GENOMICS 13, 381–388 (1992)

Expression and Chromosome Localization of the Murine Cystic Fibrosis Transmembrane Conductance Regulator

KEVIN A. KELLEY,*[,1] STEFAN STAMM,*[,†] AND CHRISTINE A. KOZAK‡

*Mount Sinai School of Medicine, Arthur M. Fishberg Research Center for Neurobiology, One Gustave Levy Place, New York, New York 10029; †Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724; and ‡National Institute of Allergy and Infectious Diseases, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20892

Received September 3, 1991; revised January 9, 1992

A 13.5-kb genomic fragment of the mouse cystic fibrosis transmembrane conductance regulator (CFTR) gene was isolated from a C57BL/6J liver DNA library, using a human CFTR exon 10 probe. This region of the human gene includes the most common cystic fibrosis mutation (deletion of the Phe^{508} residue) in the first nucleotide binding domain of CFTR. Sequence analysis demonstrated 87% identity between the predicted mouse and the normal human CFTR exon 10 sequences, including conservation of the Phe^{508} residue. Northern analysis revealed that the mouse gene is expressed in intestine, lung, stomach, kidney, and salivary gland. In contrast to human CFTR, murine CFTR transcripts were not detectable by Northern analysis in the liver or pancreas. More sensitive PCR analysis, however, revealed that the mouse CFTR gene is weakly expressed in other tissues, including liver and pancreas. During development, mouse CFTR transcripts were observed as early as Embryonic Day 13. Southern analysis of mouse × Chinese hamster somatic cell hybrid DNAs mapped the mouse CFTR locus (*Cftr*) to Chromosome 6 (Chr 6). Subsequent typing of the progeny of an interspecies backcross revealed that *Cftr* is closely linked to the proto-oncogene c-*met* locus (*Met*) in the centromeric region of mouse Chr 6, consistent with the observation that there is a conserved chromosomal segment on human chromosome 7 and mouse Chr 6. © 1992 Academic Press, Inc.

INTRODUCTION

Cystic fibrosis (CF) is a lethal autosomal recessive disorder in humans, with a carrier frequency estimated at 5% among Caucasians (Boat *et al.*, 1989). Recent attempts to understand the molecular nature of this disease have focused on the isolation of the affected gene. As a result, the human CF gene has been successfully identified using genetic and molecular approaches based on its chromosome localization (Rommens *et al.*, 1989). From the predicted protein structure, the CF gene product is similar to a number of membrane-associated active transport proteins and is likely to be involved in membrane ion transport (Riordan *et al.*, 1989). Thus, the CF gene product has been termed the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR is an N-linked glycoprotein that consists of two repeats containing a nucleotide binding domain (NBD) and six transmembrane segments; the two repeats are separated by a regulatory (R) domain (Riordan *et al.*, 1989). A number of CFTR mutations have been identified, including the deletion of a phenylalanine residue at position 508 (ΔF508) in the first NBD (Riordan *et al.*, 1989; Kerem *et al.*, 1989). In addition to the prevalent ΔF508 mutation, which is present in approximately 70% of CF patients, numerous additional mutations have been identified, many of which are also located within the first NBD (Cutting *et al.*, 1990; Kerem *et al.*, 1990).

The availability of an animal model for CF would provide a suitable system for determining the efficacy of novel treatments, including gene therapy, based on the molecular defects that have been identified in the CF gene, and allow more detailed examinations of the basic molecular defect, as well as the progression of the disease. The production of mouse models for human genetic diseases is possible through manipulation of homologous disease-related genes in pluripotent mouse embryonic stem (ES) cells and has been aided by recent advances in enrichment and analysis of gene targeting events (Frohman and Martin, 1989). The creation of a mouse CF model depends upon the presence of a mouse homologue for the gene that is affected in human CF patients. In particular, only a part of the gene that spans the region to be altered is required for homologous recombination in ES cells. To this end, we have isolated a fragment of the mouse CFTR (mCFTR) gene that is homologous to the region of the human CFTR (hCFTR) gene containing exon 10. For the present study, the isolated mCFTR sequence was used as a molecular probe to examine the expression of the mCFTR homologue and to perform definitive mapping of the mouse gene.

MATERIALS AND METHODS

Polymerase chain reaction (PCR). Genomic DNA templates were amplified as described elsewhere (Saiki *et al.*, 1988). As standard con-

[1] To whom reprint requests should be addressed.

0888-7543/92 $5.00

ditions, we used 30 cycles of PCR (denaturation for 30 s at 94°C, annealing for 1 min at 55°C, and extension for 2 min at 72°C) following an initial denaturation at 94°C for 4 min. The accuracy of the thermocycler was controlled using an external device (Stamm *et al.*, 1991). The amplified fragments were analyzed by agarose gel electrophoresis of one-tenth of the reaction mixture. For subcloning purposes, the remainder of the reaction product was extracted with phenol and precipitated. The PCR fragments were then digested with the appropriate restriction enzymes to generate the flanking restriction sites, which were synthesized on the ends of the PCR primers. The resulting fragments were purified by electrophoresis through low-melting-point agarose (FMC, Rockland, ME) and subcloned into a pSP64 vector (Promega, Madison, WI).

For isolation of partial cDNA sequences and mRNA analysis, mouse tissue RNAs were annealed with antisense oligonucleotide primers and extended with M-MLV RNase H^- reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD), according to the manufacturer's instructions, for 1 h at 37°C. The reaction volume was then adjusted to 50 μl and the cDNA template was stored at −20°C. The first-strand cDNA templates were PCR-amplified as described above. For expression analysis, the PCR reactions were modified by the addition of 2.5 μCi of 3000 Ci/mmol [α-^{32}P]dCTP (NEN/DuPont, Boston, MA) to each reaction. The PCR-generated reaction products were then analyzed by autoradiography after electrophoresis through 5% polyacrylamide, 7 *M* urea gels.

Oligonucleotide primers. Based on the published hCFTR exon 10 sequence (Riordan *et al.*, 1989), two oligonucleotides, KK009 (5′-G<u>GAATTC</u>GACTTCACTTCTAATGATGA-3′) and KK010 (5′-G<u>GGATCC</u>CTCTTCTAGTTGGCATGCTT-3′), were synthesized for PCR amplification and cloning of the 193-bp hCFTR exon 10. KK009 and KK010 were designed with *Eco*RI and *Bam*HI restriction endonuclease sites (underlined) on their 5′ ends, respectively, for subcloning of the hCFTR exon 10 fragment.

Oligonucleotides KK035 (5′-G<u>GAATTC</u>GACATCACTCCTGATGTTGA-3′) and KK036 (5′-G<u>GGATCC</u>CTGCTGTAGTTGGCAAGCTT-3′), containing *Eco*RI and *Bam*HI restriction endonuclease sites (underlined) on their 5′ ends, respectively, were synthesized to generate a PCR-amplified mCFTR exon 10 fragment from C57BL/6J genomic DNA. These oligonucleotides were based on the sequence derived from the isolated mCFTR genomic clone containing exon 10 (Fig. 2).

The oligonucleotide KK059 (5′-GGATTTGGGGAATTACTGGAG-3′) was based on the sequence at the 5′ end of mCFTR exon 9, as determined by sequence analysis of a PCR-generated partial cDNA clone (pmCF-3). KK042 is identical to KK036, except for the incorporation of an additional *Sal*I restriction site on the 5′ end. KK059 and KK042 were used to examine mCFTR expression by PCR amplification of KK042-primed, first-strand cDNA from total tissue RNAs.

The oligonucleotides KK055 (5′-G<u>GGATCC</u>TGGGTCAGAAGGACTCC-3′) and Act-1 (5′-AACATGATCTGGGTCAT-3′) were based on the published sequence of a mouse cytoskeletal β-actin cDNA (Tokunaga *et al.*, 1986). KK055 contains a *Bam*HI restriction endonuclease site (underlined) on its 5′ end. These oligonucleotides were used for PCR amplification of Act-1-primed, reverse-transcribed actin cDNA from total tissue RNAs.

PCR-generated probes. The standard PCR reaction described above was modified to produce small, double-stranded DNA probes with high specific activity (3–5 × 10^9 cpm/μg). The templates used to produce these probes were PCR products amplified from either genomic or plasmid DNA. The reaction conditions were the same as those used for the standard PCR reactions described above, except dCTP was replaced with 250 μCi (83.3 pmol, 1.7 μ*M*) of 3000 Ci/mmol [α-^{32}P]dCTP (NEN/DuPont) and the 72°C incubation was increased to 4 min. The hCFTR exon 10 probe was produced using oligonucleotides KK009 and KK010, and the mCFTR exon 10 probe was generated with oligonucleotides KK035 and KK036. The ^{32}P-labeled PCR fragments were purified through push-columns (Stratagene, La Jolla, CA), denatured at 100°C for 5 min, and used as hybridization probes for genomic library screening, as well as Southern and Northern analyses. It is somewhat surprising that we obtained full-length PCR products using a dCTP concentration of 1.7 μ*M*, which is lower than the K_m of about 10–15 μ*M* (Williams, 1989). This suboptimal concentration can probably be compensated for by the longer extension time, which was also found by other groups to be useful for obtaining full-length labeled PCR products (Schowalter and Sommer, 1989; Jansen and Ledley, 1989).

Genomic library construction and screening. A mouse genomic library was constructed by cloning *Bam*HI-digested C57BL/6J liver DNA into the *Bam*HI restriction site of a λEMBL3 vector (Stratagene, La Jolla, CA). Recombinant plaques were screened by the method of Benton and Davis (1977), using a PCR-generated hCFTR exon 10 probe. Recombinant phage DNA was prepared and analyzed as described by Kelley and Pitha (1985).

DNA sequencing. Double-stranded plasmid DNA was sequenced by a modification of the dideoxynucleotide chain-termination method (Wallace *et al.*, 1981). The chemical sequencing method of Maxam and Gilbert (1980) was used to sequence through one region of the plasmid pmCF-1, which could not be sequenced by the enzymatic chain-termination method.

Genomic DNA preparation and Southern analysis. Genomic DNAs were prepared from somatic cell hybrids and mouse liver as described previously (Heisterkamp *et al.*, 1982). The high-molecular-weight DNAs (5–10 μg) were digested with restriction endonucleases, electrophoresed through 1% agarose gels, and transferred to nitrocellulose membranes. The filters were prehybridized for 4–6 h at 37°C and then hybridized with PCR-generated probes for 48 h at 37°C in 5× SSC, 50% formamide. The filters were washed with a final stringency of 0.5× SSC at 50°C for 30 min.

Tissue RNA preparation and Northern analysis. Total RNA was isolated by extraction of various mouse tissues in guanidine thiocyanate and centrifugation through a 5.7 *M* CsCl cushion as described elsewhere (Chirgwin *et al.*, 1979; Glisin *et al.*, 1974). For the developmental study, whole embryo RNA from Days 13, 15, and 17 of gestation was isolated; the embryos were not contaminated with either extraembryonic membranes or maternal tissue. Isolated RNA was denatured and electrophoresed through 1.1% agarose, 2.2 *M* formaldehyde gels as described by Lehrach *et al.* (1977), transferred to nitrocellulose, and hybridized to a PCR-generated mCFTR exon 10 probe for 48 h at 37°C in 5× SSC, 50% formamide. The filters were washed with a final stringency of 0.5× SSC at 50°C for 30 min.

Isolation of partial cDNA clones. To verify the intron/exon boundaries of mCFTR exon 10, two partial cDNA clones, pmCF-3 and pmCF-4, spanning mCFTR exons 9–10 and 10–12, respectively, were isolated after PCR amplification of reverse-transcribed C57BL/6J intestinal RNA. The nucleotide sequence for mCFTR exons 9–12 was determined and is in complete agreement with the published BALB/c cDNA sequence of Yorifugi *et al.* (1991), while there are four positions within mCFTR exon 9 that differ from the C57BL/6J sequence published by Tata *et al.* (1991).

Chinese hamster × mouse somatic cell hybrids. The production and characterization of Chinese hamster × mouse somatic cell hybrids have been described previously (Hoggan *et al.*, 1988). Thirteen hybrids were selected from a larger panel of 76 hybrids for this study. Seven of these hybrids were typed by karyology for their mouse chromosome content; the remainder were typed for markers on specific mouse chromosomes.

Interspecies backcross. NFS/N and C57BL/6J mice (*Mus musculus*) were obtained from the Division of Natural Resources, NIH (Bethesda, MD) and The Jackson Laboratory (Bar Harbor, ME), respectively. *Mus spretus* were provided by Dr. M. Potter (NCI, NIH, Bethesda, MD) from his colony at Hazelton Laboratory (Rockville, MD). NFS/N females were mated with *M. spretus* males, and the F1 females were mated with C57BL/6J or *M. spretus* males. DNA was extracted from the livers and spleens of individual progeny, digested with various restriction endonucleases, electrophoresed through 0.4% agarose gels, and transferred to nylon membranes (Hybond N+, Amersham). Membranes were hybridized with probes labeled by the random primer method. The DNAs were typed for a *Bam*HI restriction fragment length polymorphism of the mouse *Cftr* locus, using a plasmid clone of mCFTR exon 10 (pmCF-2), and a *Bam*HI restriction enzyme variant of the *Met* locus, using a 1000-bp fragment of the MET human proto-oncogene obtained from Oncor (Gaithersberg, MD).

RESULTS

Human and Mouse CFTR Homology

A 193-bp hCFTR exon 10 fragment was PCR-amplified from human placental DNA using sense and antisense oligonucleotides from the 5′ and 3′ ends of this exon, respectively (Fig. 1A). The resulting PCR product was used as a template to prepare a PCR-generated hCFTR exon 10 probe. A majority of the PCR-generated radiolabeled fragments span the entire length of the amplified region (Fig. 1B). To assess the suitability of the hCFTR exon 10 PCR probe for the detection of homologous mouse CFTR sequences, a Southern analysis of human and mouse DNAs was performed. The human exon 10 PCR probe hybridizes to a 23-kb *Bam*HI fragment and a 4.4-kb *Eco*RI fragment in human DNA (Fig. 1C), as expected from the previously published hCFTR restriction map (Rommens *et al.*, 1989). This probe also cross-hybridizes to a 13.5-kb *Bam*HI fragment and a 2.2-kb *Eco*RI fragment in mouse DNA (Fig. 1C), indicating that there is sufficient homology to allow identification and isolation of a mouse genomic fragment containing mCFTR exon 10 sequences.

Isolation of a Genomic Fragment Containing mCFTR Exon 10

A λEMBL3 library was prepared from *Bam*HI-digested C57BL/6J liver DNA. Recombinant plaques (2.5 × 10^5) were screened with the hCFTR exon 10 PCR probe. A single positive clone was plaque purified and further characterized. This mouse genomic clone (λmCFTR10) contained a 13.5-kb *Bam*HI insert, as expected from the Southern analysis of mCFTR. A 2.1-kb *Bam*HI/*Eco*RI fragment from λmCFTR10, which hybridizes to the hCFTR exon 10 probe, was subcloned into pSP64 to generate the plasmid pmCF-1. This subcloned fragment was used as a hybridization probe for Southern analysis of human and mouse DNAs to confirm that the genomic clone contained mCFTR exon 10 sequences in comparison to hCFTR exon 10-probed mouse DNA. Unfortunately, the presence of repetitive sequence elements in the pmCF-1 subclone probe made it impossible to verify that mCFTR exon 10 sequences had been isolated (data not shown).

The entire sequence of the 2078-bp pmCF-1 insert was determined (Fig. 2). Comparison with the hCFTR exon 10 sequence revealed an analogous 193-bp region in pmCF-1. This region shares 84% homology to hCFTR exon 10 at the nucleotide level and 87% in the predicted amino acid sequence. The phenylalanine 508 (Phe^{508})

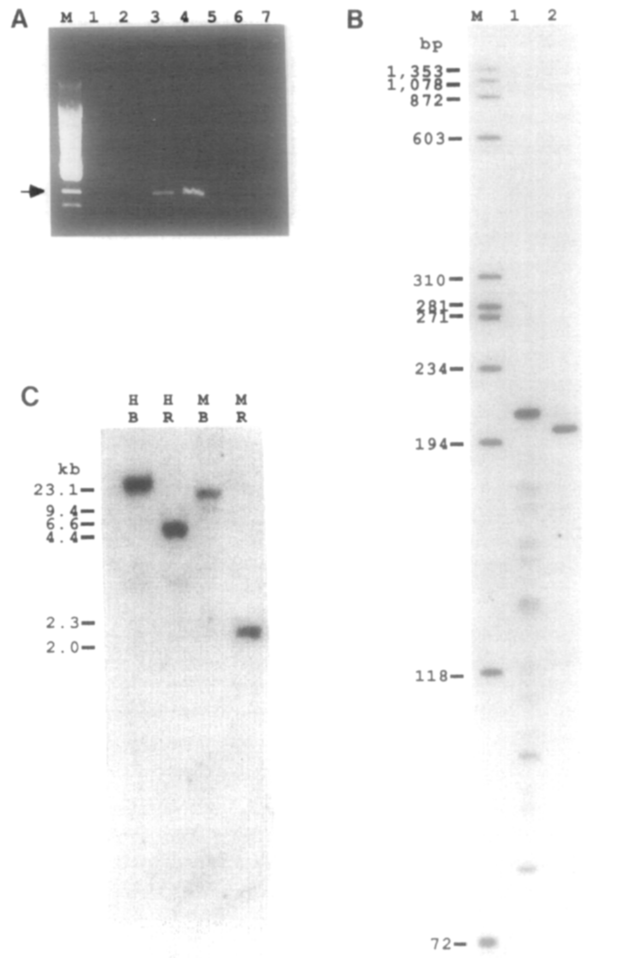

FIG. 1. (**A**) Agarose gel electrophoresis of PCR-amplified hCFTR exon 10. Oligonucleotides KK009 and KK010 were used for PCR amplification of human placental DNA (lane **3**, 100 ng; lane **4**, 500 ng) or mouse (C57BL/6J) liver DNA (lane **6**, 100 ng; lane **7**, 500 ng). These oligonucleotides specifically amplify a 207-bp fragment spanning hCFTR exon 10 from human DNA (lanes **3** and **4**). The controls were no template DNA (lane **1**) and 100 ng human placental or mouse liver DNA template in the absence of oligonucleotides (lanes **2** and **5**, respectively). M, φX174 *Hae*III marker. The 194-bp *Hae*III fragment is indicated by the arrow. (**B**) Acrylamide gel electrophoresis of hCFTR exon 10 PCR probe. Lane **1**, ^{32}P-labeled hCFTR exon 10 reaction products generated by PCR as described under Materials and Methods. The major band at 207 bp was produced by reamplification of a hCFTR exon 10 PCR fragment in the presence of [α-^{32}P]dCTP. Lane **2**, 199-bp end-labeled *Bam*HI/*Eco*RI fragment from the phCFTR10 subclone of the hCFTR exon 10 PCR fragment shown in (**A**). This fragment is slightly smaller than the PCR probe shown in lane **1** due to the removal of several nucleotides from each end of the subcloned PCR fragment after digestion with *Bam*HI and *Eco*RI. M, end-labeled φX174 *Hae*III marker; the sizes of the *Hae*III marker fragments are shown in bp. (**C**) Southern analysis of 5 μg human placental (H) DNA and 10 μg mouse liver (M) DNA digested with *Bam*HI (B) or *Eco*RI (R). After transfer, the nitrocellulose filter was hybridized with a PCR-generated hCFTR exon 10 probe for 48 h in 5× SSC, 50% formamide. The filter was washed in a final stringency of 0.5× SSC, 0.1% SDS at 55°C for 30 min. The positions of λ*Hin*dIII marker fragments are indicated in kb.

```
1    GGATCCATAACCCCACAATGAGAGGCTCAGACAGTTGGATCAAAGGAGCTCACAGGCAGCCAGTGAAGCTGAGAC
76   TGTGAGCTTCAGTTTATTGAGACACTTGGTTTGCCTCAAGCAATGGAAAGAGACATAGAAGATACTAGTATCCTG
151  CTCTGGCCTCTACATGTACACAGAAGGGTACAGGTGTCCACATGTTCACATTGTAGCTCTCTCTCTCTCTCTCTC
226  TCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCCCCACTCTGTGTGTATACACAAACTTGACATCCA
301  TCATTTCTTTTTTTATTGTAGTTTTTTTAGAAAACATTTATTAGGTTTTTTATTGGATATTTTCTTTATTTACAT
376  TTCAACTGTTATCCTCTTTCCTTGTTTCCCCTGTGAAAACTCCCCTATCCCATCTCCCCTCTCCCTGCTCACTCA
451  CCCACCCACTCCTGCTTCTCTGTCCTGGCATTCCCATACATGGAGCATCGATCCTTTACAGGACCAAGGGCCTCT
526  CCTCTCATTGATGTCCCACAAAGCCATCCTCTGCTATATATGTGGCTAGAGCCTTGAGTACCGCCTTGTATACTC
601  TGGTTGGTGGTTTAGACCCTGGGAGATCTGGGGGTACTGGTTGGTTCATTTTGTTGTTTCCCTTCAGTTTCTTGG
676  GTCCTTTCTCTAGCTCCTCCATTGAGGACCCTGTGCTCAGTCCCATATGTTTTTATAATTTCTTAAAGATGAAAG
751  CAAATTTTCATACTAGTAAAATGAAAGTACTTTCTAAGACTGAATCTGTGTTAGTTTATTATAATGAACACACTC
826  ATGTAGTTAGAGCATAGGGGCAGCCCATAGCCCAAGAGCTTTCAGCAAGTGCTCACTGTCACCAGTCTCTTACTA
901  CAAACTGATCACAGCAATTTAAGTAGGGGCTCGCTCTTCTTTGTGAACCTTAGTCCTATGTTGCCCAGATCTCTT
976  TCATCCCCTTTGTATTTTTTATGCCTAGAAAAGTCCCTGTATCATGAAGTACTAAAACATCTTTAATCAAATGAG
1051 TTACACTCTTTAAACATTGGGAGACTTGTGATTGGAATAATTGGACGCAAGAAAGGGATAAGTAATTTGATCAAA
                                 thr ser leu leu met leu ile leu gly glu leu glu
1126 CAATTTAGCTGTTGTTTTTATTTGTAG ACA TCA CTC CTG ATG TTG ATT TTG GGA GAA CTG GAA
                                   T       T   A     A       A                 G

     ala ser glu gly ile ile lys his ser gly arg val ser phe cys ser gln phe ser
1189 GCT TCA GAG GGA ATT ATT AAG CAC AGT GGA AGA GTT TCA TTC TGC TCT CAA TTT TCT
     C             T  AA                         A             T       G       C

     trp ile met pro gly thr ile lys glu asn ile ile phe gly val ser tyr asp glu
1246 TGG ATT ATG CCG GGT ACT ATC AAA GAA AAT ATC ATC TTT GGT GTT TCC TAT GAT GAG
                   T   C   C   T                   * **                        A

     tyr arg tyr lys ser val val lys ala cys gln leu gln gln
1303 TAC AGA TAT AAG AGT GTT GTC AAA GCT TGC CAA CTA CAG CAG GTAAGCATATTTATGAAAA
       T       C  GA   C   C A         A             G A G
1364 ATGCTGATTGTGTTAGCTACTTGTGTCAGTGTTGTGATAAAATTGCTTGACTACTCACCTTGAAAAGGGTTTTAT
1439 TTTAAATTCTTTTCAGGGATGATACCGTCCATCTTGGCAAAGGAGGGGCAGGAATGGGAAGATGGCGAGACATGT
1514 TATATCCATAGTCAGGAAGCAGACAGCCAGCAGGAAGTGGGGCTTCAAGGCCTAATTCTAGTAGCTTACTTTCTC
1589 CAGTAAAGCTCCAAGTTGTAAACACTGTCCTACCCCAGTGTACCCCCAACTGGAAATAATGTTTTCAAACACATG
1664 AGCCCATTGTAGGTATTTCACGTTCACACCACTACATGGATTATGCTCATTCAGTCTTCAGACTAACCAAATTAC
1739 ACAGTTAGTTCTCTATTGAGTTAATGTAAACATGTCAAGGACCCCCTAGGATTAAGCTGGAGTGGGTGGGTCAGT
1814 GAATAAAACCATGCTCCTACTTTAAGTTTACAAAATTATAAATAGATGCAGTTTATTTTTAAAGTGTGTTTGGGT
1889 GTTGTAAAAATAAAAATTCCTTATGCATGGGGTGTGGTACTTCATGAGTGCAATCCTAATACTCAAGAGACTGAA
1964 GCAAAAAGGTCATGAAGTTGAAGCCAGTCTTAGCTGTCTAATGAGTTCTAGGCCAGTCTGGATCACATGGTAGGA
2039 TCATGCTAAAAACTAACAAACCAAAAGTCTGTATGAATTC
```

FIG. 2. Nucleotide sequence and deduced amino acid sequence of the mCFTR gene spanning the region with homology to hCFTR exon 10. The sequence includes the entire 2078-bp *Bam*HI/*Eco*RI insert from pmCF-1. The underlined AG and GT at the 5′ and 3′ intron/exon boundaries represent the consensus splice donor and acceptor sites, and their locations were inferred from homology to hCFTR (Riordan *et al.*, 1989). The nucleotides beneath the mouse sequence are derived from hCFTR and represent the positions at which the human and mouse sequences are different within the protein-coding region. The CTT triplet indicated by asterisks is the sequence which is deleted in the ΔF508 mutation in human CF patients. These sequence data have been deposited in the GenBank database under Accession No. M84614.

residue, which is deleted in a majority of human CF patients, is conserved between hCFTR and mCFTR. The predicted 5′ and 3′ splice donor and acceptor sites for mCFTR exon 10, which are analogous to the sites used in the human gene, were verified by isolation and sequence analysis of two partial cDNA clones spanning mCFTR exons 9–12 (GenBank Accession No. M84613). This assignment of intron/exon boundaries was confirmed from the cDNA sequences of Tata *et al.* (1991) and Yorifugi *et al.* (1991), which were published while this study was in progress. Mouse CFTR exon 9 and exon 11 oligonucleotides were used as hybridization probes to determine the presence or absence of these exon sequences in the isolated genomic clone that contains exon 10. This analysis revealed that neither exon 9 nor exon 11 is present in the mCFTR exon 10 genomic clone (data not shown), which is consistent with the intron distances that separate these exon sequences in the human gene (Rommens *et al.*, 1989).

Genetic Mapping

Southern blot analysis identified 2.2- and 23-kb *Eco*RI fragments in mouse and hamster DNAs, respectively, which are cross-reactive with a mCFTR exon 10 probe (Fig. 3). Thirteen Chinese hamster × mouse somatic cell hybrids were typed for this restriction fragment length polymorphism. The mouse 2.2-kb *Eco*RI fragment was present in eight of these hybrids (Fig. 3). Examination of the chromosome content of these lines showed a perfect correlation with Chromosome 6 (Chr 6), indicating that the mouse gene for CFTR, designated *Cftr*, is on Chr 6 (Table 1). To provide a more specific map location, progeny of the cross (NFS/N × *M. spretus*) × C57BL/6J or *M. spretus* were typed. Southern blot analysis of the parental DNA identified CFTR-reactive *Bam*HI fragments of 12.2 kb in *M. spretus* and of 13.5 kb in NFS/N and C57BL/6J (Fig. 4). Analysis of the progeny identified the *M. spretus* 12.2-kb CFTR segment in 63 of 97 mice, and the pattern of segregation was compared with that of 90 marker loci on all linkage groups. Close linkage was observed between *Cftr* and *Met* on Chr 6 (recombination frequency was 1/97 = 1.0 ± 1.0 cM), indicating that *Cftr* is closely linked to *Met*,

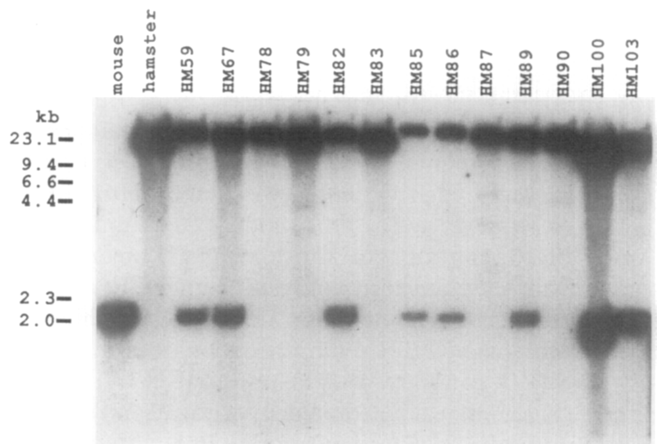

FIG. 3. Chromosome localization of mCFTR. Ten micrograms of C57BL/6J (mouse) liver DNA, Chinese hamster ovary (hamster) cell culture DNA, or various Chinese hamster × mouse (HM) somatic cell hybrid DNAs were digested with *Eco*RI, electrophoresed through 1% agarose gels, and transferred to nitrocellulose. The filter was then hybridized with a mCFTR exon 10 probe that detects a restriction fragment length polymorphism between hamster CFTR (23-kb *Eco*RI fragment) and mCFTR (2.2-kb *Eco*RI fragment). The 23-kb hamster *Eco*RI fragment is detected in all of the Chinese hamster × mouse somatic cell hybrids, as expected, while the mCFTR 2.2-kb *Eco*RI fragment is present in 8 of the 13 somatic cell hybrids examined. The presence of the mCFTR fragment in these hybrids correlates with the presence of mouse Chromosome 6 (see Table 1). The positions of λ*Hin*dIII marker fragments are indicated.

TABLE 1

Correlation between Specific Mouse Chromosomes and *Cftr* in 13 Chinese Hamster × Mouse Somatic Cell Hybrids

Chromosome	Number of hybrids[a] (hybridization/chromosome)				Percentage discordance
	+/+	−/−	+/−	−/+	
1	5	3	2	2	33
2	7	2	1	3	30
3	0	1	2	2	80
4	3	3	4	2	50
5	1	4	6	1	58
6	8	5	0	0	0
7	5	1	3	4	54
8	3	4	4	1	42
9	4	3	4	2	46
10	0	4	6	1	64
11	0	5	6	0	55
12	2	1	0	2	40
13	6	2	2	3	39
14	0	5	8	0	62
15	3	0	0	2	40
16	4	4	4	1	39
17	3	2	3	3	55
18	7	3	0	1	9
19	4	4	4	1	39
X	4	3	4	2	46

[a] Symbols represent the presence (+/) or absence (−/) of the mouse CFTR 2.2-kb *Eco*RI restriction fragment as related to the presence (/+) or absence (/−) of a particular mouse chromosome. The number of discordant observations is the sum of the +/− and −/+ observations. Seven of the hybrids were karyotyped: the remainder were typed for the presence or absence of markers on specific mouse chromosomes.

which has been positioned at the centromeric end of this chromosome (Lyon and Kirby, 1991; Hillyard *et al.*, 1991).

mCFTR Expression

Northern analysis of mouse tissue RNAs using a mCFTR exon 10 PCR probe revealed that mCFTR is expressed in intestine, lung, stomach, salivary gland, and kidney in adult C57BL/6J mice (Fig. 5) and during embryogenesis (data not shown). The size of the transcript detected by Northern analysis (6–7 kb) is in the range of what has been observed for human CFTR (Rommens *et al.*, 1989; Riordan *et al.*, 1989). The apparent lack of mCFTR transcripts in pancreas and liver was surprising since hCFTR mRNA levels are relatively high in these tissues (Riordan *et al.*, 1989).

To characterize mCFTR expression further, the pattern revealed by Northern analysis was reexamined by PCR amplification of reverse-transcribed tissue RNAs. An antisense oligonucleotide (KK042) from the 3′ end of mCFTR exon 10 was used as a primer for reverse transcription of total tissue RNAs. The newly synthesized first-strand cDNA was then PCR-amplified using KK042 and a sense oligonucleotide from the 5′ end of mCFTR exon 9 (KK059). This combination of oligonucleotides results in a PCR fragment amplified from reverse-transcribed cDNA that can be distinguished from fragments produced by amplification of any contaminating genomic DNA in the RNA sample. PCR amplification of reverse-transcribed mCFTR transcripts revealed an expression pattern similar to that detected by the Northern analysis (Fig. 6A). Use of actin primers for analysis of reverse-transcribed RNA revealed actin-specific transcripts in pancreas and liver comparable to those in other tissues, suggesting that the absence of CFTR transcripts was not due to RNA degradation (Fig. 6B). A more sensitive analysis of the PCR fragments generated from reverse-transcribed mCFTR transcripts was performed by the addition of 2.5 μCi of [α-^{32}P]dCTP to the PCR reactions. The resulting PCR fragments were analyzed by autoradiography after electrophoresis through 5% polyacrylamide gels. This analysis revealed low levels of expression in tissues that appear to be negative by Northern analysis, including the pancreas and

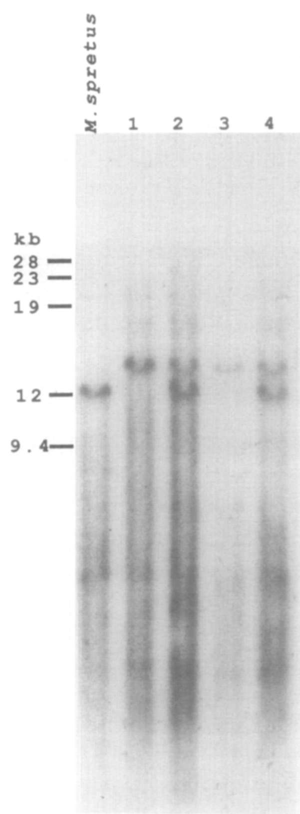

FIG. 4. Southern analysis of the progeny of an interspecies backcross. DNAs from progeny of the cross (NFS/N × *M. spretus*) × C57BL/6J were typed for the presence of a *Bam*HI restriction fragment length polymorphism that was detected between *M. spretus* and *M. musculus* (C57BL/6J and NFS/N). *M. spretus* CFTR is characterized by the presence of a 12.2-kb *Bam*HI fragment, while the C57BL/6J or NFS/N CFTR gene is identified by a 13.5-kb *Bam*HI fragment. Lanes 1–4 are four representative progeny from the (NFS/N × *M. spretus*) × C57BL/6J cross, which contain either the *M. musculus* (lanes **1** and **3**) or the *M. musculus* and *M. spretus* (lanes **2** and **4**) CFTR *Bam*HI fragments. The positions of λ *Sma*I/*Hin*dIII marker fragments are indicated.

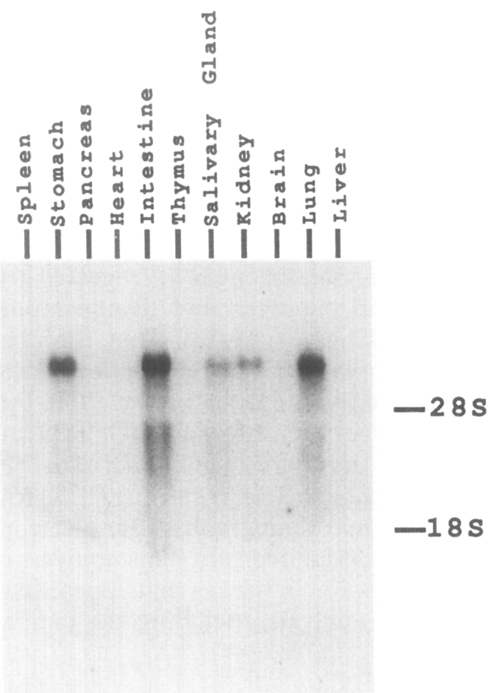

FIG. 5. Northern analysis of mCFTR expression. Total tissue RNAs isolated from an adult male C57BL/6J mouse were electrophoresed through 1.1% agarose, 2.2 *M* formaldehyde gels as described (Lehrach *et al.*, 1977). After transfer of the RNA samples to nitrocellulose, the filter was hybridized with a mCFTR exon 10 PCR probe. The positions of the 18 S and 28 S ribosomal RNAs are indicated.

liver (Fig. 6C). Mouse CFTR transcripts were also detected as early as Embryonic Day 13 (E13), which is the earliest developmental stage examined to date (Fig. 6C).

DISCUSSION

The CF gene product has two NBDs that share a high degree of homology with the NBDs of various vertebrate, invertebrate, and even prokaryotic membrane proteins (Riordan *et al.*, 1989), indicating the functional significance of this region. On this basis, we hypothesized that the first NBD of mCFTR should share significant homology with hCFTR, especially within the region flanking the Phe^{508} residue, which is deleted in a majority of CF patients. Our approach to generate a probe for detection of mCFTR was to amplify CFTR exon 10, which encodes the Phe^{508} residue and surrounding NBD sequences, from human DNA using primers predicted from the human cDNA sequence. Using a PCR-generated hCFTR probe, it was determined that the human and mouse CF genes share significant homology within exon 10, and this species cross-hybridization was used to isolate a mouse genomic fragment containing mCFTR exon 10 and flanking intron sequences. The mCFTR exon 10 homologue shares a high degree of sequence homology with hCFTR exon 10, while the absence of other mCFTR exon sequences in the isolated genomic clone is consistent with the organization of hCFTR (Rommens *et al.*, 1989).

In man, the CF locus (CFTR) has been mapped to the long arm of chromosome 7, band q31 (Tsui, 1989). Other closely linked loci, including the c-*met* proto-oncogene locus (MET), have been identified (Cutting *et al.*, 1989) and were instrumental in the identification of the cystic fibrosis gene (Rommens *et al.*, 1989). Homologues of various genes in this region of human chromosome 7 are known to map to mouse Chr 6, including *Met* (Lyon and Kirby, 1991; Hillyard *et al.*, 1991). Our somatic cell hybrid mapping data revealed that the mouse genomic clone, which was isolated on the basis of its homology to the human CFTR gene, is part of a genetic locus on mouse Chr 6. In addition to the sequence homology between hCFTR and the isolated mouse clone, this result indicated that the isolated genomic fragment was derived from the mouse CFTR homologue. This conclusion is further strengthened by the interspecies back-

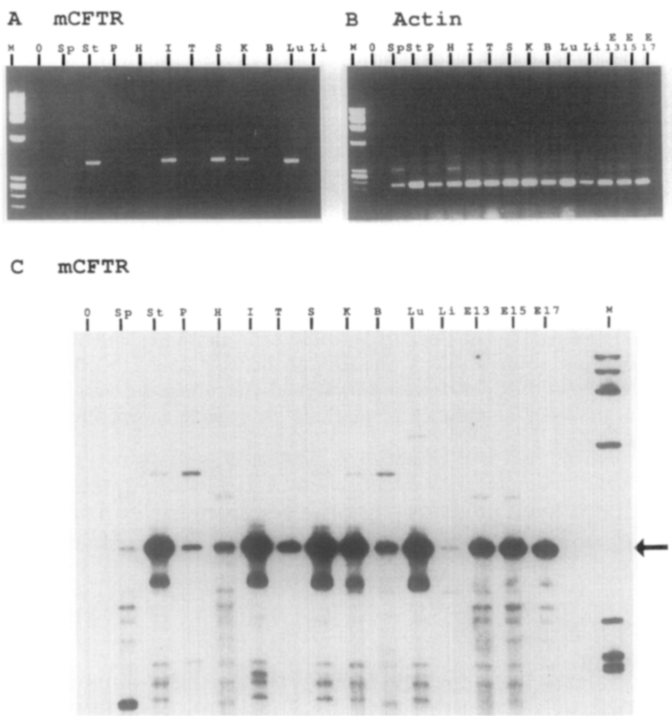

FIG. 6. PCR analysis of mCFTR expression. First-strand cDNA templates were reverse-transcribed for PCR analysis from total tissue or embryo RNAs primed with either an oligonucleotide (KK042) that is complementary to the 3′ end of mCFTR exon 10 (**A** and **C**) or one (Act-1) that is complementary to mouse cytoskeletal β-actin mRNA (**B**). Subsequent PCR amplification of the KK042-primed cDNA using KK042 and KK059 (which was derived from the sequence at the 5′ end of mCFTR exon 9) results in the production of a 387-bp fragment in samples that contain mCFTR mRNA. The mCFTR PCR reactions included [α-^{32}P]dCTP, and the amplified fragments were analyzed by electrophoresis of aliquots of each reaction through either 1.4% agarose gels (**A**) or 5% acrylamide, 7 *M* urea gels (**C**). The presence of actin mRNA in the various samples was analyzed by agarose gel electrophoresis of a 200-bp PCR fragment from Act-1-primed cDNA (**B**), using Act-1 and KK055 (see Materials and Methods). The marker (M) represents ϕX174 *Hae*III fragments (end-labeled in **C**); the marker fragments are 1353, 1078, 872, 603, 310, 284, 274, 234, 194, and 118 bp. The arrow in (**C**) indicates the 387-bp mCFTR PCR fragment. 0, no template; Sp, spleen; St, stomach; P, pancreas; H, heart; I, intestine; T, thymus; S, salivary gland; K, kidney; B, brain; Lu, lung; Li, liver.

cross that revealed that the murine *Met* locus is closely linked to the locus on mouse Chr 6 from which the isolated mouse genomic fragment was obtained. Thus, the genetic mapping provides strong evidence that the isolated genomic fragment was derived from the murine CFTR homologue and has extended the information on the comparative mapping of these regions in man and mouse.

Northern (RNA) analysis of adult mouse tissues revealed a 6- to 7-kb mRNA that is expressed in some of the same sites as hCFTR, including lung. The size and location of this transcript are similar to those observed for hCFTR and are consistent with the conclusion that the isolated mouse genomic fragment is derived from the mCFTR homologue. This same analysis, however, indicated that mCFTR and hCFTR do not share an identical tissue distribution; although hCFTR and mCFTR are both expressed in lung, intestine, and stomach, there are notable differences. While hCFTR mRNA is readily detected in pancreas and liver (Riordan *et al.*, 1989), mCFTR transcripts could not be detected in these tissues by Northern analysis. It was also previously reported by Tata *et al.* (1991) that mCFTR is not expressed in mouse liver. Upon further examination, however, we found that mCFTR transcripts are present in mouse liver and pancreas, but at levels that can be detected only by sensitive PCR analysis. This weak expression observed in some tissues, such as liver or pancreas, may reflect expression in a subpopulation of cells. The availability of mCFTR probes will allow this possibility to be examined by *in situ* hybridization studies. In addition, the initial analyses conducted by Riordan *et al.* (1989) suggested that hCFTR is not expressed in the kidney, although their subsequent experiments revealed hCFTR kidney transcripts with variability between individual samples. Kidney mCFTR transcripts were observed in the present study, as well as by Tata *et al.* (1991). The observed variability in kidney hCFTR mRNA levels may reflect normal differences due to the age of the individuals from whom the samples were obtained or allelic variations in tissue-specific *cis*- or *trans*-acting regulatory elements or factors. With the availability of isolated mCFTR sequences, it will be possible to determine the developmental expression of mCFTR to address the effect of age on the variability of expression that has been observed in human kidney.

The results of this study, as well as those of Tata *et al.* (1991) and Yorifugi *et al.* (1991), have demonstrated the existence of a mouse CFTR homologue that is very similar to hCFTR, which is a prerequisite for the production of various mouse CF mutants through gene targeting. The availability of a mouse genomic fragment containing the coding region that is analogous to hCFTR exon 10 creates the potential for targeted mutagenesis of this region of the mouse CFTR gene.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grant DK-43973 (K.A.K.) and a fellowship of the Gottlieb Daimler- and Karl Benz-Stiftung, No. 2.88.9 (S.S.). The authors thank Alexandra Sonshine for her comments on the mansucript and Dr. Mark Miller for his gift of the Act-1 oligonucleotide.

REFERENCES

Benton, W. D., and Davis, R. W. (1977). Screening λgt recombinant clones by hybridization to single plaques *in situ*. *Science* **196:** 180–182.

Boat, T. F., Welsh, M. J., and Beaudet, A. L. (1989). *In* "The Metabolic Basis of Inherited Disease" (C. L. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, Eds.), ed. 6, pp. 2649–2680, McGraw-Hill, New York.

Chirgwin, J. M., Przybla, A. E., MacDonald, R. J., and Rutter, W. J. (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18:** 5294–5301.

Cutting, G. R., Antonarakis, S. E., Buetow, K. H., Kasch, L. M., Rosenstein, B. J., and Kazazian, H. H., Jr. (1989). Analysis of DNA polymorphism haplotypes linked to the cystic fibrosis locus in North American Black and Caucasian families supports the existence of multiple mutations of the cystic fibrosis gene. *Am. J. Hum. Genet.* **44:** 307–318.

Cutting, G. R., Kasch, L. M., Rosenstein, B. J., Zielenski, J., Tsui, L-C., Antonarakis, S. E., and Kazazian, H. H., Jr. (1990). A cluster of cystic fibrosis mutations in the first nucleotide-binding fold of the cystic fibrosis conductance regulator protein. *Nature* **346:** 366–369.

Frohman, M. A., and Martin, G. R. (1989). Cut, paste, and save: New approaches to altering specific genes in mice. *Cell* **56:** 145–147.

Glisin, V., Crkvenjakov, R., and Byus, C. (1974). Ribonucleic acid isolation by cesium chloride centrifugation. *Biochemistry* **13:** 2633–2637.

Heisterkamp, N., Groffen, J., Stephenson, J. R., Spurr, N. K., Goodfellow, P. N., Sollomon, E., Casritt, B., and Bodmer, W. F. (1982). Chromosomal localization of human cellular homologues of two viral oncogenes. *Nature* **299:** 747–749.

Hillyard, A. L., Doolittle, D. P., Davisson, M. T., and Roderick, T. H. (1991). Locus map of mouse. *Mouse Genome* **89:** 16–30.

Hoggan, M. D., Halden, N. F., Buckler, C. E., and Kozak, C. A. (1988). Genetic mapping of the mouse c-*fms* proto-oncogene to chromosome 18. *J. Virol.* **62:** 1055–1056.

Jansen, R., and Ledley, F. D. (1989). Production of discrete high specific activity DNA probes using the polymerase chain reaction. *Gene Anal. Tech.* **6:** 79–83.

Kelley, K. A., and Pitha, P. M. (1985). Characterization of a mouse interferon gene locus. I. Isolation of a cluster of four α-interferon genes. *Nucleic Acids Res.* **13:** 805–823.

Kerem, B.-S., Rommens, J. M., Buchanan, J. A., Markiewicz, D., Cox, T. K., Chakravarti, A., Buchwald, M., and Tsui, L.-C. (1989). Identification of the cystic fibrosis gene: Genetic analysis. *Science* **245:** 1073–1080.

Kerem, B.-S., Zielenski, J., Markiewicz, D., Bozon, D., Gazit, E., Yahav, J., Kennedy, D., Riordan, J. R., Collins, F. S., Rommens, J. M., and Tsui, L.-C. (1990). Identification of mutations in regions corresponding to the two putative nucleotide (ATP) binding-folds of the cystic fibrosis gene. *Proc. Natl. Acad. Sci. USA* **87:** 8447–8451.

Lehrach, H., Diamond, D., Wozney, J. M., and Boedtker, H. (1977). RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. *Biochemistry* **16:** 4743–4751.

Lyon, M. F., and Kirby, M. C. (1991). Mouse chromosome atlas. *Mouse Genome* **89:** 37–59.

Maxam, A. M., and Gilbert, W. (1980). Sequencing end-labeled DNA with base-specific chemical cleavages. *In* "Methods in Enzymology" (R. Wu, L. Grossman, and K. Moldave, Eds.), Vol. 65, pp. 499–560, Academic Press, New York.

Riordan, J. R., Rommens, J. M., Kerem, B.-S., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J.-L., Drumm, M. L., Iannuzzi, M. C., Collins, F. S., and Tsui, L.-C. (1989). Identification of the cystic fibrosis gene: Cloning and characterization of complementary DNA. *Science* **245:** 1066–1073.

Rommens, J. M., Iannuzzi, M. C., Kerem, B.-S., Drumm, M. L., Melmer, G., Dean, M., Rozmahel, R., Cole, J. L., Kennedy, D., Hidaka, N., Zsiga, M., Buchwald, M., Riordan, J. R., Tsui, L.-C., and Collins, F. S. (1989). Identification of the cystic fibrosis gene: Chromosome walking and jumping. *Science* **245:** 1059–1065.

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., and Erlich, H. A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239:** 487–491.

Schowalter, D. B., and Sommer, S. (1989). The generation of radiolabeled DNA and RNA probes with polymerase chain reaction. *Anal. Biochem.* **177:** 90–94.

Stamm, S., Gillo, B., and Brosius, J. (1991). Temperature recording from thermocyclers used for PCR. *Biotechniques* **10:** 430–435.

Tata, F., Stanier, P., Wicking, C., Halford, S., Kruyer, H., Lench, N. J., Scambler, P. J., Hansen, C., Braman, J. C., Williamson, R., and Wainwright, B. J. (1991). Cloning the mouse homolog of human cystic fibrosis transmembrane conductance regulator. *Genomics* **10:** 301–307.

Tokunaga, K., Taniguchi, H., Yoda, K., Shimzu, M., and Sakiyama, S. (1986). Nucleotide sequence of a full-length cDNA for mouse cytoskeletal β-actin mRNA. *Nucleic Acids Res.* **14:** 2829.

Tsui, L.-C. (1989). Tracing the mutations in cystic fibrosis by means of closely linked DNA markers. *Am. J. Hum. Genet.* **44:** 303–306.

Wallace, R. B., Johnson, M. J., Suggs, S. V., Ken-ichi, M., Bhatt, R., and Itakura, K. (1981). A set of synthetic oligodeoxyribonucleotide primers for DNA sequencing in the plasmid vector pBR322. *Gene* **16:** 21–26.

Williams, J. F. (1989). Optimization strategies for the polymerase chain reaction. *Biotechniques* **7:** 762–769.

Yorifugi, T., Lemna, W. K., Ballard, C. A., Rosenbloom, C. L., Rozmahel, R., Plavsic, N., Tsui, L.-C., and Beaudet, A. L. (1991). Molecular cloning and sequence analysis of the murine cDNA for the cystic fibrosis transmembrane conductance regulator. *Genomics* **10:** 547–550.