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Smarca4 Cas9-CKO Strategy

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Reviewer: Miaomiao Cui

Design Date: 2023-8-29

Overview

Target Gene Name

- Smarca4

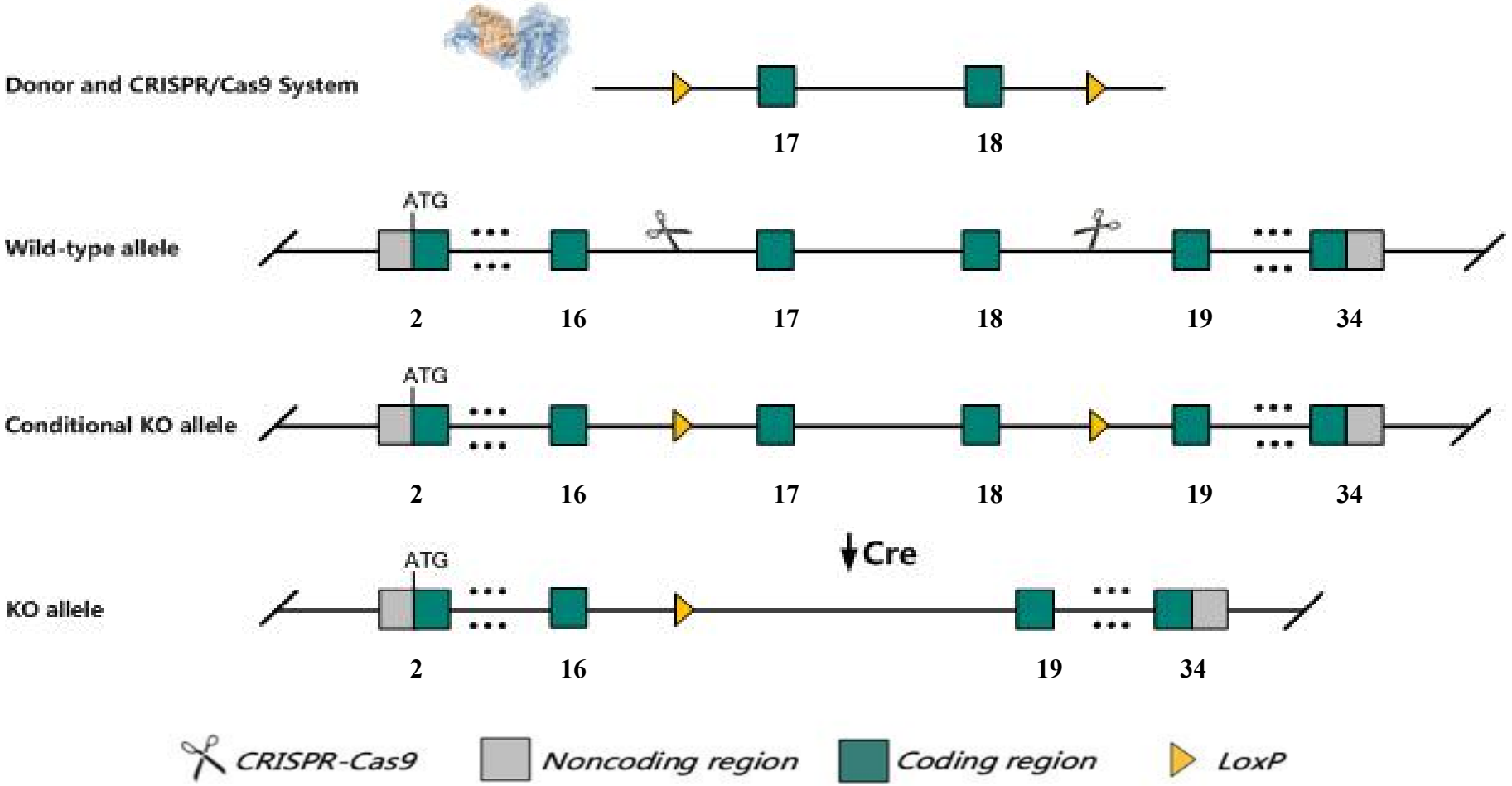
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Smarca4* gene.

Technical Information

- The *Smarca4* gene has 4 transcripts. According to the structure of *Smarca4* gene, exon17-exon18 of *Smarca4*-201 (ENSMUST00000034707.15) transcript is recommended as the knockout region. The region contains 178bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Smarca4* 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

Smarca4 SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 [Mus musculus (house mouse)]

Gene ID: 20586, updated on 31-May-2023

Summary

Official Symbol	Smarca4 provided by MGI
Official Full Name	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 provided by MGI
Primary source	MGI:MGI:88192
See related	Ensembl:ENSMUSG00000032187
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	BAF190A, Brg1, HP1-BP72, SNF2beta, SW1/SNF, b2b508.1Clo, b2b692Clo
Summary	Enables several functions, including ATP hydrolysis activity; DNA polymerase binding activity; and nucleic acid binding activity. Involved in nervous system development; positive regulation of Wnt signaling pathway; and positive regulation of transcription by RNA polymerase II. Acts upstream of or within with a negative effect on gene expression. Acts upstream of or within several processes, including animal organ development; blastocyst development; and circulatory system development. Located in euchromatin; heterochromatin; and perichromatin fibrils. Part of SWI/SNF complex; nBAF complex; and npBAF complex. Is expressed in several structures, including alimentary system; early conceptus; genitourinary system; nervous system; and sensory organ. Used to study breast cancer. Human ortholog(s) of this gene implicated in Coffin-Siris syndrome 4; hepatocellular carcinoma; lung non-small cell carcinoma; and rhabdoid cancer. Orthologous to human SMARCA4 (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Ubiquitous expression in whole brain E14.5 (RPKM 48.7), CNS E14 (RPKM 45.5) and 28 other tissues See more
Orthologs	human all

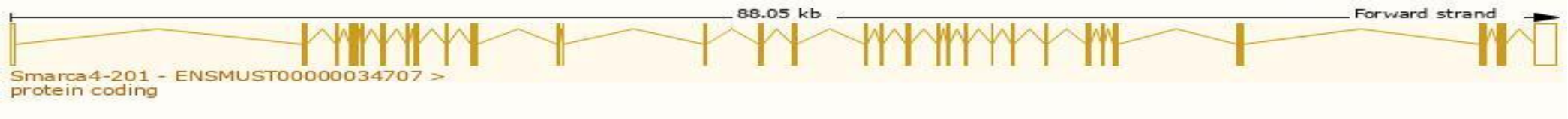
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

The gene has 4 transcripts,all transcripts are shown below:

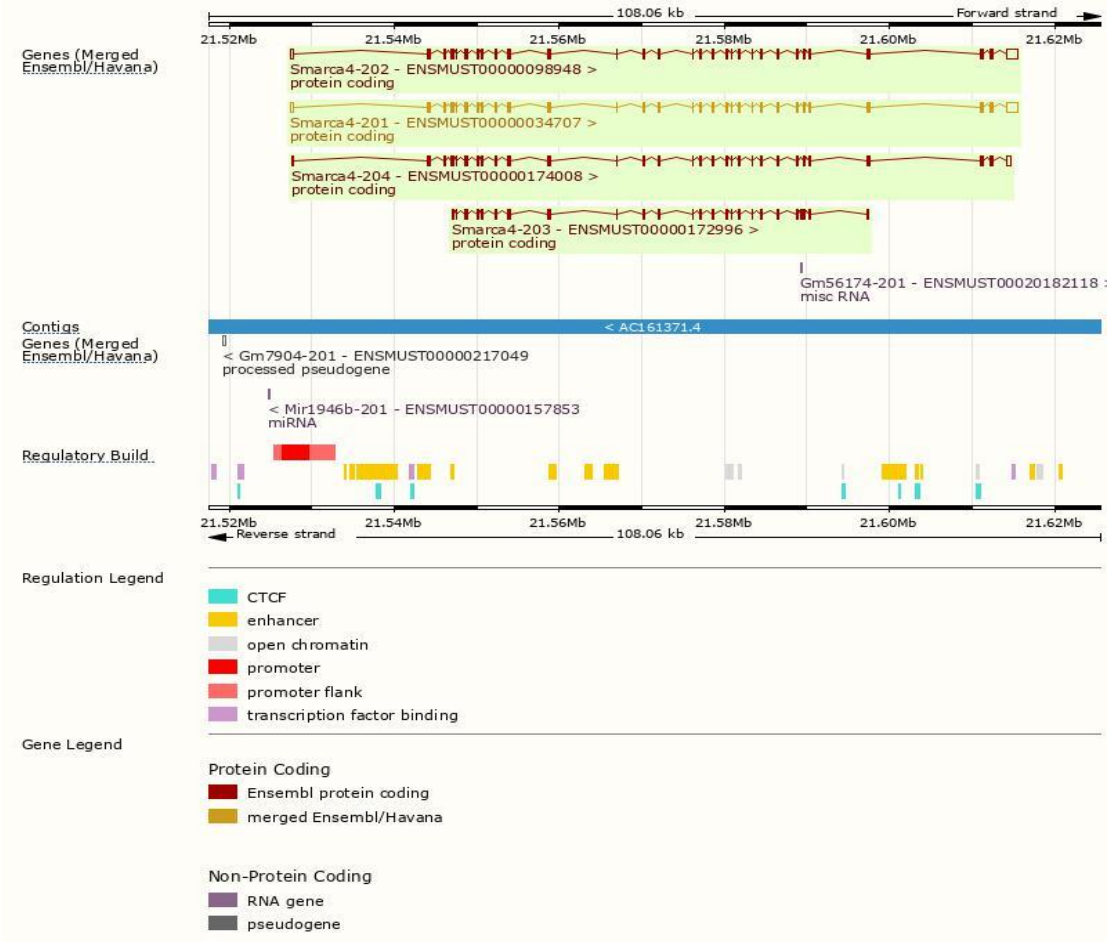
Transcript ID	Name	bp	Protein	Biotype ▲	CCDS	UniProt Match	Flags
ENSMUST00000098948.10	Smarca4-202	6376	1617aa	Protein coding	CCDS52737	A0A0R4J170	Ensembl Canonical GENCODE basic APPRIS ALT1 TSL:1
ENSMUST00000034707.15	Smarca4-201	6354	1614aa	Protein coding	CCDS22909	Q3TKT4-2	GENCODE basic APPRIS P5 TSL:1
ENSMUST00000174008.8	Smarca4-204	5405	1613aa	Protein coding	CCDS57662	Q3TKT4-1	GENCODE basic APPRIS ALT1 TSL:1
ENSMUST00000172996.2	Smarca4-203	3800	1267aa	Protein coding		G3UX35	TSL:1 CDS 5' and 3' incomplete

The strategy is based on the design of *Smarca4*-201 transcript, the transcription is shown below:



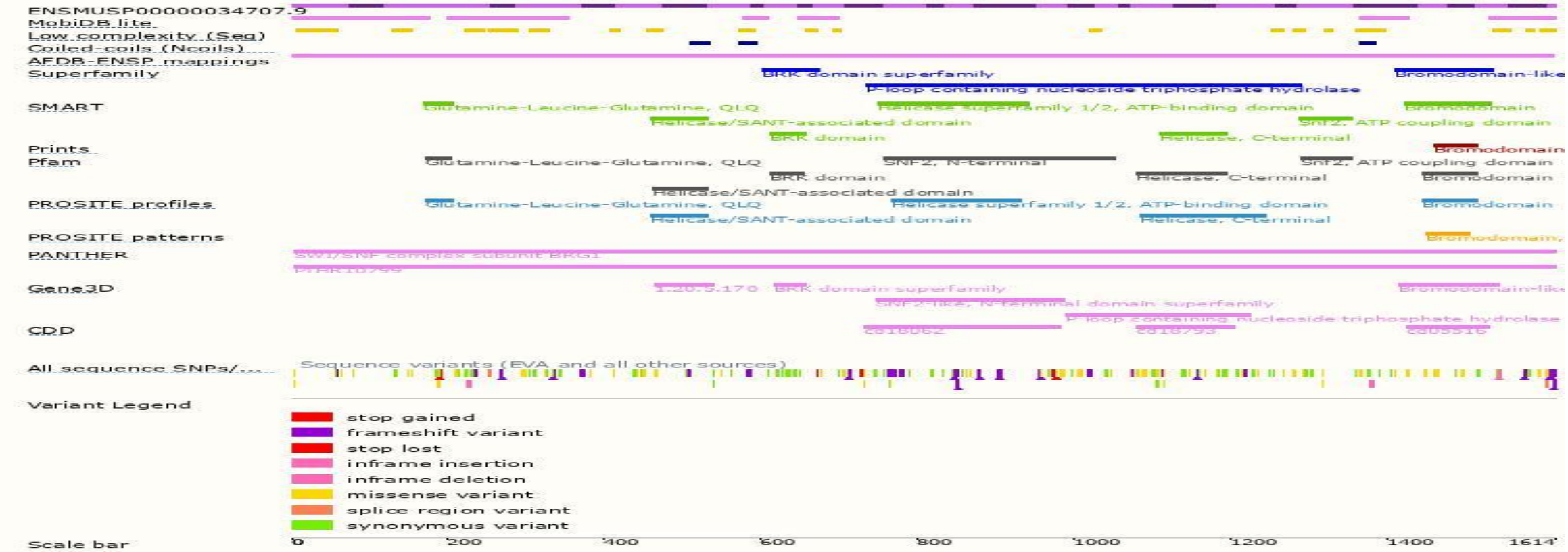
Source: https://www.ensembl.org

Genomic Information



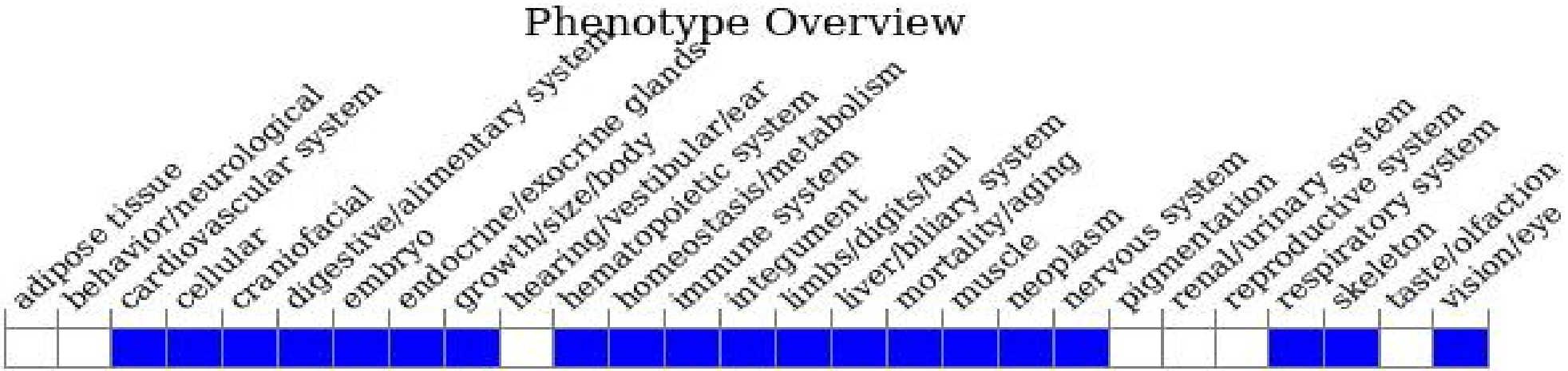
Source: : https://www.ensembl.org

Protein Information



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

Mouse Phenotype Information (MGI)



- Homozygotes for a null allele die in utero before implantation. Embryos heterozygous for this null allele and an ENU-induced allele show impaired definitive erythropoiesis, anemia and lethality during organogenesis. Heterozygotes for a different null allele show cyanosis and cardiovascular defects.

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

Important Information

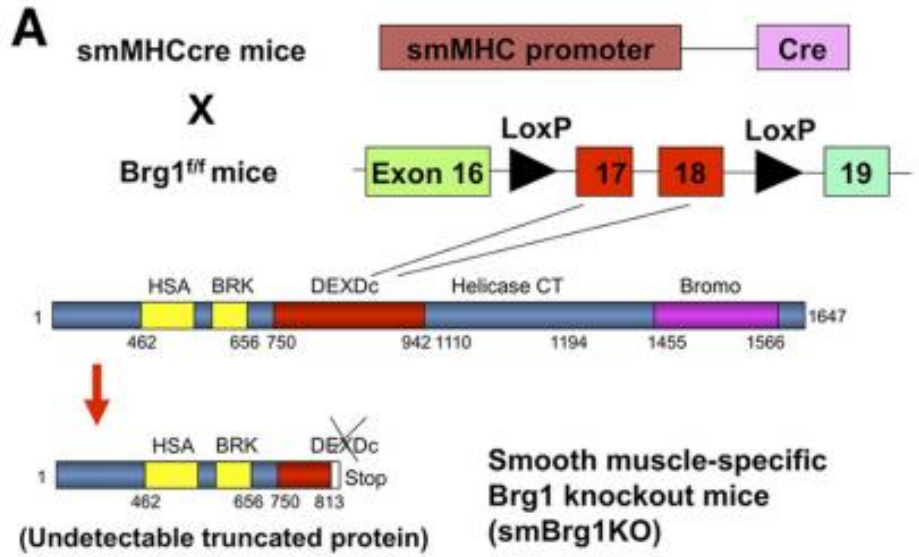
- *Smarca4* is located on Chr9. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the 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 1

MOLECULAR AND CELLULAR BIOLOGY, July 2011, p. 2618–2631 Vol. 31, No. 13
0270-7306/11/$12.00 doi:10.1128/MCB.01338-10

SWI/SNF Complexes Containing Brahma or Brahma-Related Gene 1 Play Distinct Roles in Smooth Muscle Development▽†

Min Zhang,[1] Meng Chen,[1] Ju-Ryoung Kim,[1] Jiliang Zhou,[1]‡ Rebekah E. Jones,[1] Johnathan D. Tune,[1] Ghassan S. Kassab,[1] Daniel Metzger,[2] Shawn Ahlfeld,[3] Simon J. Conway,[3] and B. Paul Herring[1]*



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Reference 2

MOLECULAR AND CELLULAR BIOLOGY, Oct. 1997, p. 5976–5986 Vol. 17,
0270-7306/97/$04.00+0

SNF2β-BRG1 Is Essential for the Viability of F9 Murine Embryonal Carcinoma Cells

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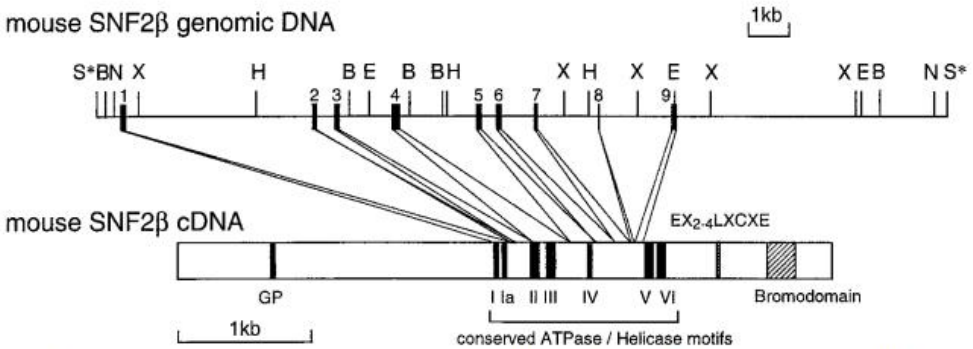


FIG. 1. Organization of the m*SNF2*β gene region encompassing the conserved ATPase-helicase motifs. The nine exons identified in a 20.9-kb genomic clone are indicated as black boxes. A partial restriction enzyme map is given. The m*SNF2*β cDNA (Fig. 3) is represented below the structure of the gene. S, *Sal*I; B, *Bam*HI; N, *Nhe*I; X, *Xba*I; E, *Eco*RI; GP, glycine-proline-rich region; I, Ia, II, III, IV, V, and VI, ATPase-helicase motifs; $EX_{2-4}LXCXE$, putative retinoblastoma protein interaction site; S*, *Sal*I site not present in genomic DNA.

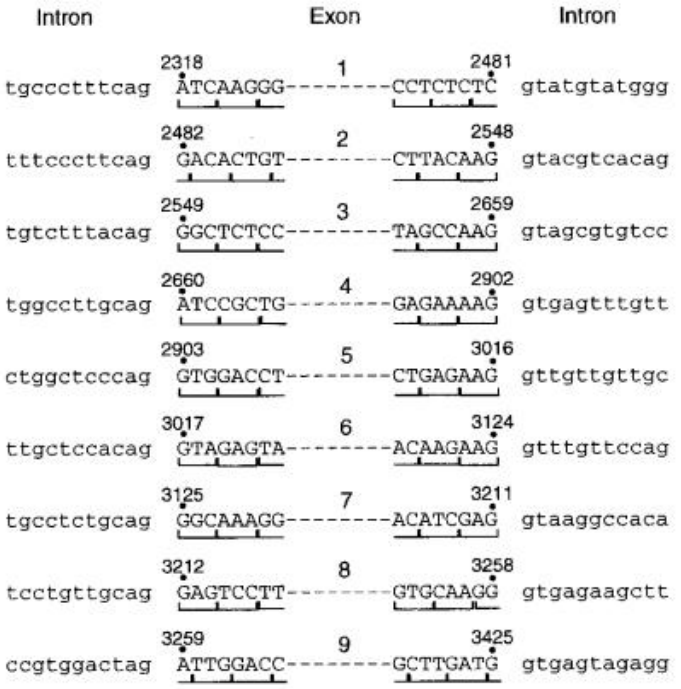
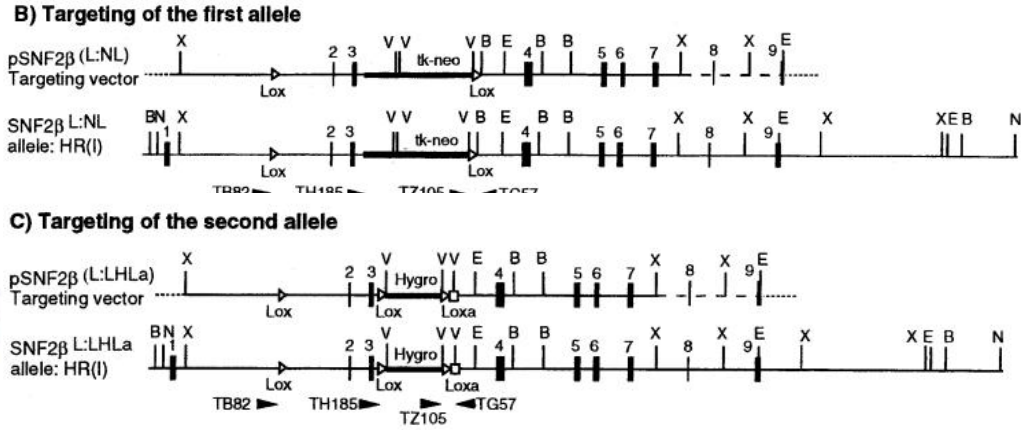


FIG. 4. Exon-intron boundaries of the m*SNF2*β gene region encoding the conserved ATPase-helicase motifs (Fig. 1 and 3). The codons are bracketed. The coordinates of the first and last nucleotides of each exon refer to the cDNA sequence presented in Fig. 3.



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