Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 2975–2979, April 1994
Genetics

Targeted gene walking by low stringency polymerase chain reaction: Assignment of a putative human brain sodium channel gene (*SCN3A*) to chromosome 2q24-31

(DNA/hybrid panel mapping/yeast artificial chromosome/*in situ* hybridization)

MADHU S. MALO*†, KAKUL SRIVASTAVA*, JOSHUA M. ANDRESEN*, XIAO-NING CHEN‡, JULIE R. KORENBERG‡, AND VERNON M. INGRAM*§

*Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139; and ‡Ahmansohn Department of Pediatrics and Medical Genetics Birth Defects Center, Cedars-Sinai Medical Center, University of California, Los Angeles, CA 90048

Communicated by Leonard S. Lerman, November 8, 1993

ABSTRACT **We have developed a low stringency polymerase chain reaction (LSPCR) to isolate the unknown neighboring region around a known DNA sequence, thus allowing efficient targeted gene walking. The method involves the polymerase chain reaction (PCR) with a single primer under conditions of low stringency for primer annealing (40°C) for the first few cycles followed by more cycles at high stringency (55°C). This enables the amplification of a targeted DNA fragment along with other nontargeted fragments. High stringency (55°C) nested PCRs with end-labeled primers are then used to generate a ladder of radioactive bands, which accurately identifies the targeted fragment(s). We performed LSPCR on human placental DNA using a highly conserved sodium channel-specific primer for 5 cycles at 40°C followed by 27 cycles at 55°C for primer annealing. Subsequently, using higher stringency (55°C) PCR with radiolabeled nested primers for 8 cycles, we have isolated a 0.66-kb fragment of a putative human sodium channel gene. Partial sequence (325 bp) of this fragment revealed a 270-bp region (exon) with homology to the rat brain sodium channel IIIα (RBIII) gene at the nucleotide (87%) and amino acid (92%) levels. Therefore, we putatively assign this sequence as a part of a gene coding the α-subunit of a human brain type III sodium channel (*SCN3A*). Using PCR on two human/rodent somatic cell hybrid panels with primers specific to this putative *SCN3A* gene, we have localized this gene to chromosome 2. Fluorescence *in situ* hybridization to human metaphase chromosomes was used to sublocalize the *SCN3A* gene to chromosome at 2q24-31. In conclusion, LSPCR is an efficient and sensitive method for targeted gene walking and is also useful for the isolation of homologous genes in related species.**

Sodium channels are voltage-gated transmembrane proteins that are involved in the generation of action potentials in electrically excitable cells (1). These channels are typically composed of a large (230–270 kDa) transmembrane α-subunit and one or two smaller (33–38 kDa) accessory β-subunits (1, 2). Abnormal sodium channels have been shown to be responsible for some diseases. For example, mutations of the human muscle sodium channel Iα gene, mapping on chromosome 17q23.1-25.3 (3, 4), cause hyperkalemic periodic paralysis (4, 5) and paramyotonia congenita (6). A rat epithelial sodium channel (7) has been shown to be related to *Caenorhabditis elegans* "degenerin" genes *deg-1* and *mec-4*, mutants of which are touch-insensitive. Neither the human counterpart nor any mutation of this rat epithelial sodium channel gene has yet been identified. Isolation of more human sodium channel genes and their subchromosomal localization would provide candidate loci to be evaluated in families with genetic disorders. Moreover, sodium channels are also the targets of many toxins and various clinically important drugs, such as anticonvulsants, antiarrhythmics, and local anesthetics (1).

The polymerase chain reaction (PCR) has revolutionized the isolation of a known targeted DNA sequence from minute amounts of DNA and RNA samples (8–10). Different modifications of PCR, such as inverse PCR, adaptor-ligated PCR, anchor PCR, and targeted gene walking PCR, have described the isolation of unknown DNA sequences adjacent to a known sequence (11–17). However, our attempts for targeted gene walking were unsuccessful when we tried these methods to isolate some sodium channel gene sequences from the human placental DNA. Because annealing of a few bases at the 3′ end is all that is required for a primer to be functional in PCR (18), nested PCR, or primer extension with only one radiolabeled "internal primer," as used by Parker *et al.* (17) for targeted gene walking, does not eliminate the possibility of misidentification of a fragment. Indeed, when we applied the targeted gene walking PCR (17) for the isolation of a few targeted sequences from human placental DNA, we obtained many radiolabeled bands that did not contain the targeted sequence (unpublished observations). To eliminate such misidentification of a targeted fragment we have modified the targeted gene walking PCR (17) and developed a low stringency polymerase chain reaction (LSPCR) for targeted gene walking. We applied the LSPCR to isolate a putative human brain sodium channel IIIα (*SCN3A*) gene sequence. We have localized this putative *SCN3A* gene to the chromosome band 2q24-31 by fluorescence *in situ* hybridization (FISH).

MATERIALS AND METHODS

Materials. Two complete human/rodent somatic cell hybrid panels were from the NIGMS, Coriell Institute for Medical Research (Camden, NJ). Most of the hybrids of panel 1 contained multiple human chromosomes, but the majority of the hybrids of panel 2 carried only one human chromosome.

LSPCR. For the amplification of human sodium channel genes by LSPCR the primer V17 was designed based on the rat brain sodium channel Iα (RBI) gene sequence (V17: 5′-GTTGTGAATGCCCTGTTAGGAGCAATTCC-3; see ref. 19, nt 4006–4034; also see Fig. 3). Using the primer V17 and another downstream reverse primer we PCR-amplified four short 55-bp (excluding primer sites) human sodium channel gene sequences (M.S.M., B. J. Blanchard, J.M.A.,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PCR, polymerase chain reaction; LSPCR, low stringency polymerase chain reaction; YAC, yeast artificial chromosome; FISH, fluorescence *in situ* hybridization.

†Present address: Department of Oncology and Immunology, Lederle Laboratories, Pearl River, NY 10965.
§To whom reprint requests should be addressed.

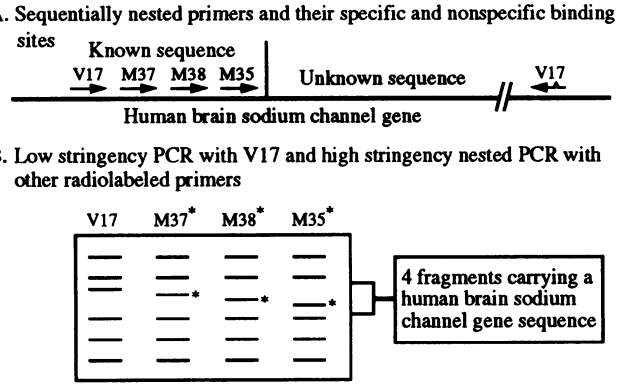

FIG. 1. Strategy of LSPCR. (*A*) Specific and nonspecific binding sites of a primer. A straight arrow indicates the specific binding site where the primer anneals at high stringency conditions (55°C). A wavy arrow indicates a nonspecific binding site where the primer anneals at low stringency conditions (40°C) with some nucleotides mismatched. Sequences of the primers are described in the text and are also shown in Fig. 3. (*B*) Isolation of a targeted gene fragment by generation of a ladder with nested PCR products. The radiolabeled primers and expected bands of the ladder are indicated by asterisks (*). Because of carried over V17 the original banding pattern (Fig. 2*A*) will be observed when the gel is stained with ethidium bromide.

X.-N.C., X. Li, E. W. Jabs, J.R.K., and V.M.I., unpublished observations). To identify these multiple sodium channel genes, the following degenerate nested primers (Fig. 3) were designed. The primers are M35, 5′-TTCTGG(T,C)TGAT-(C,A)TT(C,T)AGCATC-3′; M37, 5′-CAATTCC(A,T,C)-TC(C,T)ATCATGAATGTG-3′; and M38, 5′-CATGAATG-TG(C,T)T(G,A,T)(C,T)T(G,T)GT(C,G,T)TGTC-3′. LSPCR was performed in 25 μl of 1× *Taq* DNA polymerase buffer containing 0.2 mM of each dNTP, 75 pmol of primer V17, 0.25–2.5 μg of placental DNA, and 2.5 units of *Taq* DNA polymerase (Promega). Two-step cycling protocols were followed in the case of LSPCR. The first step was 94°C for 2 min and then 5 cycles of 94°C for 1 min, 40°C for 1 min (low stringency), and 72°C for 5 min followed by the second step of 27 cycles of 94°C for 1 min, 55°C for 1 min (high stringency), and 72°C for 5 min. The final extension was for 5 min at 74°C. The PCR products were analyzed by 10% polyacrylamide or 1.5% agarose gel electrophoresis (20).

Nested PCRs were performed on 2 μl of the LSPCR sample (Fig. 2*A*, lane 1) using 5–10 pmol of the end-labeled primers (M37, M38, and M35; specific activity, $>10^7$ cpm/μg of primer) for 8 cycles at the high annealing temperature of 55°C with no additional primer V17 added. Each nested PCR reaction contained >5.0 pmol of the original V17 primer. The nested PCR products were electrophoresed through a 10% polyacrylamide gel as well as through a 1.5% agarose gel. The gels were autoradiographed and the desired radioactive band from the polyacrylamide gel was cut out and eluted overnight at 37°C in an elution buffer [10 mM Tris·HCl, pH 8.0/1 mM EDTA/100 mM NaCl/1% (vol/vol) phenol].

Cloning and Sequencing. The eluted DNA was reamplified by PCR and sequenced by cycle sequencing with modified Vent DNA polymerase (exo⁻) (New England Biolabs) as described in Sears *et al.* (21). The preliminary sequence obtained was used to design the primer M40 (5′-ATCTTCCT-TGGAAACATCTTGA-3′; Fig. 3). PCR was performed on human placental DNA using primers V17 and M40. An expected 325-bp PCR fragment (VM1740) was amplified and cloned into the vector pCRII (TA Cloning System, Invitrogen). The clone pSC3 obtained was subsequently sequenced with a Sequenase kit (United States Biochemicals).

Mapping of Human/Rodent Somatic Cell Hybrid Panels. The human/rodent somatic cell hybrid panels were mapped by PCR amplification of a 303-bp *SCN3A* gene sequence using primers M37 and M40 (Fig. 3). The PCR product from cell line GM/NA10826B (carrying only human chromosome 2) was partially sequenced by cycle sequencing (21).

FISH. A yeast artificial chromosome (YAC), ySC3, carrying the putative *SCN3A* sequence was isolated (David Patterson, Denver, YAC address: A212A5) by performing PCR with primers M37 and M40 on a YAC library containing human genomic DNA partial *Eco*RI fragments (22). The presence of the *SCN3A* sequence in ySC3 was confirmed by sequencing the PCR product MM3740 obtained from ySC3 using primers M37 and M40. The YAC ySC3 was used as probe for *in situ* hybridization. The probe was labeled with biotin-11-dUTP (Sigma) using nick-translation (23) and hybridized to metaphase chromosomes prepared from normal male peripheral blood lymphocytes by the bromodeoxyuridine synchronization method (24). *In situ* hybridization was performed essentially according to the method described by Lichter *et al.* (25) with some modifications. Briefly, the hybridization solution contained 140 ng of probe, 7 μg of total human DNA, and 3 μg of sonicated salmon sperm DNA per 10 μl of hybridization mix. After preannealing at 37°C for 1 hr, the probe was applied to the slide and incubated overnight at 37°C. Posthybridization washes were at 44°C in 2× SSC/50% formamide (three times) followed by 1× SSC at 60°C (three times). Hybridized DNA was detected with avidin-conjugated fluorescein isothiocyanate (Vector Laboratories).

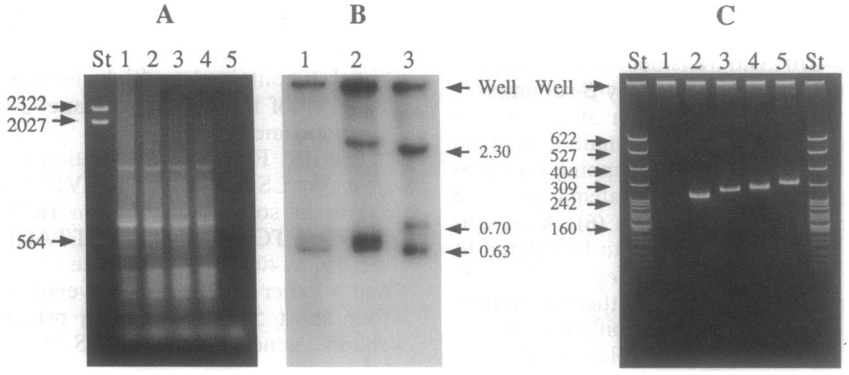

FIG. 2. PCR amplification of human sodium channel gene sequences. (*A*) LSPCR with rat-specific primer V17 on different quantities of human placental DNA. Lanes: St, standard size markers containing 0.5 μg of λ DNA *Hin*dIII; 1–4, LSPCR with V17 on 0.25, 0.5, 1.0, and 2.5 μg of DNA, respectively; 5, no DNA. (*B*) Autoradiograph of the polyacrylamide gel resolving the nested PCR products. Lanes: 1–3, nested PCRs with radiolabeled primers M37, M38, and M35, respectively. (*C*) PCR on human placental DNA with primers specific to the *SCN3A* gene sequence. Lanes: St, standard size markers containing 0.5 μg of pBR322 DNA *Msp* I; 1, no primer; 2, PCR product with primers M35 and M40; 3, PCR product with primers M38 and M40; 4, PCR product with primers M37 and M40; 5, PCR product with primers V17 and M40.

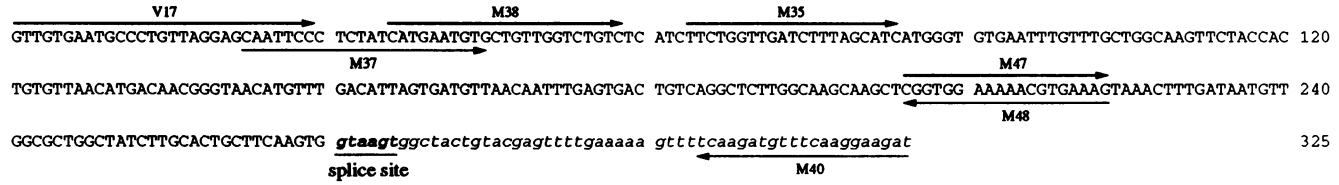

FIG. 3. Genomic nucleotide sequence of a part of a putative human brain sodium channel IIIα (*SCN3A*) gene containing a part of an exon. The exon sequence is indicated by uppercase letters and the intron sequence is shown by lowercase italics. A putative splice site is marked and underlined. The consensus nucleotides of the splice site are indicated boldface. The related primer sequences are marked and arrows indicate the direction of DNA synthesis. The V17 region corresponds to the rat brain sodium channel Iα (RBI) gene sequence.

One amplification was carried out using biotinylated-anti-avidin. To generate clear reverse bands, metaphase chromosomes were counterstained with chromomycin A3 followed by distamycin A, a modification of the procedure of Magenis *et al.* (26). The image was captured by using a Photometrics cooled charged-coupled device (CCD) camera (CH250) and BDS (Biological Detection System) image software.

RESULTS

Strategy for Targeted Gene Walking by LSPCR. We targeted the gene walking from four short 55-bp sodium channel gene sequences (see *Materials and Methods*). The strategy of LSPCR is depicted in Fig. 1. Under the conditions of low stringency for annealing (40°C), the primer V17 (specific to four human sodium channel gene sequences mentioned above) binds to the specific binding site as well as to some other nonspecific sites with some possible mismatches. Annealing of only a few bases at the 3′ end is required for a primer to be functional in PCR (18) and hence LSPCR amplifies multiple fragments of DNA, one of which should carry the desired specific priming site—i.e., a sodium channel gene sequence. The desired specific fragment is identified later by performing nested PCRs on the LSPCR products with end-labeled primers (M37, M38, M35; see Figs. 1 and 3) under conditions of high stringency for primer annealing (55°C). A ladder of radioactive bands of sequentially decreasing size, as indicated in Fig. 1, accurately identifies the desired specific fragments.

Isolation of a Human Brain Sodium Channel IIIα (*SCN3A*) Gene Sequence. The α-subunits (type I) of the sodium channel

```
SCN3A                GTTGTGAATGCCCTGTTAGGAGCAATTCCCTCTATCATGAATGTGCTGTTGGTCTGTCTCATCTTCTGGTTGATCTTTAGCATCATG  87
SCN1A  tttgtgtattagGTGGTTGTGAATGCCCTTTTAGGAGCAATTCCATCCATCATGAATGTGCTTCTGGTTTGTCTTATATTCTGGCTAATTTTCAGCATCATG  127
SCN2A  gaaggaatgaggGCTGTTGTAAATGCTCTTTTAGGAGCCATTCCATCTATCATGAATGTACTTCTGGTTTGTCTGATCTTTTGGCTAATATTCAGTATCATG  4166
RBI    gaagggatgaggGTGGTTGTGAATGCCCTGTTAGGAGCAATTCCATCCATCATGAATGTGCTTCTGGTTTGCCTTATATTCTGGCTAATTTTCAGCATCATG  4092
RBII   gaaggaatgaggGTTGTTGTAAACGCTCTCTTAGGCGCCATCCCCTCCATAATGAACGTACTTCTGGTCTGCCTGATCTTTTGGCTAATATTCAGTATCATG  4062
RBIII  gaaggcatgaggGTGGTTGTAAATGCTCTTGTTGGTGCAATTCCCTCCATCATGAATGTGTTATTGGTGTGTCTCATCTTCTGGCTGATTTTTAGCATCATG  3906
SCN3A                  V  V  N  A  L  L  G  A  I  P  S  I  M  N  V  L  L  V  C  L  I  F  W  L  I  F  S  I  M
SCN1A               V  V  V  N  A  L  L  G  A  I  P  S  I  M  N  V  L  L  V  C  L  I  F  W  L  I  F  S  I  M
SCN2A               A  V  V  N  A  L  L  G  A  I  P  S  I  M  N  V  L  L  V  C  L  I  F  W  L  I  F  S  I  M
RBI                 V  V  V  N  A  L  L  G  A  I  P  S  I  M  N  V  L  L  V  C  L  I  F  W  L  I  F  S  I  M
RBII                V  V  V  N  A  L  L  G  A  I  P  S  I  M  N  V  L  L  V  C  L  I  F  W  L  I  F  S  I  M
RBIII               V  V  V  N  A  L  V  G  A  I  P  S  I  M  N  V  L  L  V  C  L  I  F  W  L  I  F  S  I  M
                                                                                  IIIS5 transmembrane segment

SCN3A  GGTGTGAATTTGTTTGCTGGCAAGTTCTACCACTGTGTTAACATGACAACGGGTAACATGTTTGACATTAGTGATGTTAACAATTTGAGTGACTGTCAGGCT  189
SCN1A  GGCGTAAATTTGTTTGCTGGCAAATTCTACCACTGTATTAACACCACAACTGGTGACAGGTTTGACATCGAAGACGTGAATAATCATACTGATTGCCTAAAA  229
SCN2A  GGAGTGAATCTCTTTGCTGGCAAGTTTTACCATTGTATTAATTACACCACCTGGAGAGATGTTTGATGTAAGCGTGGTCAACAACTACAGTGAGTGCAAAGCT  4268
RBI    GGCGTAAATTTGTTTGCTGGCAAATTCTACCACTGTGTTAACACCACAACTGGTGACACATTTGAGATCACCGAAGTCAATAATCATTCTGATTGCCTAAAA  4194
RBII   GGGGTGAATCTCTTTGCTGGCAAGTTCTACCATTGCATCAACTACACCACGGGGGAAATGTTTGATGTGAGCGTGGTCAACAACTACAGTGAATGCCAAGCT  4164
RBIII  GGTGTGAATCTGTTTGCTGGAAAGTTCTATCACTGTGTTAACACGACAACAGGCAACATGTTTGAAATAAAAGAAGTGAACAATTTCAGTGACTGTCAGGCT  4008
SCN3A  G  V  N  L  F  A  G  K  F  Y  H  C  V  N  M  T  T  G  N  M  F  D  I  S  D  V  N  N  L  S  D  C  Q  A
SCN1A  G  V  N  L  F  A  G  K  F  Y  H  C  I  N  T  T  T  G  D  R  F  D  I  E  D  V  N  N  H  T  D  C  L  K
SCN2A  G  V  N  L  F  A  G  K  F  Y  H  C  I  N  Y  T  T  G  E  M  F  D  V  S  V  V  N  N  Y  S  E  C  K  A
RBI    G  V  N  L  F  A  G  K  F  Y  H  C  V  N  T  T  T  G  D  T  F  E  I  T  E  V  N  N  H  S  D  C  L  K
RBII   G  V  N  L  F  A  G  K  F  Y  H  C  I  N  Y  T  T  G  E  M  F  D  V  S  V  V  N  N  Y  S  E  C  Q  A
RBIII  G  V  N  L  F  A  G  K  F  Y  H  C  V  N  T  T  T  G  D  M  F  E  I  K  E  V  N  N  F  S  D  C  Q  A

SCN3A  CTTGGCAAGCAA---------GCTCGGTGGAAAAACGTGAAAGTAAACTTTGATAATGTTGGCGCTGGCTATCTTGCACTGCTTCAAGTGgtaagtggctac  282
SCN1A  CTAATAGAAAGAAATGAGACTGCTCGATGGAAAAATGTGAAAGTAAACTTTGATAATGTAGGATTTGGGTATCTCTCTTTGCTTCAAGTgtaagtgaacac  331
SCN2A  CTCATTGAGAGCAATCAAACTGCCAGGTGGAAAAATGTGAAAGTAAACTTTGATAACGTAGGACTTGGATATCTGTCTCTACTTCAAGTAgccacgtttaag  4370
RBI    CTAATAGAAAGAAATGAGACTGCCCGGTGGAAAAATGTGAAAGTAAACTTTGATAATGTAGGATTTGGGTATCTTTCTTTGCTTCAAGTTgcgacgtttaag  4296
RBII   CTCATTGAGAGCAATCAGACGGCCAGGTGGAAGAACGTGAAGGTCAACTTCGATAACGTGGGACTCGGATACCTTTCTCTGCTCCAAGTAgccacctttaaa  4266
RBIII  CTTGGCAAGCAA---------GCCCGGTGGAAGAATGTGAAAGTCAACTTTGACAACGTTGGGGCTGGCTACCTGGCATTGCTGCAAGTGgccacattcaaa  4101
SCN3A  L  G  K  Q  -  -  -  A  R  W  K  N  V  K  V  N  F  D  N  V  G  A  G  Y  L  A  L  L  Q  V
SCN1A  L  I  E  R  N  E  T  A  R  W  K  N  V  K  V  N  F  D  N  V  G  F  G  Y  L  S  L  L  Q  V
SCN2A  L  I  E  S  N  Q  T  A  R  W  K  N  V  K  V  N  F  D  N  V  G  L  G  Y  L  S  L  L  Q  V
RBI    L  I  E  R  N  E  T  A  R  W  K  N  V  K  V  N  F  D  N  V  G  F  G  Y  L  S  L  L  Q  V
RBII   L  I  E  S  N  Q  T  A  R  W  K  N  V  K  V  N  F  D  N  V  G  L  G  Y  L  S  L  L  Q  V
RBIII  L  G  K  Q  -  -  -  A  R  W  K  N  V  K  V  N  F  D  N  V  G  A  G  Y  L  A  L  L  Q  V
```

FIG. 4. Primary structure of a part of an exon carrying the IIIS5 region of the putative human brain sodium channel IIIα (*SCN3A*) gene. The rows of amino acid sequences are shown below the rows of nucleotide sequences in chronological order. The sequences are *SCN3A* (human brain sodium channel IIIα gene genomic DNA sequence; this report), *SCN1A* (human brain sodium channel Iα gene genomic DNA sequence; M.S.M., B. J. Blanchard, J.M.A., X.-N.C., X. Li, E. W. Jabs, J.R.K., and V.M.I., unpublished observations), *SCN2A* (human brain sodium channel IIα gene cDNA sequence HBA; ref. 28), RBI (rat brain sodium channel Iα gene cDNA sequence; ref. 19), RBII (rat brain sodium channel IIα gene cDNA sequence; ref. 19), and RBIII (rat brain sodium channel IIIα gene cDNA sequence; ref. 27). Numbers on the right correspond to nucleotide positions. The *SCN3A* exon sequence and the corresponding *SCN1A*, *SCN2A*, and rat cDNA sequences are shown in uppercase letters. The italic uppercase letters indicate the V17 region (Fig. 3), which corresponds to the RBI sequence. To simplify calculations of homology, this region has been counted as a part of the *SCN3A* sequence. The intron sequences of *SCN3A* and *SCN1A* genes are shown by lowercase italics and the rest of the cDNA sequences of the other genes are shown by lowercase nonitalic letters. A bold uppercase letter represents a nucleotide or an amino acid that differs from the *SCN3A* sequence. A gap of 9 nucleotides has been introduced in RBIII sequence after nucleotide position 4023 for maximum homology. The transmembrane region IIIS5 (19) has been underlined and marked.

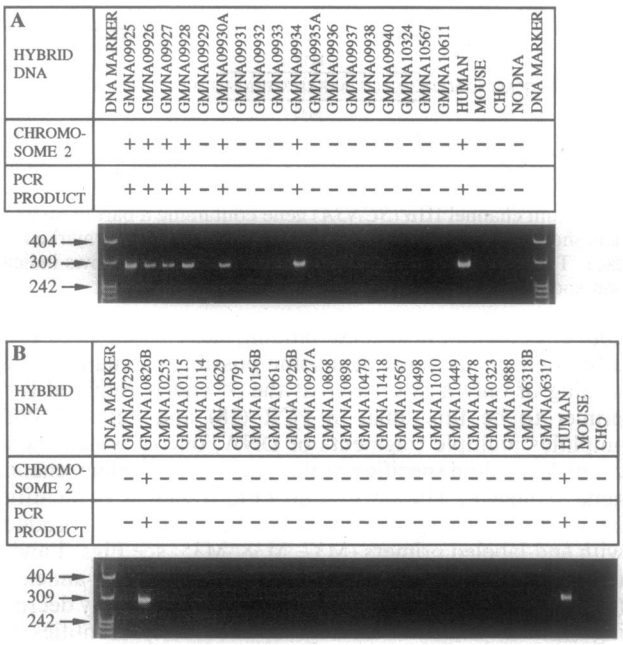

FIG. 5. Chromosomal assignment of the *SCN3A* gene by PCR mapping of the NIGMS human/rodent hybrid somatic cell lines. (*A*) PCR on panel 1 hybrids using exon-specific primer M37 and intron-specific primer M40 (see Fig. 3). (*B*) PCR on hybrids of panel 2 using the same primers. The expected PCR product is 303 bp.

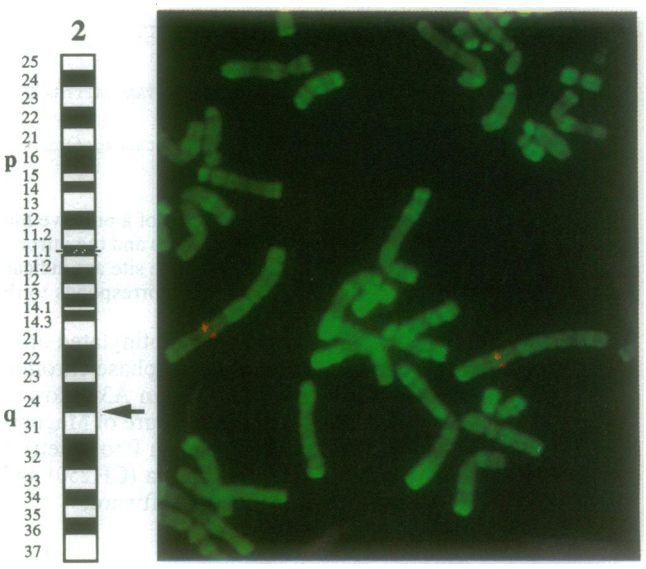

FIG. 6. FISH mapping of the *SCN3A* gene to chromosomal region 2q24-31. The red dots indicate the fluorescein isothiocyanate signals and the bold arrow along the side of chromosome 2 bands indicates the location of the *SCN3A* gene.

proteins are highly conserved; the single polypeptide chain consists of four homologous repeats (I–IV), each of which contains six transmembrane α-helices (S1–S6) (19). The IIIS5 regions of the rat sodium channel genes are very highly conserved among types I, II, IIA, and III (2, 19, 27). The PCR primer V17 was designed from this region to use in LSPCR, thus allowing possible amplification of many sodium channel gene sequences. LSPCR was performed on human placental DNA using the primer V17 and the result is shown in Fig. 2*A*. It is observed that LSPCRs on DNA concentrations of 0.25–2.5 μg generate the same banding patterns. The autoradiography of the polyacrylamide gel resolving the nested PCR products shows three bands of sizes 2.30 kb, 0.70 kb, and 0.63 kb in lane 3 (Fig. 2*B*). The 0.63-kb band (Fig. 2*B*, lane 3) is accompanied by bands of sequentially increasing sizes in lanes 2 and 1 (Fig. 2*B*), whereas, the 0.70-kb band is not accompanied by similar bands. The 2.3-kb band is apparently accompanied by bands of sequentially increasing sizes in lanes 2 and 1 (Fig. 2*B*). However, the size difference of 13–28 bp (see primer sites in Fig. 3) among the double-stranded DNA fragments of these large sizes should not generate this ladder in a polyacrylamide gel. Therefore, these bands may be single-stranded DNA of the targeted fragments. After electrophoresis through a 1.5% agarose gel, subsequent autoradiography shows that the 2.3-kb band (Fig. 2*B*, lane 3) as well as the other two bands forming the ladder (Fig. 2*B*, lanes 1 and 2; in polyacrylamide gel) have disappeared and three new bands of about 0.42 kb appear in the agarose gel (data not shown). However, the 0.63-, 0.65-, and 0.66-kb bands remained constant in both cases. We thus inferred that the 0.63-, 0.65-, and 0.66-kb bands were the targeted fragments (double-stranded) of a sodium channel gene.

The 0.66-kb band in lane 1 (Fig. 2*B*) was cut out of the gel, eluted, and reamplified by PCR using primers M37 and V17; it was then partially sequenced by cycle sequencing using the radiolabeled primer M35. The preliminary sequence information obtained was used to design a reverse primer, M40 (Fig. 3). Fig. 2*C* shows the result of PCR amplifications on human placental DNA using the primer M40 in conjunction with other primers. This result confirms the predicted ladder-forming pattern of PCR fragments (also see Fig. 3 for primer sites). The 325-bp PCR product VM1740 produced by primers V17 and M40 (Fig. 2*C*, lane 5) was subcloned into the vector pCRII to obtain the plasmid pSC3. Both strands of the insert VM1740 in pSC3 were then sequenced (Fig. 3).

Comparison of this sequence of VM1740 with the human and rat brain sodium channel cDNA sequences shows that the isolated sequence contains a part (270 bp) of an exon (Fig. 4) that is 78%, 75%, 78%, 73%, and 87% homologous at the nucleotide level and 84%, 86%, 84%, 87%, and 92% homologous at the amino acid level with the *SCN1A*, *SCN2A*, RBI, RBII, and RBIII sequences, respectively. Therefore, we putatively recognize the sequence VM1740 as a fragment of a human brain sodium channel IIIα (*SCN3A*) gene. The identified intron sequence is rich (65%) in A·T bases.

Localization of the *SCN3A* Gene to Chromosome 2 by Somatic Cell Hybrid Panel Mapping. The result of PCR mapping of the hybrid panels is shown in Fig. 5 and the discordance analysis is shown in Table 1. There was 100% concordance between the presence of the human sodium channel gene PCR product (303 bp; MM3740) and chromosome 2 when the complete NIGMS human/rodent somatic cell hybrid panels 1 and 2 were analyzed. There was greater

Table 1. Mapping of the putative *SCN3A* gene to chromosome 2: Analysis of two complete human/rodent somatic cell hybrid panels

	No. of discordant hybrids for each chromosome*																							
Hybrid panel	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
1 (18 hybrids)	4	0	8	9	6	8	8	7	7	9	7	9	4	7	6	5	9	4	7	7	7	5	6	7
2 (24 hybrids)	2	0	2	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	2

*Discordant hybrids are hybrid DNAs that on amplification produced the PCR product and did not contain the human chromosome or did not produce the PCR product and contained the chromosome. Concordant hybrids are hybrid DNAs that on amplification produced the PCR product and contained the human chromosome or did not produce the PCR product and did not contain the chromosome.

than 22% and 8% discordances for all other chromosomes in panel 1 and panel 2, respectively. We also partially sequenced the PCR product obtained from the hybrid cell line GM/NA10826B (carrying only human chromosome 2), which revealed that the product contained the *SCN3A* sequence. Thus the *SCN3A* gene mapped to chromosome 2.

Sublocalization of the *SCN3A* Gene to Chromosome 2q24-31 by FISH. The probe ySC3 carrying the *SCN3A* genomic sequence mapped to 2q24-31 (Fig. 6). Two independent experiments were carried out and >200 metaphase cells were examined. Signals were noted on two chromatids of at least one chromosome 2 in 50% of cells. No other chromosome bands with signals were detected in >0.5% of cells.

DISCUSSION

The successful amplification from placental DNA of a DNA sequence belonging to a human sodium channel gene using the LSPCR shows the efficiency and sensitivity of the method for targeted gene walking in the case of a large mammalian genome when other methods failed. Compared to linear amplification obtained by primer extension (17), our method achieves exponential amplification of a targeted fragment using nested PCR with a radiolabeled primer. Therefore, our method is more sensitive for the identification of a desired fragment than a protocol based on linear amplification. However, in a PCR a fraction of the template molecules is not primed and the sensitivity of this method may identify these single-stranded template molecules with different conformations as different bands. These potentially confusing single-stranded DNA bands were easily excluded by electrophoresing through polyacrylamide and agarose gels.

When we tried targeted gene walking PCR (17) to amplify a few targeted sequences, we obtained many radiolabeled bands that, when sequenced, did not carry the targeted sequence (unpublished observations). These bands were probably due to nonspecific annealing and subsequent extension (18) of the radiolabeled nested primer. The ladder-forming pattern of the desired nested PCR products as reported here accurately identifies a desired fragment. We expected that because of high homology among the sodium channel genes a few additional human sodium channel gene sequences would be amplified using LSPCR with the primer V17; however, we were able to amplify only one sodium channel gene sequence. This limitation of the method was probably due to the absence of a nonspecific binding site for V17 within the amplifiable distance from the V17 specific binding site in the other sodium channel genes. To isolate the other sodium channel genes, a lower temperature for annealing or a primer specific to another region may be used in an LSPCR. Alternatively, V17 in conjunction with a "walking" primer as used in Parker *et al.* (17) could be used in an LSPCR, and subsequently the LSPCR products could be subjected to nested PCRs with different radiolabeled primers for the identification of a ladder(s) of the desired fragments.

We mapped the *SCN3A* gene to chromosome 2 with 100% concordance using PCR on human/rodent somatic cell hybrid panels. This is a useful alternative protocol to conventional mapping of somatic cell hybrid panels by Southern hybridization (29). The result of FISH mapped the gene to chromosome 2q24-31. The human brain sodium channel IIα (*SCN2A*) gene has been mapped to 2q23-24.3 (28–30) and we have mapped the human brain sodium channel Iα (*SCN1A*) gene to chromosome 2q24 (M.S.M., B. J. Blanchard, J.M.A., X.-N.C., X. Li, E. W. Jabs, J.R.K., and V.M.I., unpublished observations). Mouse brain sodium channel genes form a cluster on chromosome 2 (31). The localization of three human brain sodium channels to 2q24-31 strongly suggests that the human brain sodium channel genes might also constitute a cluster. This could be of evolutionary significance. Finally, three human brain sodium channel genes have now been subchromosomally localized (ref. 30; this report; unpublished observations mentioned above), providing candidate loci to be evaluated in families with genetic disorders.

This work was supported by the grants from the John & Dorothy Wilson Fund and the Markey Foundation to V.M.I. and by grants from the National Institutes of Health (SSS-2 HG00037 to J.R.K.) and the Department of Energy (DE-FG03-93ER61402 to J.R.K.).

1. Hille, B. (1992) *Ionic Channels of Excitable Membranes* (Sinauer, Sunderland, MA).
2. Auld, V. J., Goldin, A. L., Krafte, D. S., Marshall, J., Dunn, J. M., Catterall, W. A., Lester, H. A., Davidson, N. & Dunn, R. J. (1988) *Neuron* **1**, 449–461.
3. George, A. L., Jr., Ledbetter, D. H., Kallen, R. G. & Barchi, R. L. (1991) *Genomics* **9**, 555–556.
4. Fontaine, B., Khurana, T. S., Hoffman, E. P., Bruns, G. A. P., Haines, J. L., Trofatter, J. A., Hanson, M. P., Rich, J., McFarlane, H., McKenna-Yasek, D., Romano, D., Gusella, J. F. & Brown, R. H., Jr. (1990) *Science* **250**, 1000–1003.
5. Rojas, C. V., Wang, J., Schwartz, L. S., Hoffman, E. P., Powell, B. R. & Brown, R. H., Jr. (1991) *Nature (London)* **354**, 387–389.
6. McClatchey, A. I., VandenBergh, P., Pericak-Vance, M. A., Raskund, W., Verellen, C., McKenna-Yasek, D., Rao, K., Haines, J. L., Bird, T., Brown, R. H. & Gusella, J. F. (1992) *Cell* **68**, 769–774.
7. Canessa, C. M., Horisberger, J. & Rossier, B. C. (1993) *Nature (London)* **361**, 467–470.
8. Saiki, R. K., Sharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. & Arnheim, N. (1985) *Science* **230**, 1350–1354.
9. Todd, J. A., Bell, J. I. & McDevit, H. O. (1987) *Nature (London)* **329**, 599–604.
10. Stoflet, E. S., Koeberl, D. D., Sarkar, G. & Sommer, S. S. (1988) *Science* **239**, 491–494.
11. Collasius, M., Puchta, H., Schlenker, S. & Valet, G. (1991) *J. Virol. Methods* **32**, 115–119.
12. Copley, C. G., Boot, C., Bundell, K. & McPheat, W. L. (1991) *Bio/Technology* **9**, 74–79.
13. Ochman, H., Gerber, A. S. & Hartl, D. L. (1988) *Genetics* **120**, 621–623.
14. Frohman, M. A., Dush, M. K. & Martin, G. R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8998–9002.
15. Triglia, T., Peterson, M. G. & Kemp, D. J. (1988) *Nucleic Acids Res.* **16**, 8186.
16. Loh, E. Y., Elliott, J. F., Cwirla, S., Lanier, L. L. & Davis, M. M. (1989) *Science* **243**, 217–220.
17. Parker, J. D., Rabinovitch, P. S. & Burmer, G. C. (1991) *Nucleic Acids Res.* **19**, 3055–3060.
18. Sommer, R. & Tautz, D. (1989) *Nucleic Acids Res.* **17**, 6749.
19. Noda, M., Ikeda, T., Kayano, T., Suzuki, H., Takeshima, H., Kurasaki, M., Takahashi, H. & Numa, S. (1986) *Nature (London)* **320**, 188–192.
20. Sambrook, J., Fristch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
21. Sears, L. E., Morgan, L. S., Kissinger, C., Creasey, T., Perry-O'Keefe, H., Roskey, M., Sutherland, E. & Slatko, B. E. (1992) *BioTechniques* **13**, 626–633.
22. Brownstein, B. H., Silverman, G. A., Little, R. D., Burke, D. T., Korsmeyer, S. J., Schlessinger, D. & Olson, M. V. (1989) *Science* **244**, 1348–1351.
23. Langer-Safer, P. R., Levine, M. & Ward, D. C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4381–4385.
24. Zabel, B. U., Naylor, S. L., Sakaguchi, A. Y., Bell, G. I. & Shows, T. B. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6932–6936.
25. Lichter, P., Tang, C., Call, K., Hermanson, G., Evans, G. A., Housman, D. & Ward, D. C. (1990) *Science* **247**, 64–69.
26. Magenis, R. E., Donlon, T. A. & Tomar, D. R. (1985) *Hum. Genet.* **69**, 300–303.
27. Kayano, T., Noda, M., Flockerzi, V., Takahashi, H. & Numa, S. (1988) *FEBS Lett.* **228**, 187–194.
28. Ahmed, C. M. I., Ware, D. H., Lee, S. C., Patten, C. D., Ferrer-Montiel, A. V., Schinder, A. F., McPherson, J. D., Wagner-McPherson, C. B., Wasmuth, J. J., Evans, G. A. & Montal, M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 8220–8224.
29. Litt, M., Luty, J., Kwak, M., Allen, L., Magenis, R. E. & Mandel, G. (1989) *Genomics* **5**, 204–208.
30. Han, J., Lu, C., Brown, G. B. & Rado, T. A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 335–339.
31. Malo, D., Schurr, E., Dorfman, J., Canfield, V., Levenson, R. & Gros, P. (1991) *Genomics* **10**, 666–672.