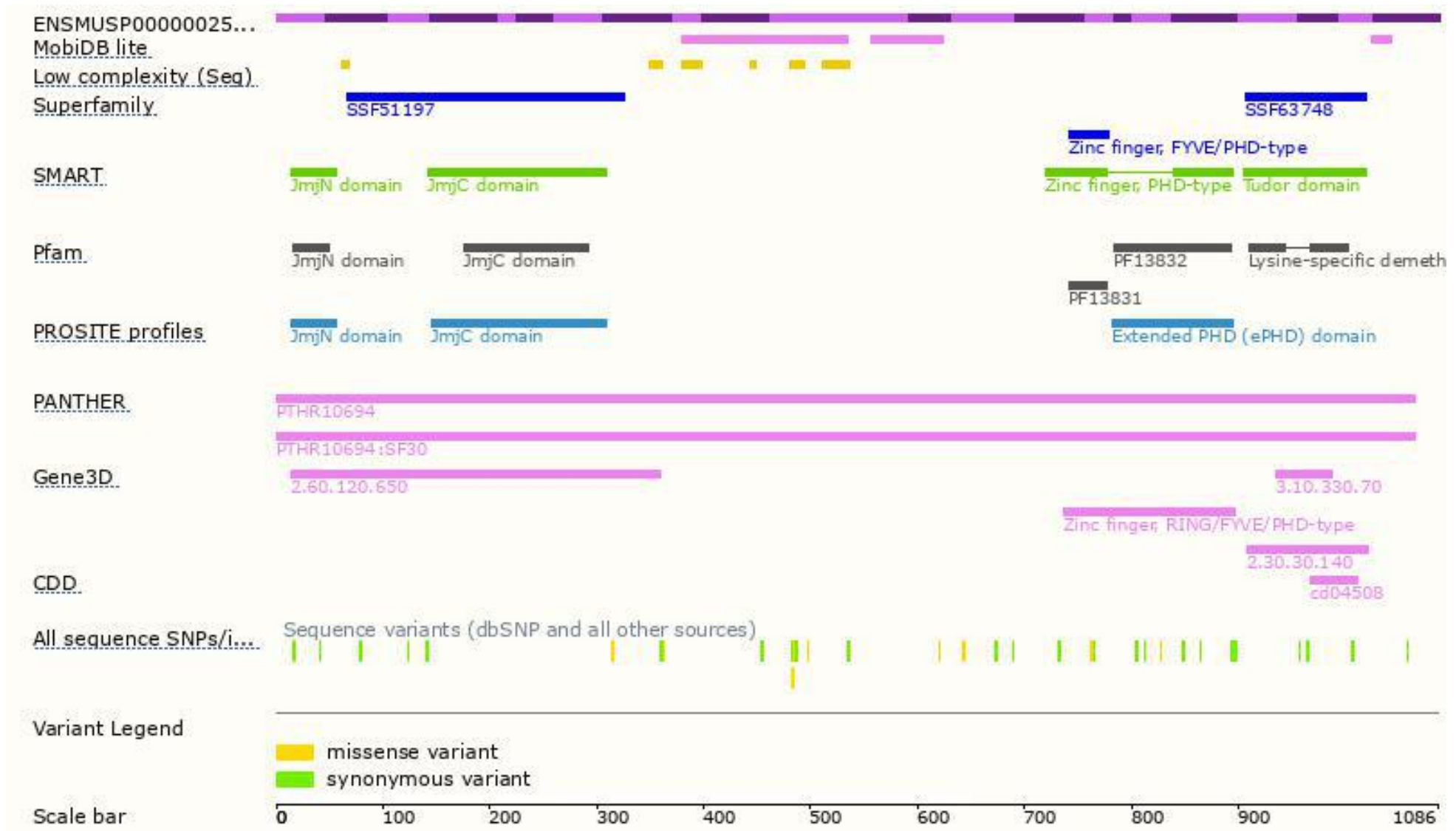


# Genomic location distribution





# 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 targeted allele lacking demethylase activity exhibit no gross abnormalities. Mice homozygous for a conditional allele activated in mammary gland epithelial cells exhibit delayed mammary gland development with reduced branching.

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