

Kdm3a Cas9-CKO Strategy

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Design Date: 2019-9-11

Reviewer: JiaYu

Project Overview



Project Name

Kdm3a

Project type

Cas9-CKO

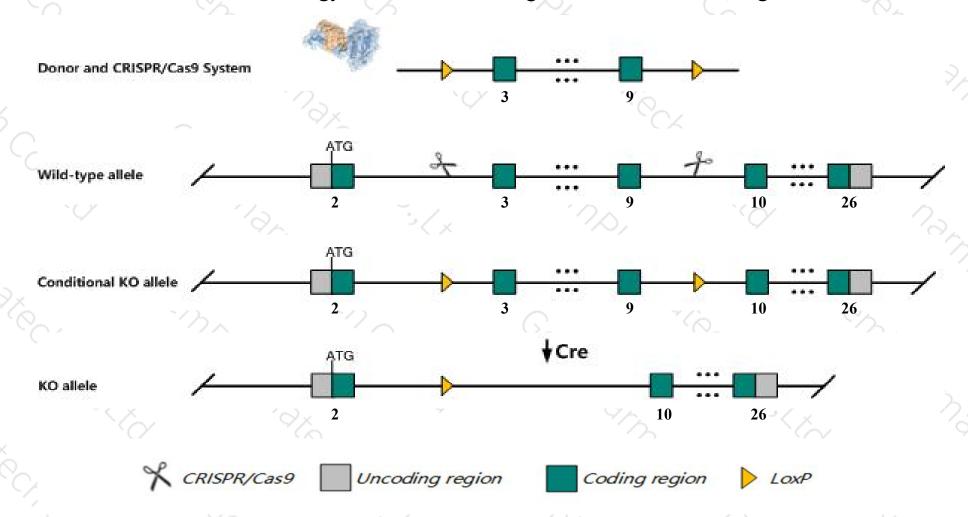
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Kdm3a* gene. The schematic diagram is as follows:



Technical routes



- The *Kdm3a* gene has 14 transcripts. According to the structure of *Kdm3a* gene, exon3-exon9 of *Kdm3a-202* (ENSMUST00000167220.3) transcript is recommended as the knockout region. The region contains 821bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Kdm3a* 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, Male mice homozygous for a hypomorphic allele display infertility, oligoasthenoteratozoospermia, small testis, and impaired spermiogenesis. Mice homozygous for a null allele exhibit abnormal spermatogenesis and obesity associated with hyperlipidemia.
- > The *Kdm3a* gene is located on the Chr6. 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)



Kdm3a lysine (K)-specific demethylase 3A [Mus musculus (house mouse)]

Gene ID: 104263, updated on 19-Feb-2019

Summary

☆ ?

Official Symbol Kdm3a provided by MGI

Official Full Name lysine (K)-specific demethylase 3A provided byMGI

Primary source MGI:MGI:98847

See related Ensembl: ENSMUSG00000053470

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 1700105C21Rik, C230043E16Rik, JHDM2a, Jmjd1, Jmjd1a, KDM2A, TGSA, Tsga

Expression Ubiquitous expression in CNS E11.5 (RPKM 18.6), testis adult (RPKM 15.8) and 25 other tissuesSee more

Orthologs <u>human all</u>

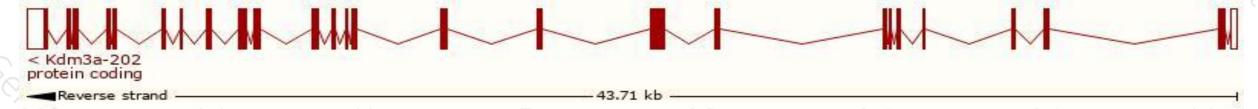
Transcript information (Ensembl)



The gene has 14 transcripts, all transcripts are shown below:

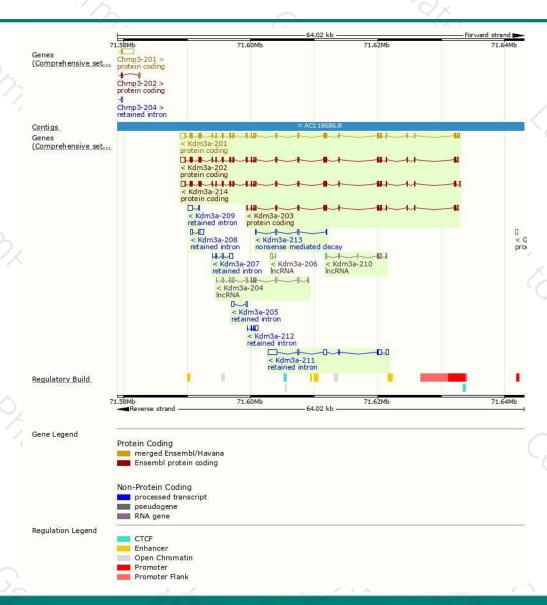
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Kdm3a-202	ENSMUST00000167220.3	4853	<u>1323aa</u>	Protein coding	CCDS20233	Q6PCM1	TSL:1 GENCODE basic APPRIS P1
Kdm3a-201	ENSMUST00000065509.10	4816	<u>1323aa</u>	Protein coding	CCDS20233	Q6PCM1	TSL:1 GENCODE basic APPRIS P1
Kdm3a-214	ENSMUST00000207023.1	4753	<u>1323aa</u>	Protein coding	CCDS20233	Q6PCM1	TSL:5 GENCODE basic APPRIS P1
Kdm3a-203	ENSMUST00000205289.1	2723	<u>834aa</u>	Protein coding	-	A0A0U1RNV6	CDS 3' incomplete TSL:1
Kdm3a-213	ENSMUST00000206916.1	679	<u>43aa</u>	Nonsense mediated decay		A0A0U1RPI3	CDS 5' incomplete TSL:3
Kdm3a-204	ENSMUST00000205470.1	1718	No protein	Processed transcript	į.	· -	TSL:5
Kdm3a-210	ENSMUST00000206597.1	828	No protein	Processed transcript		120	TSL:3
Kdm3a-206	ENSMUST00000206050.1	341	No protein	Processed transcript	-	750	TSL:3
Kdm3a-211	ENSMUST00000206704.1	3137	No protein	Retained intron		181	TSL:1
Kdm3a-208	ENSMUST00000206357.1	917	No protein	Retained intron	·) 4)	TSL:2
Kdm3a-207	ENSMUST00000206339.1	815	No protein	Retained intron	· ·	120	TSL:2
Kdm3a-209	ENSMUST00000206582.1	785	No protein	Retained intron	-	750	TSL:3
Kdm3a-212	ENSMUST00000206798.1	743	No protein	Retained intron	ē	121	TSL:2
Kdm3a-205	ENSMUST00000205505.1	541	No protein	Retained intron			TSL:2

The strategy is based on the design of Kdm3a-202 transcript, The transcription is shown below



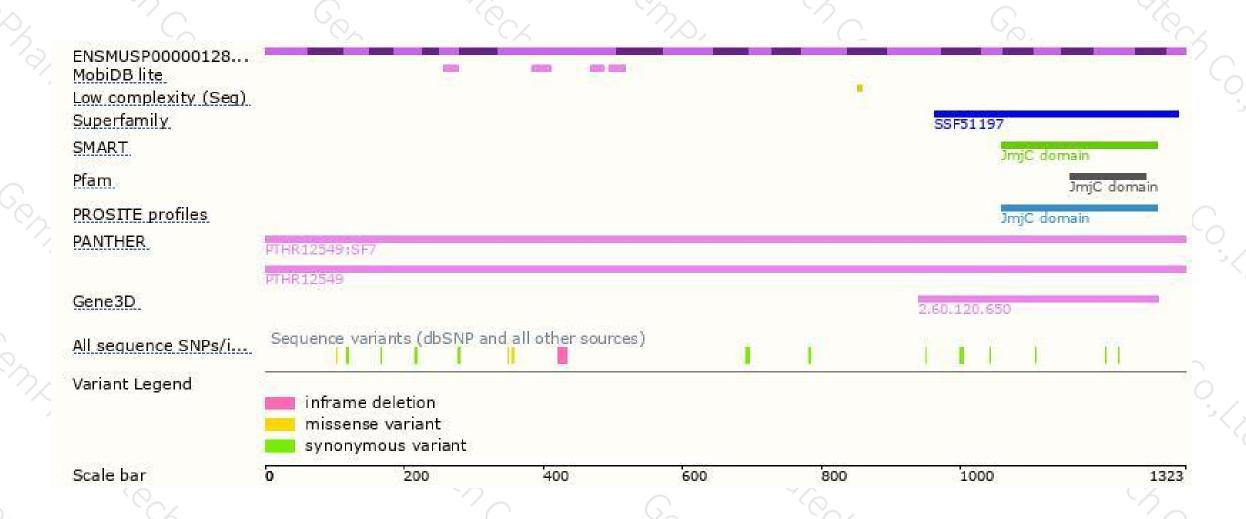
Genomic location distribution





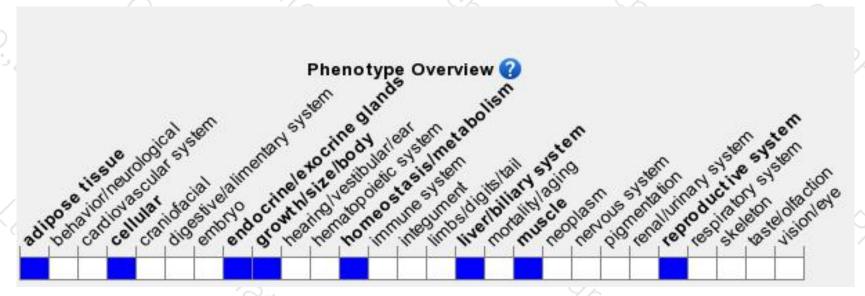
Protein domain





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, Male mice homozygous for a hypomorphic allele display infertility, oligoasthenoteratozoospermia, small testis, and impaired spermiogenesis. Mice homozygous for a null allele exhibit abnorma spermatogenesis and obesity associated with hyperlipidemia.



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





