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Hnrnpa2b1 Cas9-CKO Strategy

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Reviewer: Ruirui Zhang

Design Date: 2023-9-13

Overview

Target Gene Name

- Hnrnpa2b1

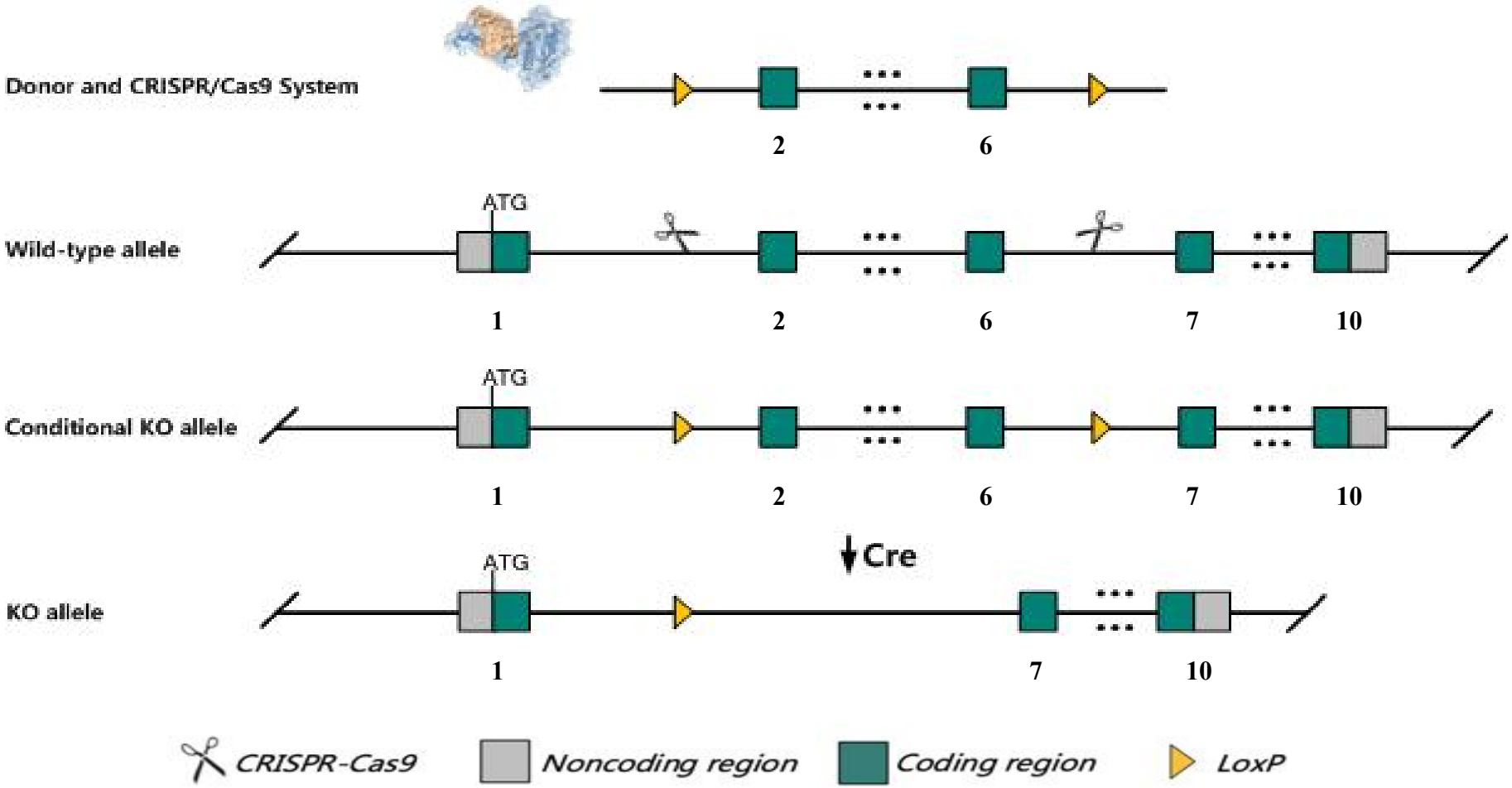
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Hnrnpa2b1* gene.

Technical Information

- The *Hnrnpa2b1* gene has 13 transcripts. According to the structure of *Hnrnpa2b1* gene, exon2-exon6 of *Hnrnpa2b1*-202 (ENSMUST00000090002.10) transcript is recommended as the knockout region. The region contains 652bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Hnrnpa2b1* 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

Hnrnpa2b1 heterogeneous nuclear ribonucleoprotein A2/B1 [Mus musculus (house mouse)]

Gene ID: 53379, updated on 31-May-2023

Summary

Official Symbol	Hnrnpa2b1 provided by MGI
Official Full Name	heterogeneous nuclear ribonucleoprotein A2/B1 provided byMGI
Primary source	MGI:MGI:104819
See related	Ensembl:ENSMUSG00000004980
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	9130414A06Rik, Hnrpa2, Hnrpa2b1, hnrnp-A
Summary	Enables pre-mRNA intronic binding activity. Acts upstream of or within negative regulation of mRNA splicing, via spliceosome and negative regulation of transcription by RNA polymerase II. Located in nucleus. Is expressed in telencephalon. Human ortholog(s) of this gene implicated in inclusion body myopathy with Paget disease of bone and frontotemporal dementia and inclusion body myopathy with early-onset Paget disease of bone with or without frontotemporal dementia 2. Orthologous to human HNRNPA2B1 (heterogeneous nuclear ribonucleoprotein A2/B1). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Broad expression in CNS E11.5 (RPKM 290.0), liver E14 (RPKM 181.3) and 16 other tissuesSee more
Orthologs	human all

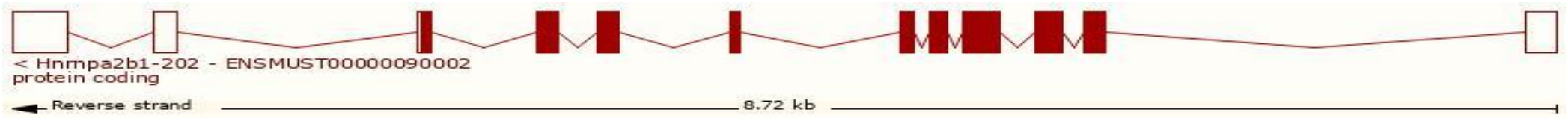
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

The gene has 13 transcripts,all transcripts are shown below:

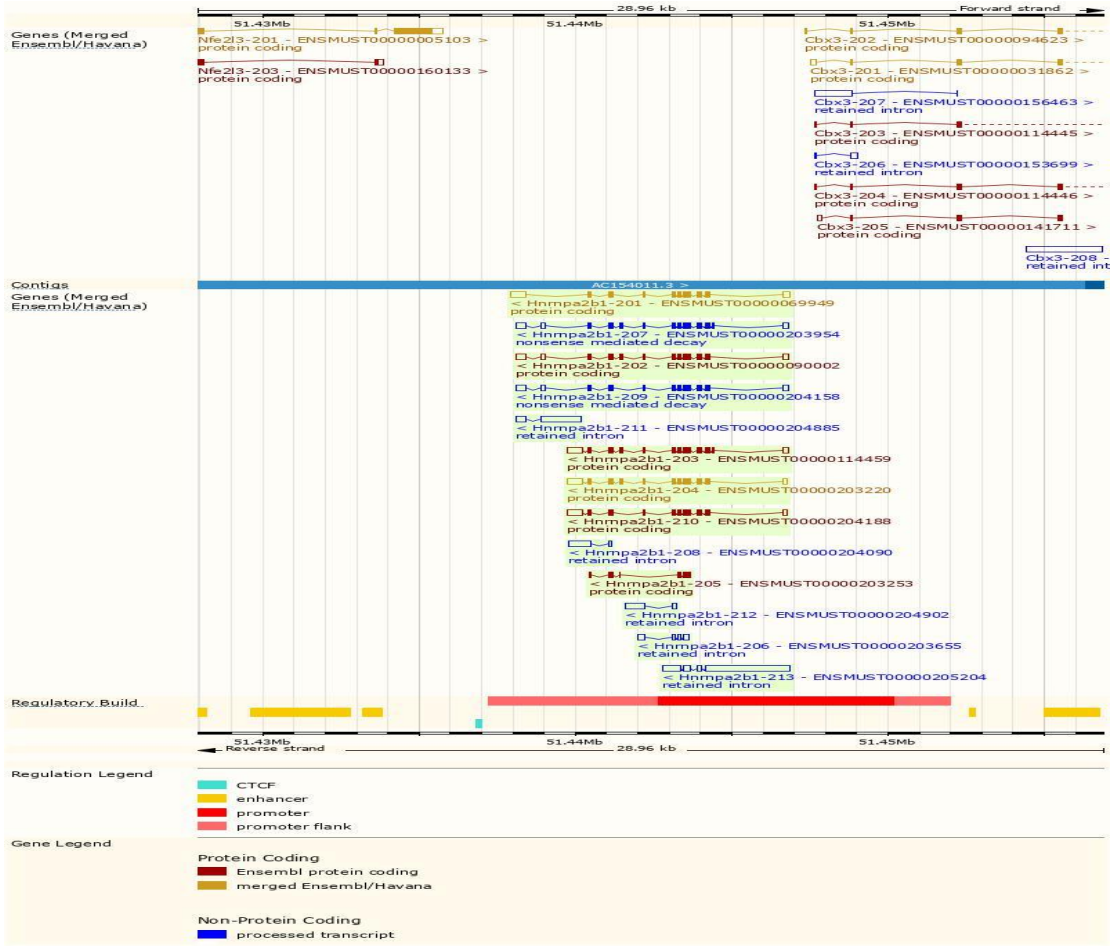
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000114459.8	Hnrnpa2b1-203	1728	353aa	Protein coding		O88569-1	Ensembl Canonical, GENCODE basic, APPRIS ALT1, TSL:5
ENSMUST00000203954.3	Hnrnpa2b1-207	1703	353aa	Nonsense mediated decay		O88569-1	TSL:5
ENSMUST00000090002.10	Hnrnpa2b1-202	1662	341aa	Protein coding	CCDS51774	O88569-2	GENCODE basic, APPRIS P4, TSL:5
ENSMUST00000203220.3	Hnrnpa2b1-204	1638	341aa	Protein coding	CCDS51774	O88569-2	GENCODE basic, APPRIS P4, TSL:1
ENSMUST00000069949.13	Hnrnpa2b1-201	1647	301aa	Protein coding	CCDS51773	O88569-3	GENCODE basic, TSL:1
ENSMUST00000204188.3	Hnrnpa2b1-210	1518	301aa	Protein coding	CCDS51773	O88569-3	GENCODE basic, TSL:5
ENSMUST00000204158.3	Hnrnpa2b1-209	1542	301aa	Nonsense mediated decay	CCDS51773	O88569-3	TSL:1
ENSMUST00000203253.2	Hnrnpa2b1-205	466	155aa	Protein coding		A0A0N4SUM2	TSL:3, CDS 5' and 3' incomplete
ENSMUST00000205204.2	Hnrnpa2b1-213	3645	No protein	Retained intron		-	TSL:1
ENSMUST00000204885.2	Hnrnpa2b1-211	1554	No protein	Retained intron		-	TSL:1
ENSMUST00000204090.2	Hnrnpa2b1-208	766	No protein	Retained intron		-	TSL:2
ENSMUST00000204902.2	Hnrnpa2b1-212	706	No protein	Retained intron		-	TSL:2
ENSMUST00000203655.2	Hnrnpa2b1-206	596	No protein	Retained intron		-	TSL:2

The strategy is based on the design of *Hnrnpa2b1*-202 transcript, the transcription is shown below:



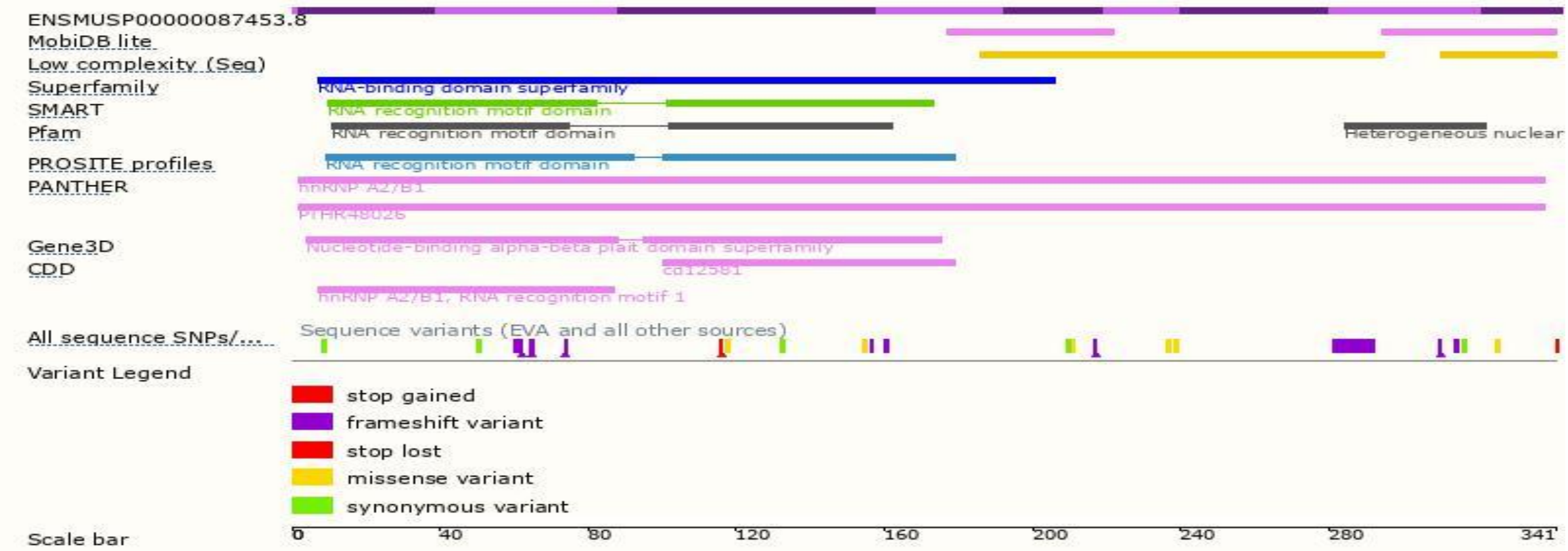
Source: https://www.ensembl.org

Genomic Information



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

Protein Information



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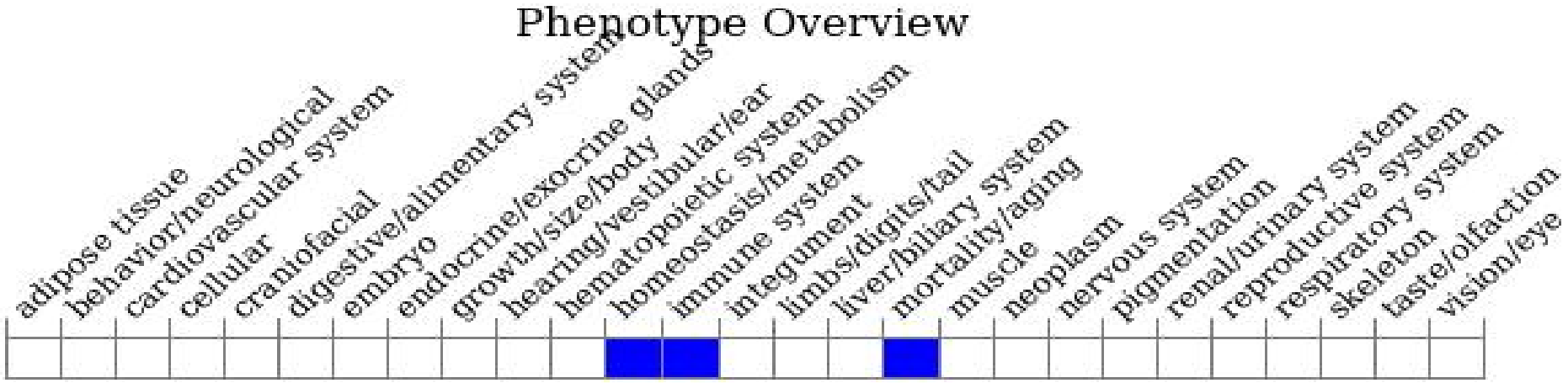
Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)

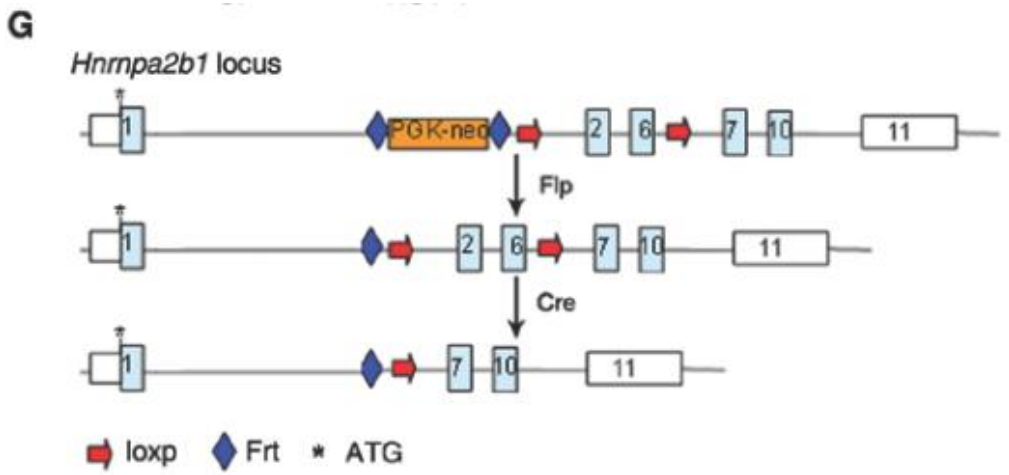


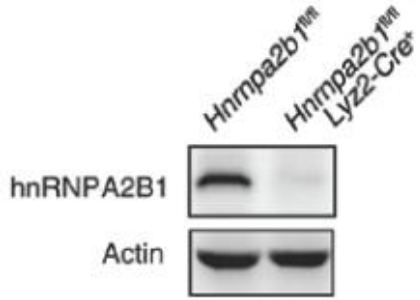
Source: https://www.informatics.jax.org

Important Information

- The lethality of *Hnrnpa2b1* knockout is unknown.
- The KO region is about 2.5kb away from *Cbx3* gene. Knockout the region may affect the function of *Cbx3* gene.
- The effect on transcript-205 is unknown.
- *Hnrnpa2b1* is located on Chr6. 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





Materials and methods

Mice

C57BL/6 mice were purchased from Joint Ventures Sipper BK Experimental Animal (Shanghai, China). *Lyz2*-Cre mice and *Irf3*$^{-/-}$ mice were purchased from The Jackson Laboratory. To establish *Hnrnpa2b1*-conditional-knockout mice, exons 2–6 of the *Hnrnpa2b1* gene were trapped by insertion of loxP sequences which can be specifically recognized by CRE recombinase. *Hnrnpa2b1*$^{fl/fl}$ mice were backcrossed onto C57BL/6J background, and then crossed with *Lyz2*-Cre mice. Exons 2–6 were excised by CRE recombinase in myeloid cells. *Hnrnpa2b1*$^{fl/fl}$*Lyz2-Cre*$^{+/-}$ mice were mated with *Hnrnpa2b1*$^{fl/fl}$*Lyz2-Cre*$^{-/-}$ mice to generate *Hnrnpa2b1*$^{fl/fl}$*Lyz2-Cre*$^{+}$ and littermate control mice for further experiments. The mice were bred in specific pathogen-free conditions. Mice bearing a *Mettl3*^{fl} allele (*Mettl3*^{fl} mice) were from Dr. Q. Zhou (Chinese Academy of Sciences, China) and were crossed with *Lyz2*-Cre mice to obtain *Mettl3*$^{fl/fl}$*Lyz2-Cre*$^{+}$ mice. Mice at 8 weeks of age were used for in vivo experiments.

Wang L, et al., Nuclear hnRNPA2B1 initiates and amplifies the innate immune response to DNA viruses. Science. 2019 Aug 16;365(6454)