Smarca4 Cas9-KO Strategy

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Reviewer: Lingyan Wu

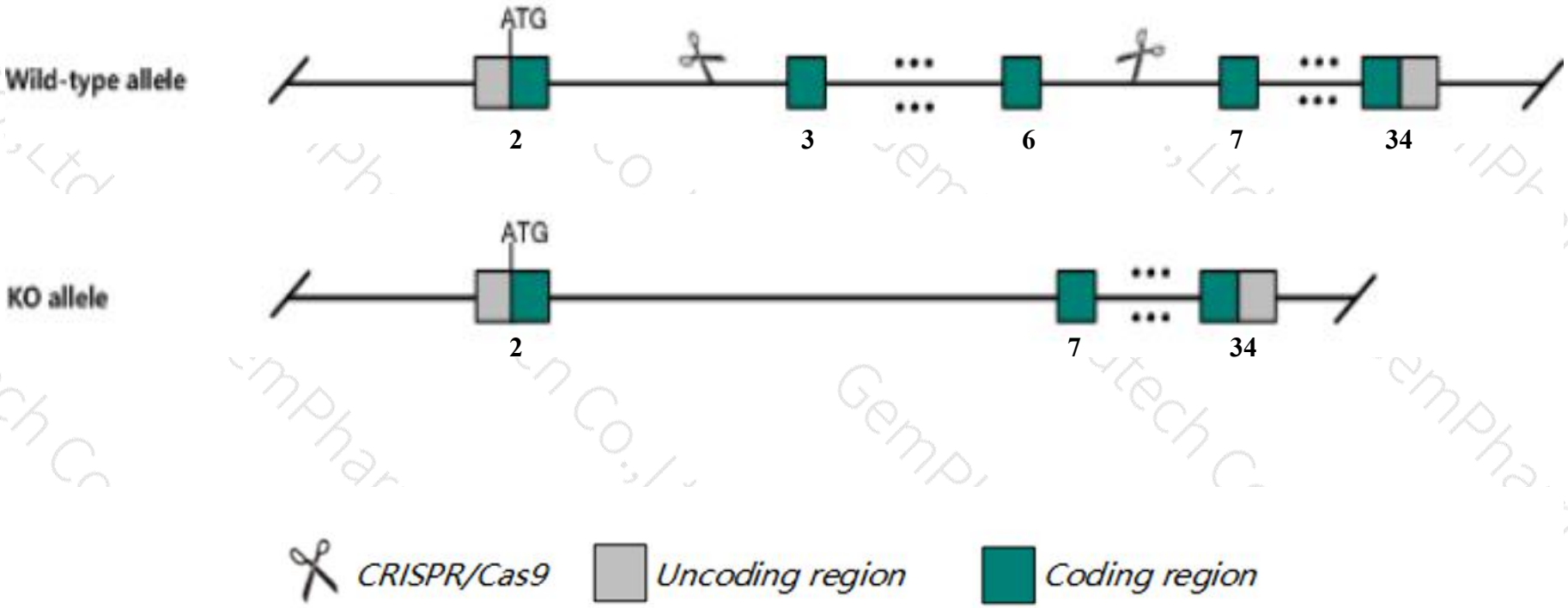
Design Date: 2018-9-20

Project Overview

Project Name	***Smarca4***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Smarca4* gene. The schematic diagram is as follows:



Technical routes

➢ The *Smarca4* gene has 4 transcripts. According to the structure of *Smarca4* gene, exon3-exon6 of *Smarca4-201*(ENSMUST00000034707.14) transcript is recommended as the knockout region.The region contains 896bp coding sequence. Knock 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 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.

Notice

➢ According to the existing MGI data,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.

➢ The *Smarca4* gene is located on the Chr9. 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）

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

Gene ID: 20586, updated on 21-Mar-2020

Summary

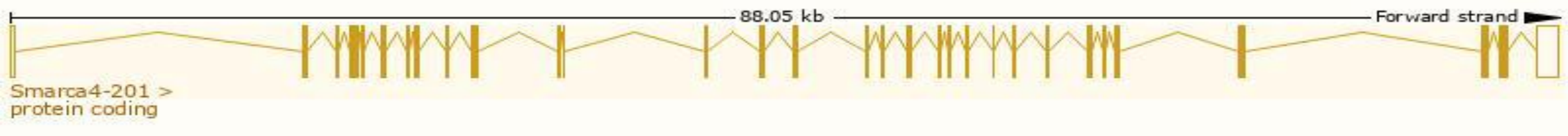
Official Symbol	Smarca4 provided by MGI
Official Full Name	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 provided by MGI
Primary source	MGI:MGI:88192
See related	Ensembl:ENSMUSG00000032187
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus 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
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

Transcript information（Ensembl）

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

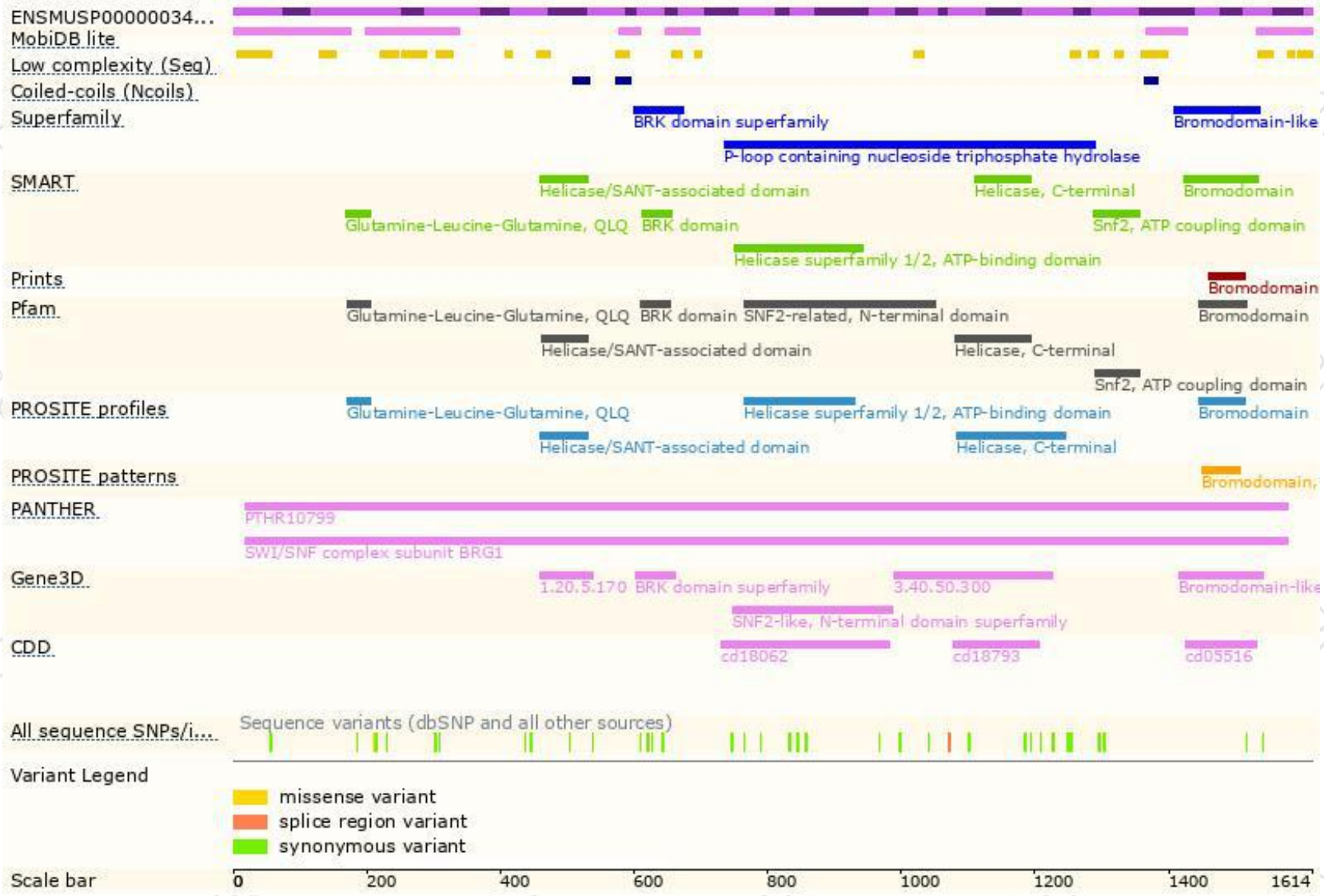
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Smarca4-202	ENSMUST00000098948.9	6376	1617aa	Protein coding	CCDS52737	A0A0R4J170	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT1
Smarca4-201	ENSMUST00000034707.14	6354	1614aa	Protein coding	CCDS22909	Q3TKT4	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Smarca4-204	ENSMUST00000174008.7	5405	1613aa	Protein coding	CCDS57662	Q3TKT4	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT1
Smarca4-203	ENSMUST00000172996.1	3800	1267aa	Protein coding	-	G3UX35	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:1

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

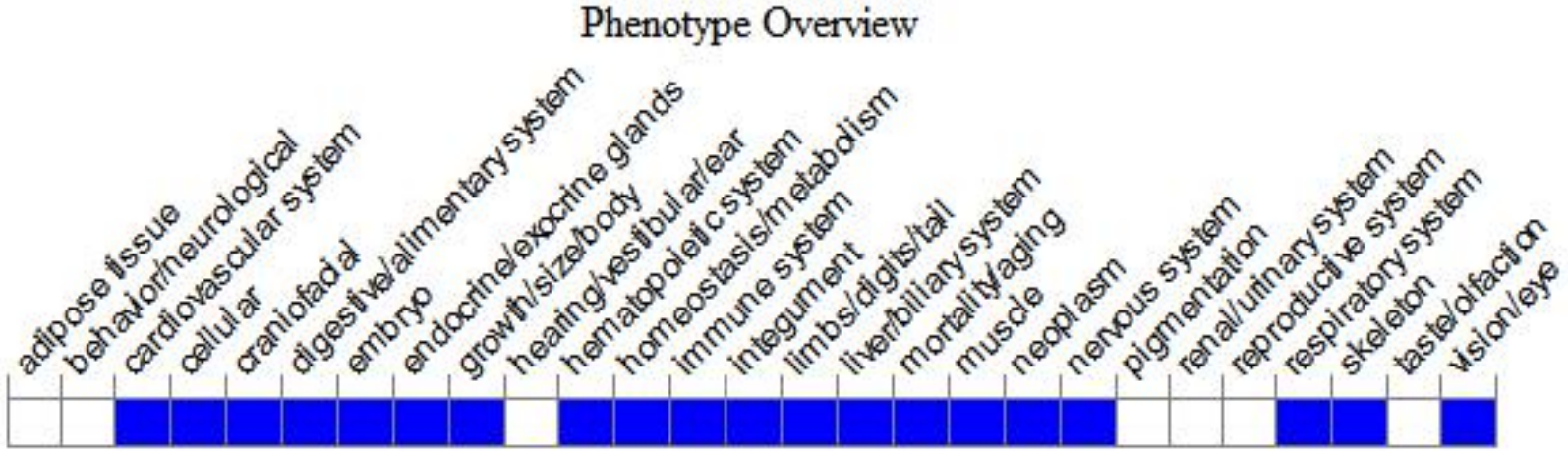


Genomic location distribution

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,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.

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

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