Kdm2b Cas9-KO Strategy

Designer: Qiong Zhou

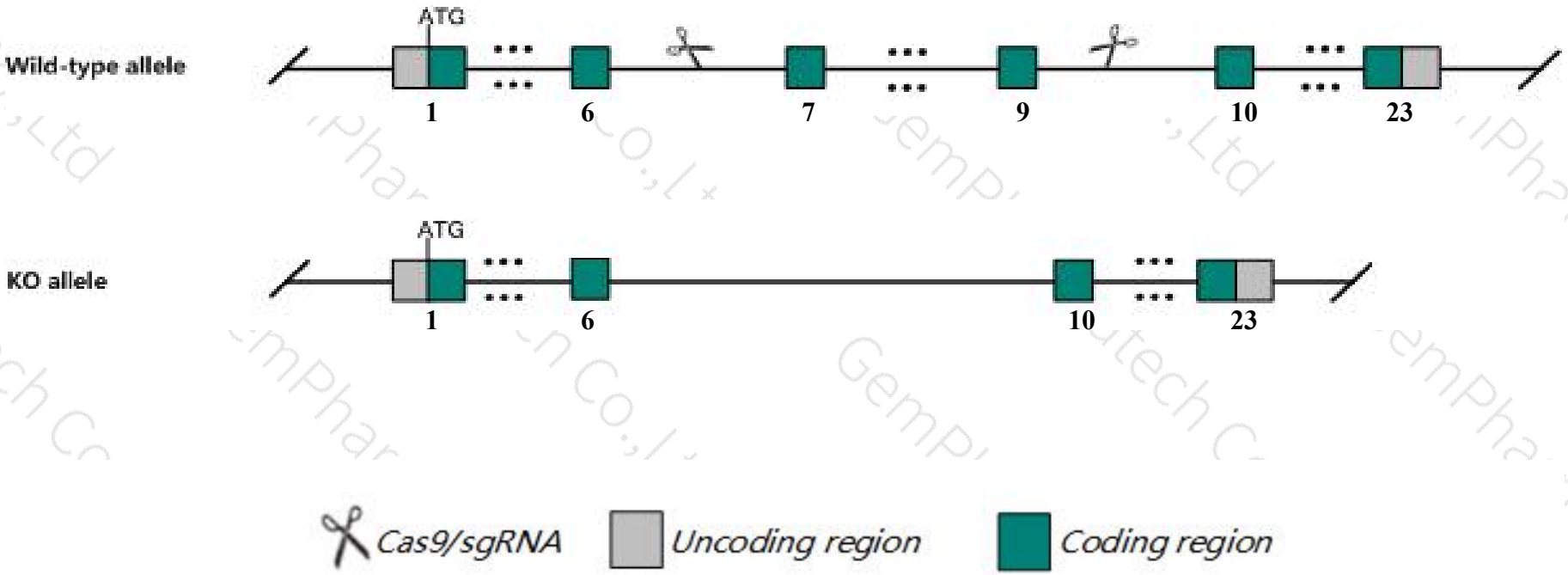
Design Date: 2018/6/4

Project Overview

Project Name	***Kdm2b***
Project type	**Cas9-KO**
Strain background	**C57BL/6J**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Kdm2b* gene. The schematic diagram is as follows:



Technical routes

➢ The *Kdm2b* gene has 20 transcripts. According to the structure of *Kdm2b* gene, exon7-exon9 of *Kdm2b-202* (ENSMUST00000046073.15) transcript is recommended as the knockout region.The region contains 364bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Kdm2b* 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 targeted allele that does not express the long form protein exhibit exencephaly, fetal and postnatal lethality, coloboma, curly tail, oligozoospermia, increased apoptosis, and increased neuronal precursor proliferation.

- The *Kdm2b-201* transcript is unaffected.

- The *Kdm2b* gene is located on the Chr5. 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）

集萃药康 GemPharmatech

Kdm2b lysine (K)-specific demethylase 2B [Mus musculus (house mouse)]

Gene ID: 30841, updated on 31-Jan-2019

Summary

Official Symbol	Kdm2b provided by MGI
Official Full Name	lysine (K)-specific demethylase 2B provided byMGI
Primary source	MGI:MGI:1354737
See related	Ensembl:ENSMUSG00000029475
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Cxxc2, Fbl10, Fbxl10, Jhdm1b, PCCX2
Summary	The protein encoded by this gene is a H3K36-specific histone demethylase, which contains an N-terminal jumonji C domain, a CxxC zinc finger domain, a plant homeodomain finger, an F-box, and eight leucine-rich repeats. Amongst its demonstrated functions, this protein plays roles in the suppression of premature cellular senescence, leukemia maintenance and development, maintenance of mouse embryonic stem cell pluripotency, and induced pluripotent stem cell generation. Mice homozygous for a targeted deletion of the zinc finger domain display embryonic lethality with development ceasing at approximately 7 to 8 days post coitum, demonstrating an essential role in early development. A pseudogene of this gene is found on chromosome 4. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Oct 2014]
Expression	Ubiquitous expression in CNS E14 (RPKM 10.9), whole brain E14.5 (RPKM 10.7) and 28 other tissuesSee more
Orthologs	human all

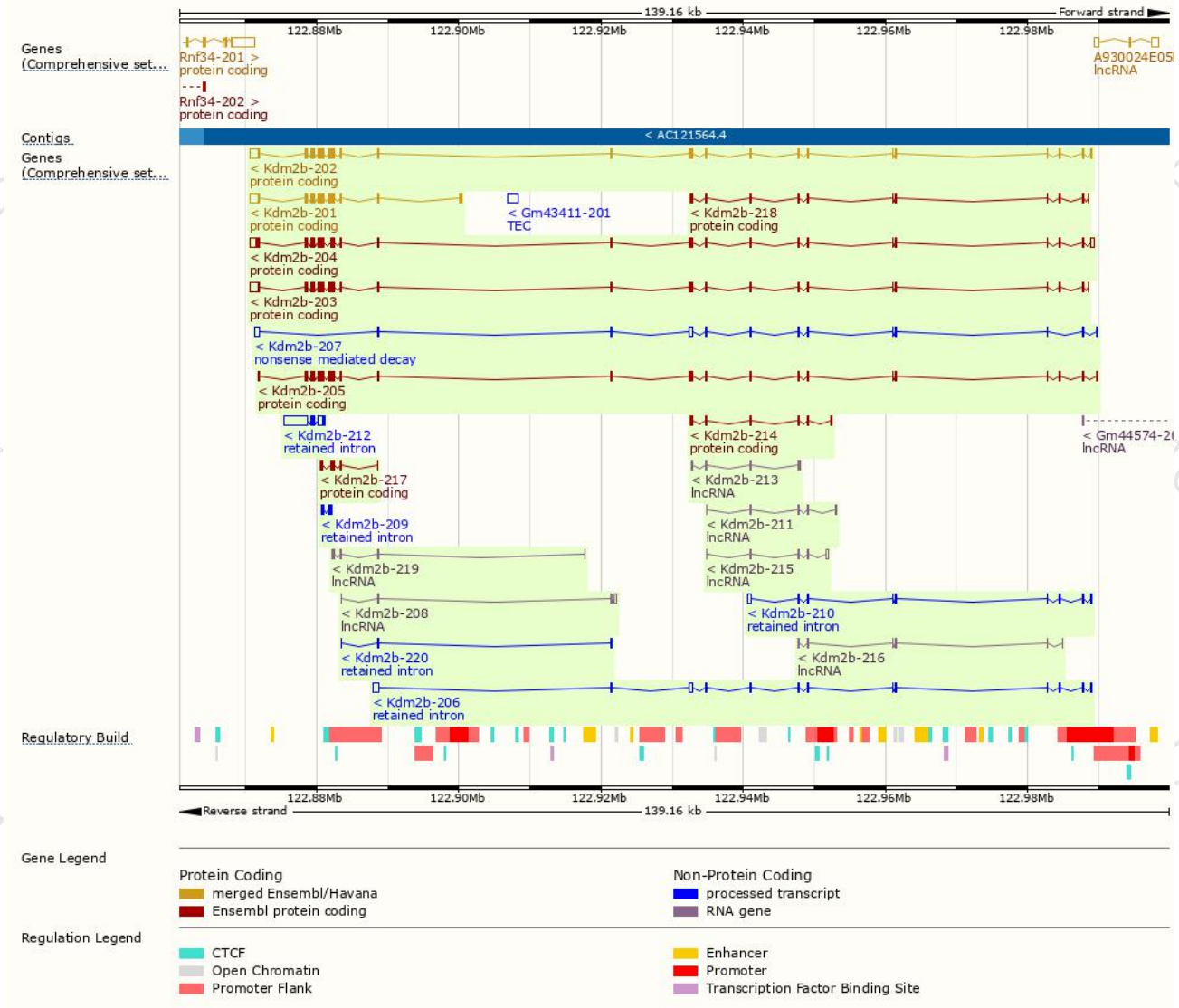
Transcript information（Ensembl）

The gene has 20 transcripts,all transcripts are shown below:

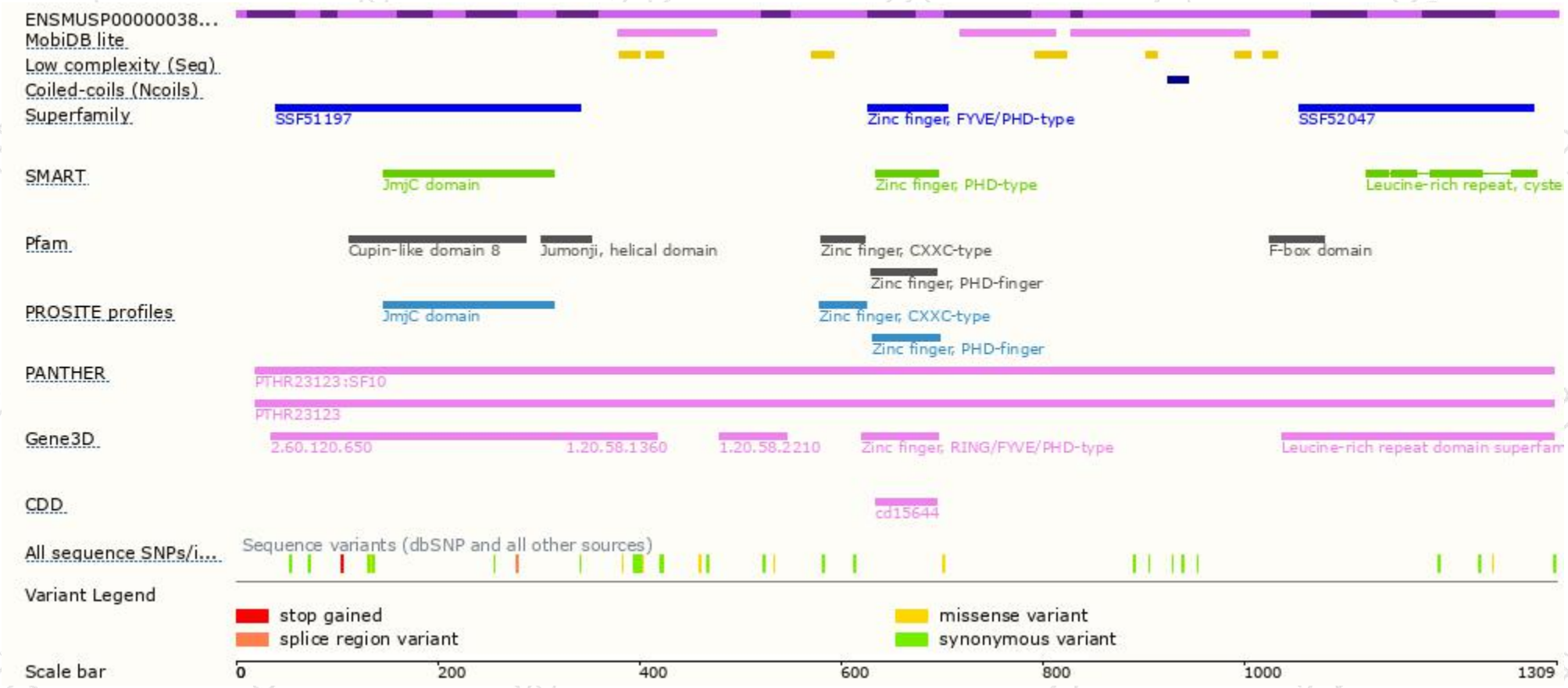
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Kdm2b-202	ENSMUST00000046073.15	5180	1309aa	Protein coding	CCDS39259	Q6P1G2	TSL:1 GENCODE basic APPRIS P2
Kdm2b-201	ENSMUST00000031435.13	3532	776aa	Protein coding	CCDS19657	Q6P1G2	TSL:1 GENCODE basic
Kdm2b-204	ENSMUST00000118027.7	5062	1266aa	Protein coding	-	D3YVU4	TSL:5 GENCODE basic
Kdm2b-203	ENSMUST00000086200.10	4999	1303aa	Protein coding	-	E9QL25	TSL:5 GENCODE basic APPRIS ALT2
Kdm2b-205	ENSMUST00000121739.7	4107	1254aa	Protein coding	-	D3YVU7	TSL:5 GENCODE basic APPRIS ALT2
Kdm2b-218	ENSMUST00000156474.7	1598	514aa	Protein coding	-	D3YUE3	CDS 3' incomplete TSL:1
Kdm2b-214	ENSMUST00000145082.1	1043	286aa	Protein coding	-	D3YV31	CDS 3' incomplete TSL:2
Kdm2b-217	ENSMUST00000152872.1	888	296aa	Protein coding	-	F6QTG9	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
Kdm2b-207	ENSMUST00000127403.7	2543	38aa	Nonsense mediated decay	-	D6RHM8	TSL:5
Kdm2b-213	ENSMUST00000143273.7	724	No protein	Processed transcript	-	-	TSL:5
Kdm2b-216	ENSMUST00000150543.1	697	No protein	Processed transcript	-	-	TSL:3
Kdm2b-215	ENSMUST00000147544.7	625	No protein	Processed transcript	-	-	TSL:5
Kdm2b-208	ENSMUST00000129998.7	550	No protein	Processed transcript	-	-	TSL:3
Kdm2b-219	ENSMUST00000173355.7	516	No protein	Processed transcript	-	-	TSL:3
Kdm2b-211	ENSMUST00000138929.7	464	No protein	Processed transcript	-	-	TSL:3
Kdm2b-212	ENSMUST00000139674.1	4244	No protein	Retained intron	-	-	TSL:1
Kdm2b-206	ENSMUST00000123479.7	2753	No protein	Retained intron	-	-	TSL:1
Kdm2b-210	ENSMUST00000134501.7	1491	No protein	Retained intron	-	-	TSL:1
Kdm2b-209	ENSMUST00000132419.1	641	No protein	Retained intron	-	-	TSL:2
Kdm2b-220	ENSMUST00000174357.1	414	No protein	Retained intron	-	-	TSL:5

The strategy is based on the design of *Kdm2b-202* transcript,The transcription is shown below

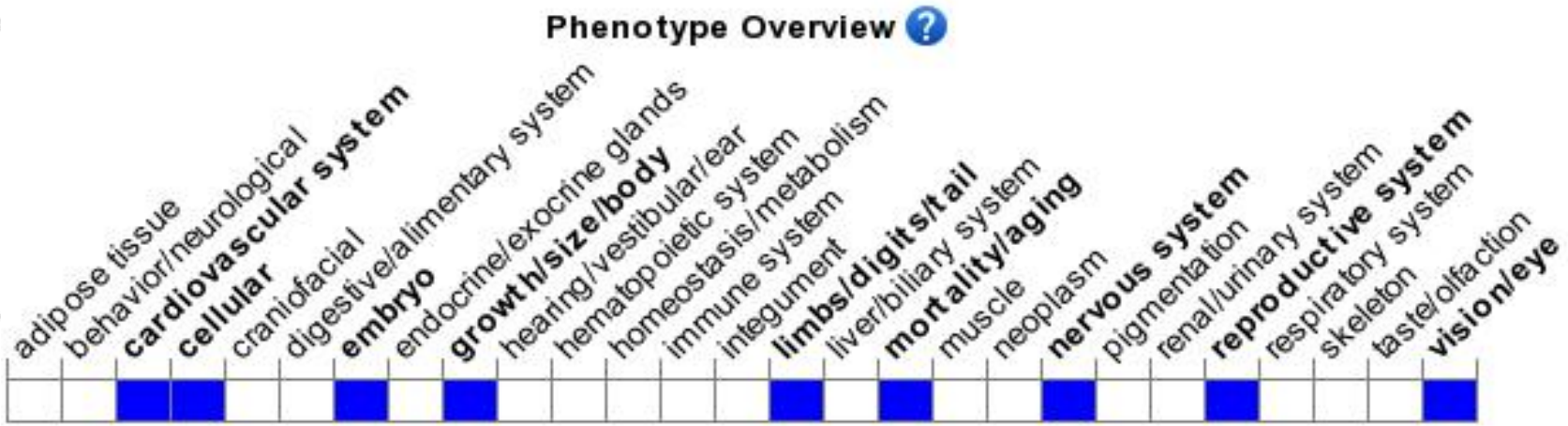
Genomic location distribution



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,Mice homozygous for a targeted allele that does not express the long form protein exhibit exencephaly, fetal and postnatal lethality, coloboma, curly tail, oligozoospermia, increased apoptosis, and increased neuronal precursor proliferation.

集萃药康
GemPharmatech

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

Tel: 025-5864 1534

集萃药康生物科技
GemPharmatech Co.,Ltd