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Kcnj16 Cas9-KO Strategy

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Reviewer: Ruirui Zhang

Design Date: 2023-10-31

Overview

Target Gene Name

- Kcnj16

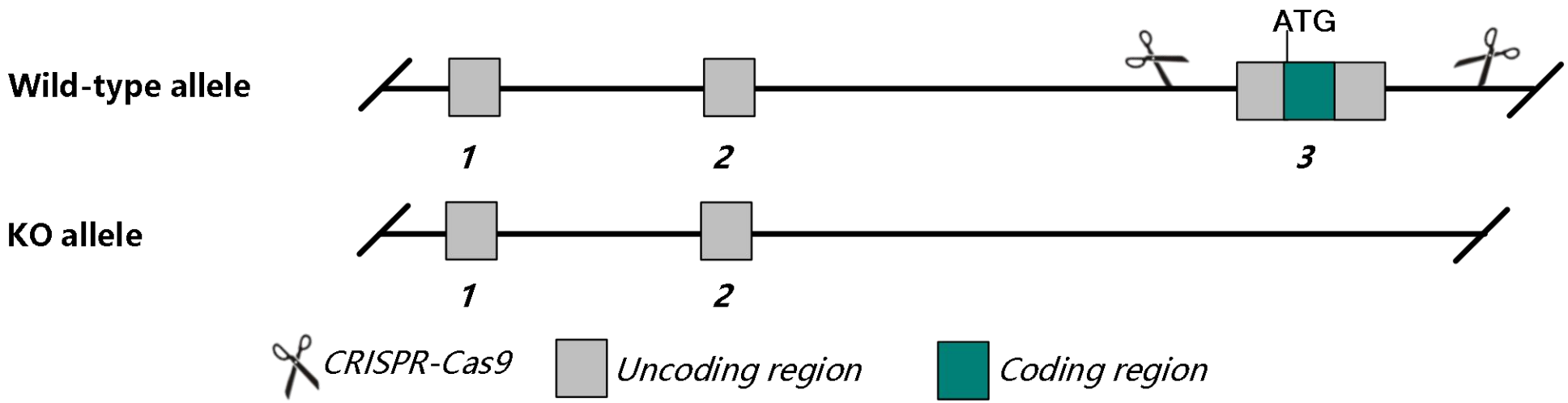
Project Type

- Cas9-KO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Technical Information

- The *Kcnj16* gene has 6 transcripts. According to the structure of *Kcnj16* gene, exon3 of *Kcnj16*-206 (ENSMUST00000180023.8) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Kcnj16* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs 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.

Gene Information

Kcnj16 potassium inwardly-rectifying channel, subfamily J, member 16 [Mus musculus (house mouse)]

Gene ID: 16517, updated on 13-Mar-2020

Summary

Official Symbol	Kcnj16 provided by MGI
Official Full Name	potassium inwardly-rectifying channel, subfamily J, member 16 provided by MGI
Primary source	MGI:MGI:1314842
See related	Ensembl:ENSMUSG00000051497
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	6430410F18Rik, AI132396, Kir5.1
Expression	Biased expression in kidney adult (RPKM 63.6), frontal lobe adult (RPKM 5.2) and 3 other tissues See more
Orthologs	human all

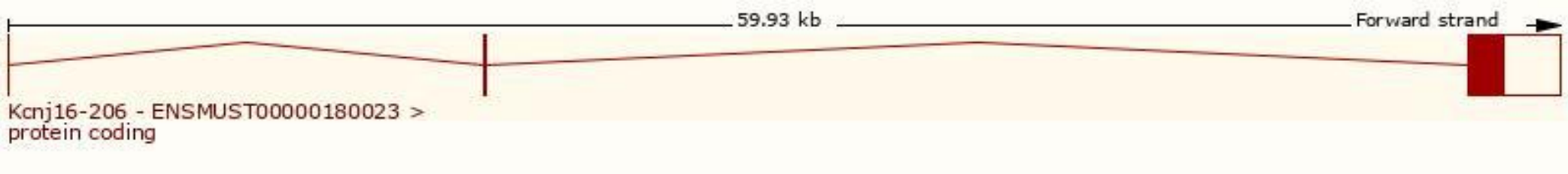
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

The gene has 6 transcripts,all transcripts are shown below:

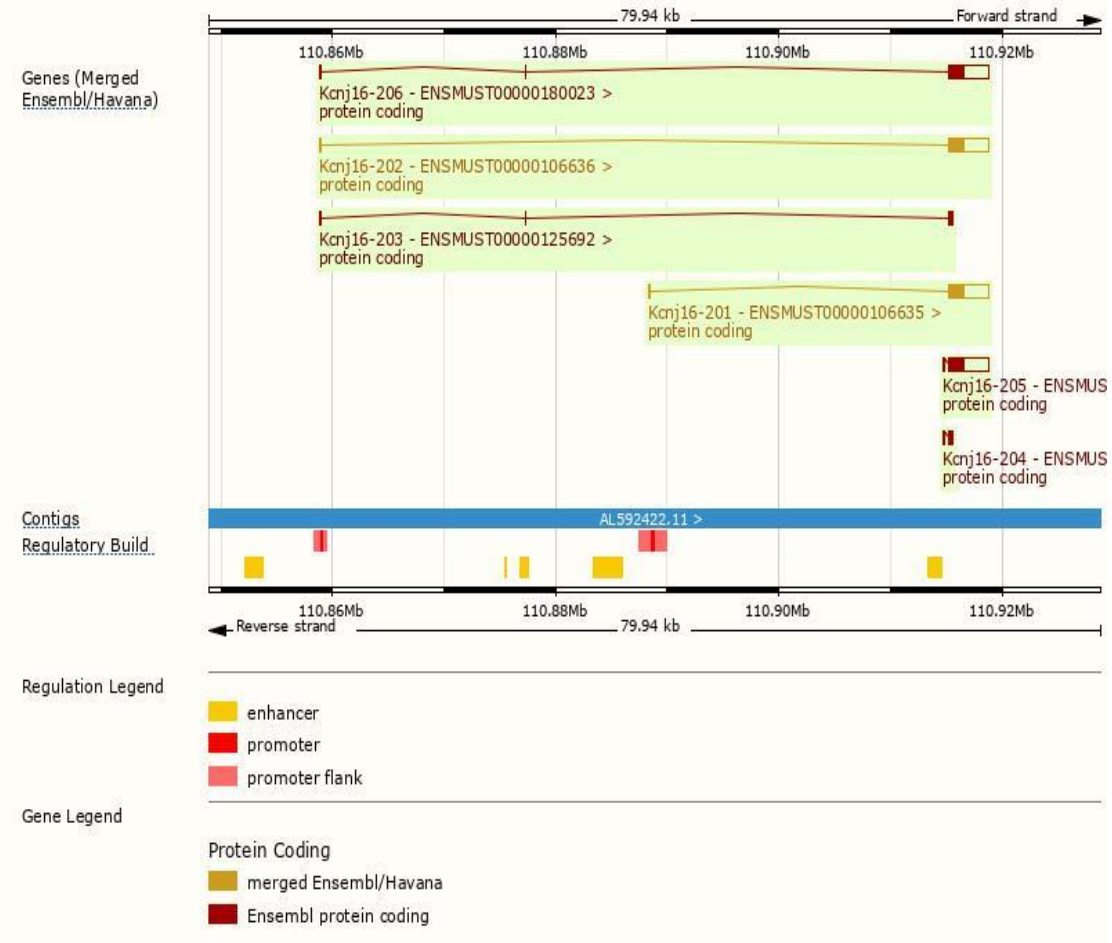
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000180023.8	Kcnj16-206	3701	419aa	Protein coding	CCDS25593	Q9Z307	Ensembl Canonical, GENCODE basic, APPRIS P1, TSL:3
ENSMUST00000178798.2	Kcnj16-205	3678	419aa	Protein coding	CCDS25593	Q9Z307	GENCODE basic, APPRIS P1, TSL:3
ENSMUST00000106635.2	Kcnj16-201	3671	419aa	Protein coding	CCDS25593	Q9Z307	GENCODE basic, APPRIS P1, TSL:1
ENSMUST00000106636.8	Kcnj16-202	3623	419aa	Protein coding	CCDS25593	Q9Z307	GENCODE basic, APPRIS P1, TSL:1
ENSMUST00000125692.2	Kcnj16-203	527	103aa	Protein coding		A2A5W8	TSL:3, CDS 3' incomplete
ENSMUST00000150902.8	Kcnj16-204	424	66aa	Protein coding		A2A5W7	TSL:3, CDS 3' incomplete

The strategy is based on the design of *Kcnj16*-206 transcript, the transcription is shown below:



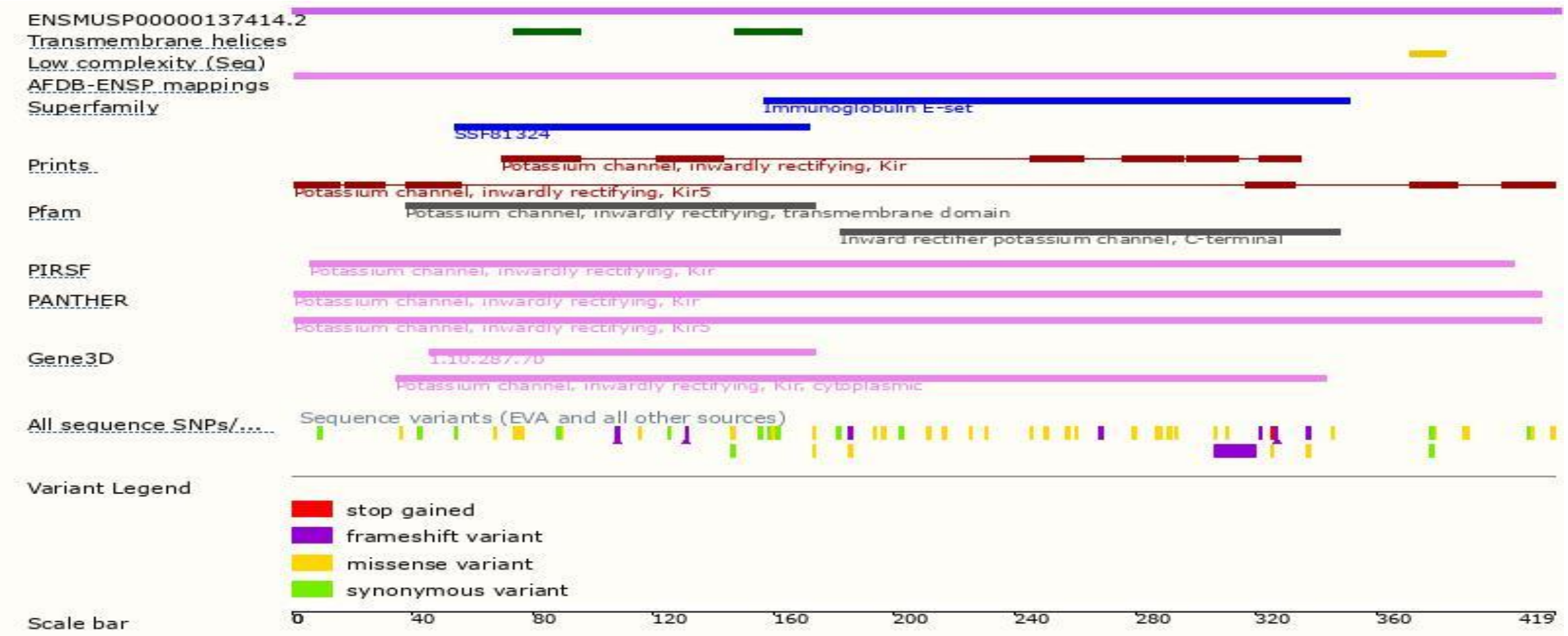
Source: https://www.ensembl.org

Genomic Information



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

Protein Information



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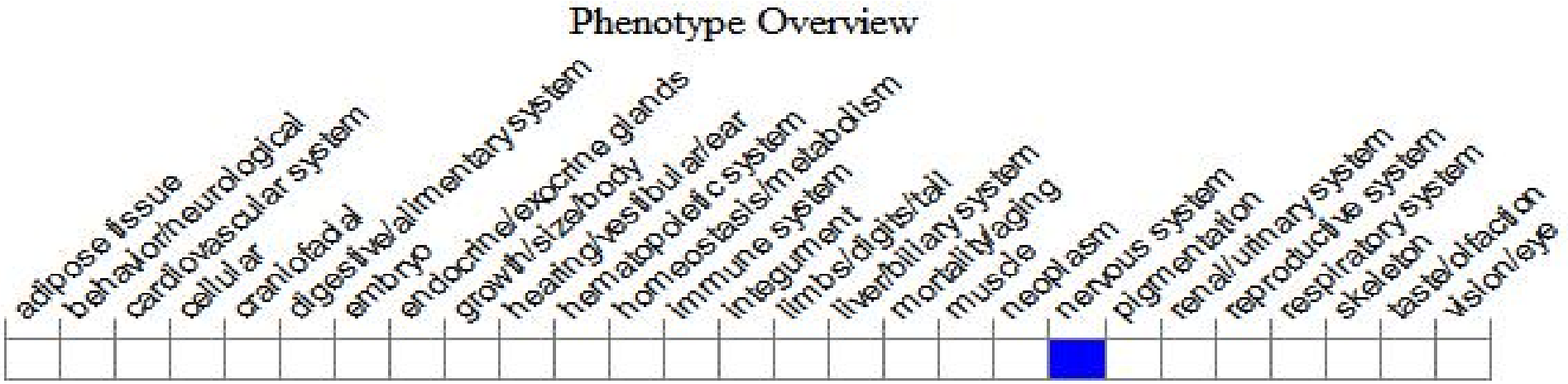
Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



- Mice homozygous for a knock-out allele exhibit abnormal neuron response to ammonium chloride withdrawal and carbon dioxide treatment.

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

Important Information

- According to the existing MGI data,mice homozygous for a knock-out allele exhibit abnormal neuron response to ammonium chloride withdrawal and carbon dioxide treatment.
- The effect on transcript-203&204 is unknwon.
- *Kcnj16* is located on Chr11. 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 risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Reference

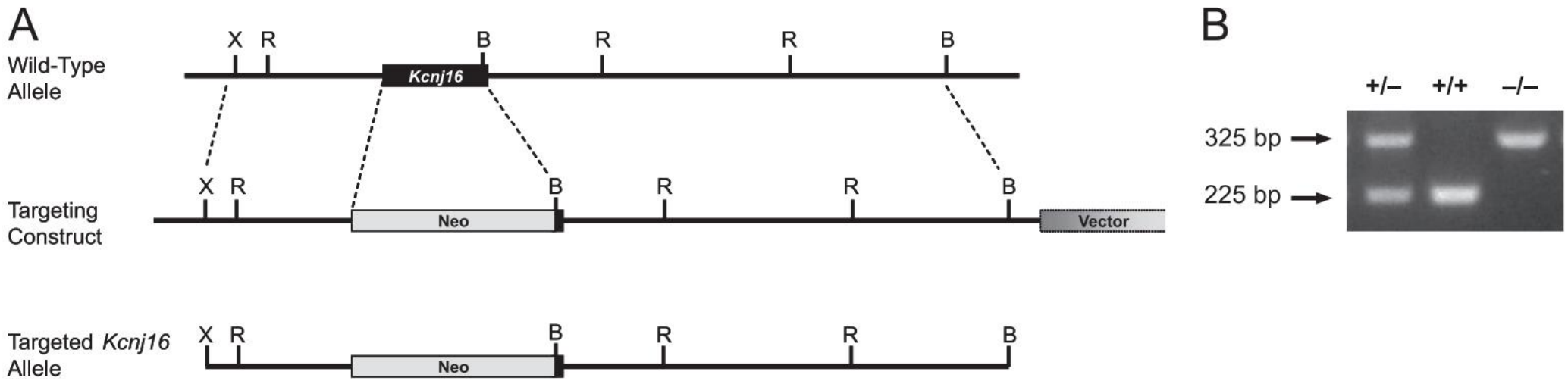


FIGURE 1. **Deletion of the Kcnj16 gene.** *A*, targeting strategy. *Top*, restriction map of the WT Kir5.1 (*Kcnj16*) gene used to construct the targeting vector. The entire 419-amino acid sequence of Kir5.1 is encoded on a single exon. *Dotted lines* define the limits of the recombinagenic arms. *Middle*, targeting construct in which a neomycin resistance gene (*Neo*) was inserted to replace amino acids 1 to 360 of the Kir5.1 open reading frame. *Bottom*, targeted *Kcnj16* allele. Restriction enzyme sites: *B*, BamHI; *X*, XbaI; *R*, EcoRI. *B*, genotype analysis. DNA from ear or tail biopsies was analyzed by PCR using a three-primer set. A 225-bp fragment from the WT Kir5.1 gene was amplified using a forward primer (5′-CTGCTTGCAGTTTGAAGGAAG-3′). This corresponds to codons 325–331 of the mouse Kir5.1 gene and a reverse primer (5′-CATTCATCTTGTGGGGACAGGACGGTCT-3′) corresponding to anticodons 389–397. A 325-bp PCR product from the successfully targeted gene was amplified using the reverse primer from the Kir5.1 gene and a forward primer (5′-AGGGGGAGGATTGG-GAAGACAATAGCA-3′) complementary to sequences in the 3′ region of the integrated neomycin resistance gene. PCR cycle parameters were 94 °C for 30 s then 30 cycles of 94 °C for 15 s, 60 °C for 20 s, and 72 °C for 40 s. Samples were run on a 1.8% agarose gel.

D'Adamo MC, et al., Genetic inactivation of Kcnj16 identifies Kir5.1 as an important determinant of neuronal PCO2/pH sensitivity. J Biol Chem. 2011 Jan 7;286(1):192-8