Structure, Organization, and Expression of the Human Band 7.2b Gene, a Candidate Gene for Hereditary Hydrocytosis*

Patrick G. Gallagher and Bernard G. Forget†

From the Departments of Pediatrics, Internal Medicine, and Genetics, Yale University School of Medicine, New Haven, Connecticut 06520-8021

Band 7.2b is an integral membrane phosphoprotein absent from the erythrocyte membranes of patients with hereditary hydrocytosis, a hemolytic anemia inherited in an autosomal dominant fashion and characterized by stomatocytic red blood cells with abnormal permeability to Na+ and K+. The precise role of band 7.2b is unknown, but it may interact with other proteins of the junctional complex of the membrane skeleton. To gain additional insight into the structure and function of this protein and to provide the necessary tools for further genetic studies of hydrocytosis patients, we determined the sequence of the full-length human band 7.2b cDNA, characterized the genomic structure of the band 7.2b gene, studied its pattern of expression in different tissues, and characterized the promoter of the gene. The composite band 7.2b gene cDNA was 3047 base pairs in length. Northern blot analysis revealed a wide tissue distribution of expression of the band 7.2b gene, with utilization of alternative polyadenylation signals generating transcripts of 2.2 and 3.1 kilobases. Cloning of the band 7.2b chromosomal gene revealed that it is composed of seven exons distributed over 40 kilobases of DNA. The band 7.2b gene promoter was identified as a TATA-less, (G + C)-rich promoter with a typical InR recognition sequence and a single transcription initiation site. It directed high level expression of a reporter gene in both erythroid and nonerythroid cells. An imperfect simple sequence repeat polymorphism was identified in the 5′-flanking DNA, and an assay was developed for its analysis by PCR.

Hereditary stomatocytosis consists of a heterogeneous group of disorders characterized by the presence of mouth-shaped (stomatocytic) erythrocytes on peripheral blood smears. The clinical severity of hereditary stomatocytosis is variable; some patients experience hemolysis and anemia, while others are asymptomatic (1). The red cell membranes of these patients usually exhibit abnormal permeability to the univalent cations sodium and potassium, with resultant modification of intracellular water content (2–5).

Hereditary hydrocytosis is one subset of the stomatocytosis syndromes. Hydrocytic erythrocytes are characterized by an increased intracellular sodium and decreased intracellular potassium content (6, 7). The transport rate of potassium via both the Na+/K+ pump and the Na+/K+/2Cl– cotransport system is increased. It has been hypothesized that there is an ion leak in these erythrocytes, with an inadequate compensatory increase in transport by the Na+/K+ pump.

The red cell membranes of many hydrocytosis patients lack a 31-kDa protein, band 7.2b (5, 8–13). Band 7.2b is an integral membrane phosphoprotein whose function is not completely understood. It has been hypothesized that band 7.2b may support, activate, or regulate an yet unidentified associated ion channel (7). Recent evidence showing a potential interaction between band 7.2b and the membrane skeleton protein β-adducin suggests that band 7.2b may be a part of the junctional complex of the membrane skeleton (14). In this capacity, band 7.2b may participate in a variety of specialized cellular functions (15). Protein immunoblotting has shown the presence of band 7.2b reactivity in a wide variety of tissues and in a wide cross-species distribution (12, 16). The human band 7.2b protein has been purified, and partial cDNAs corresponding to its coding region have been cloned (12, 13, 16, 17). Band 7.2b shares no sequence homology to other known proteins. To gain additional insight into the structure and function of this protein and provide the necessary tools for further genetic studies of hydrocytosis patients, we constructed a composite full-length human band 7.2b cDNA containing the 5′- and 3′-untranslated sequences compared with previously cloned cDNAs. We also cloned the chromosomal gene encoding band 7.2b, characterized the genomic structure of the band 7.2b gene, studied its pattern of expression in different tissues, and identified its promoter. An imperfect simple sequence repeat (SSR) polymorphism in the band 7.2b gene was identified, and a PCR-based assay for its analysis was developed.

MATERIALS AND METHODS

Genomic Cloning—Human band 7.2b cDNA fragments corresponding to the 5′ end of the coding region (Fig. 1, probe 4) or the 3′ end of the coding region (Fig. 1, probe 3) (18) were generated by PCR using primers A and B or primers C and D (Table I), respectively, using a human bone marrow cDNA library (Clontech, Palo Alto, CA) as template. These fragments were used as hybridization probes to screen a human genomic DNA library. The library is a Charon 4A bacteriophage library containing fragments of genomic DNA partially digested with AluI and HaeIII with EcoRI linkers added (19). Selected recombinants that hybridized to the screening probes were purified and subcloned into pGEM-7Z plasmid vectors (Promega Corp., Madison, WI). Subcloned fragments were analyzed by restriction endonuclease digestion, Southern blotting, and nucleotide sequencing.

Nucleotide Sequencing—Nucleotide sequencing was performed using the dye-deoxy chain termination method of Sanger et al. (20) with T7 DNA polymerase (Sequenase, U.S. Biochemical Corp.). The sequencing primers were the Sp6 or T7 vectors of the pGEM-7Z plasmid vector or, for some reactions, synthetic oligonucleotides corresponding to known cDNA sequences (Table I). Deoxyxynosine triphosphate was substituted for dATP during sequencing reactions.

The abbreviations used are: SSR, simple sequence repeat; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; bp, base pair(s); kb, kilobases.
for deoxyguanosine triphosphate to resolve band compressions and ambiguities (21).

Oligonucleotide Synthesis—Oligonucleotides were synthesized using an automated synthesizer (Applied Biosystems, Foster City, CA) and purified by oligonucleotide purification column (OPC) chromatography (Applied Biosystems).

RNA Preparation—Total RNA was prepared from human liver tissue or from the human tissue culture cell lines K562 (chronic myelogenous leukemia in blast crisis with erythroid characteristics; ATCC, CCL 243), HEL (erythroleukemia; ATCC, TIB 180), or HL60 (promyelocytic leukemia; ATCC, CCL 240) using the guanidinium-thiocyanate-chloroform method as described (22).

Primer Extension Analyses—The transcription start site of the band 7.2b gene was determined using primer extension analysis. Primers B or E (Table I) were used in primer extension reactions as described (23). Templates in these reactions were 10 µg of total RNA from the human cell lines K562, HEL, and HL60 or tRNA.

5′ Rapid Amplification of cDNA Ends (RACE) (Fig. 1).—1 µg of total human liver RNA was reverse transcribed using primer E (Table I) and avian myeloblastosis virus reverse transcriptase (Promega Corp.). Single-stranded oligonucleotide ligation and PCR amplification were carried out as described using primers B and E (24, 25). Amplification products were subcloned and sequenced.

Detection of a Polymorphism of the Band 7.2b Gene—An imperfect SSR polymorphism was identified in the 5′-flanking DNA of the band 7.2b gene (see below), and an assay for this polymorphism was developed. Oligonucleotide primers 1 and J (Table I), corresponding to sequences that flank the SSR, were synthesized for use in PCR. Primer I was end-labeled with [32P]ATP using polynucleotide kinase. PCR amplification was carried out using labeled primer I and unlabeled primer J with 100–250 ng of genomic DNA/reaction as template. Amplification conditions were as follows: denaturation at 94°C for 45 s, annealing at 60°C for 45 s, and extension at 72°C for 25 cycles of amplification were performed. Amplification products were mixed 1:1 with formamide-based loading dye, heated to 94°C for 5 min, and then electrophoresed in a 6% acrylamide gel. After electrophoresis, the gel was dried, and autoradiography of the gel for 6 h at −80°C using an intensifying screen was performed.

Northern Blot Analyses—Multiple tissue Northern blots containing 2 µg of poly(A)+ mRNA were obtained from Clontech. The locations of probes used in Northern blotting are shown in Fig. 1. Probe 1 was obtained by PCR amplification of human bone marrow cDNA using primers D + F (Fig. 1). Probe 2 is a 0.7-kb EcoRI fragment present in the 3′-untranslated region of the band 7.2b gene. A 2.0-kb human β-actin cDNA probe was used as a control for loading in Northern blot analyses (26).

Cell Culture—Two cell lines, K562 (erythroid) and NIH3T3 (mouse fibroblast, nonerythroid) were used to study expression of the putative promoter of the band 7.2b gene. K562 cells were maintained in RPMI 1640 medium containing 10% fetal calf serum. NIH3T3 cells were maintained in Eagle’s minimal essential medium supplemented with 10% fetal calf serum.

Preparation of Reporter Plasmids—A 568-bp DNA fragment corresponding to −531 to +37 bp of the band 7.2b gene 5′-flanking DNA was amplified using primers G and H (Table I) and genomic DNA done PCR amplification (see Fig. 4) as template. Test plasmids were prepared by inserting the amplified DNA fragment upstream of the firefly luciferase reporter gene in the plasmid pGL2B (Promega Corp.) in both orientations. These plasmids were designated pH87-forward and pH87-reverse, respectively. All test plasmids were sequenced to exclude cloning or PCR-generated artifacts.

Transient Transfection Analyses—All plasmids tested were purified using Qiagen columns (Qiagen, Inc., Chatsworth, CA), and at least two preparations of each plasmid were tested. 107 K562 cells were transfected by electroporation with a single pulse of 300 V at 960 microfarads with 20 µg of test plasmid and 0.5 µg of pCMVβ, a mammalian reporter plasmid expressing β-galactosidase driven by the human cytomegalovirus immediate early gene promoter (Clonetech). 106 NIH3T3 cells were transfected with 2.0 µg of test plasmid and 0.25 µg of the pCMVβ plasmid by lipofection using 4 µl of Lipofectamine (Life Technologies, Inc.). Twenty-four hours after transfection, cells were harvested and lysed, and the activity of both luciferase and β-galactosidase activity was determined in cell extracts. All assays were performed in triplicate. Differences in transfection efficiency were determined by co-transfection with the pCMVβ plasmid.

Computer Analyses—Computer-assisted analyses of derived nucleotide and predicted amino acid sequences were performed utilizing the sequence analysis software package of the University of Wisconsin Genetics Computer Group (UW GCG; Madison, WI) (27) and the BLAST algorithm (National Center for Biotechnology Information, Bethesda, MD) (28).

RESULTS

Mapping the 5′ End of the Human Band 7.2b cDNA—To identify the 5′ end of the human band 7.2b cDNA, primer extension experiments were performed. These experiments (Fig. 2) predicted the presence of an additional 61 bp in the mRNA upstream from the putative initiator codon, previously identified in cDNA cloning. These additional 61 bp of upstream 5′-untranslated sequence were obtained by 5′ RACE (Figs. 1 and 3). These sequences agreed with the 17 of 21 nucleotides of 5′-untranslated sequence previously reported (13). Sequences
obtained by RACE were verified by comparison to corresponding genomic DNA sequences (see below). These sequences around the translational start site match important consensus sequences, $A$ at $\text{3}^{\text{a}}$ and $G$ at $\text{1}^{\text{b}}$ (29). No additional ATGs were present in the 5$\text{9}$-untranslated sequences. Taken together, these data suggest that this sequence is at or very near the 5$\text{9}$ end of the human band 7.2b cDNA.

Cloning of Chromosomal Gene: Isolation and Analysis of Recombinant Clones—Primary screening of a human genomic DNA library with a cDNA probe yielded over a dozen hybridization-positive plaques. Selected recombinants were analyzed, and five overlapping clones were identified that spanned 40 kb of DNA containing the band 7.2b gene. An $\text{EcoRI}$ restriction map of this 40-kb region is shown in Fig. 4.

Mapping the Exon/Intron Junctions of the Band 7.2b Gene—The human band 7.2b gene is encoded by seven exons (Table II). Five of the seven exons are relatively small, 135 bp or less in length, with two of the exons (73 and 82 bp) at the lower limit usually observed for exon size (30). The first and seventh exons contain untranslated sequences; the 5$\text{9}$-untranslated region is 61 bp in length, and the 3$\text{9}$-untranslated is 2120 bp in length. Comparison of the exon/intron boundaries with reported consensus sequences reveals that the ag$\text{g}$ rule was not violated at any splice junction (31, 32). There are no AG dinucleotides within the 15 bp upstream of the 3$\text{9}$ (acceptor) splice junctions. There is an $\text{Alu}$ repeat in reverse orientation in the 3$\text{9}$-untranslated region from positions 1373 to 1675 of the cDNA that is 83% similar to a consensus $\text{Alu}$ sequence (33).

Secondary structure predictions of the band 7.2b protein predict the presence of three domains, a highly charged NH2-terminal domain, a hydrophobic stretch that encodes a potential membrane-spanning domain, and a COOH-terminal domain composed of $\beta$ sheet and $\alpha$ helix. There is no concordance between the exon organization of the gene and the location of

---

**Fig. 3.** Nucleotide sequence with encoded amino acid sequence of human band 7.2b cDNA. The composite nucleotide sequence shown was determined from 5' RACE products and limited sequencing of genomic DNA clones. The composite band 7.2b cDNA is 3047 bp in length, predicting a 288-amino acid polypeptide of 31 kDa, consistent with the mobility previously observed on SDS-polyacrylamide gel electrophoresis gels. The initiator methionine, ATG, the termination codon, TAG, and both polyadenylation signals, AATAAA, are underlined. The locations of exons are denoted by triangles.
Fig. 4. Genomic organization of the human band 7.2b gene. Five overlapping clones containing the band 7.2b gene were isolated from a human genomic DNA library. These clones spanned a distance of over 40 kb. A restriction map for EcoRI (E) is shown. Individual exons (not to scale) are denoted by closed boxes. The location of an imperfect simple sequence polymorphism in the 5'-flanking DNA of the band 7.2b gene is indicated by an arrow and SSR.

Table II

| Exon/ intron boundaries of the human band 7.2b gene |
|-----------------|-----------------|
| 3' acceptor site | Exon (bp)       | 5' donor site |
| 1               | CTTCCTCAAGggtgaggctcc (122) | S F K |
| 2               | GTGATADAAGgttaaagaca | |
| 3               | AAAGAAGCTGtctagctactg | |
| 4               | CTCCTCAGGAttaagtttt | |
| 5               | CAAATGAGgttggagaga | |
| 6               | CAACGCGCCAAGgtaacttttt | |
| 7               | TTTTTCCTCAAGgttaaagaca | |

a Eukaryotic consensus: (c/t)n(c/t)ag:G.

b Eukaryotic consensus: (C/A)AG-gt(a/g)a.

these three domains in the protein.

Table II

| Exon/ intron boundaries of the human band 7.2b gene |
|-----------------|-----------------|
| 3' acceptor site | Exon (bp)       | 5' donor site |
| 1               | CTTCCTCAAGggtgaggctcc (122) | S F K |
| 2               | GTGATADAAGgttaaagaca | |
| 3               | AAAGAAGCTGtctagctactg | |
| 4               | CTCCTCAGGAttaagtttt | |
| 5               | CAAATGAGgttggagaga | |
| 6               | CAACGCGCCAAGgtaacttttt | |
| 7               | TTTTTCCTCAAGgttaaagaca | |

a Eukaryotic consensus: (c/t)n(c/t)ag:G.

b Eukaryotic consensus: (C/A)AG-gt(a/g)a.

The Human Band 7.2b Gene

The human band 7.2b gene is indicated by an arrow and SSR.

Band 7.2b mRNA Exhibits Wide Tissue Distribution of Expression—Northern blot analysis using probe 1 (Fig. 1) detected an abundant mRNA of 3.1 kb in all tissues examined except brain, colon, and ovary (Fig. 6A). A 2.2-kb signal was also detected in most tissues but in lesser amount compared with the 3.1-kb mRNA, particularly in spleen, where almost no 2.2-kb signal was observed.

Alternate Polyadenylation of the Band 7.2b mRNA—Polyadenylation signals are located at positions 2034–2039 bp and 3012–3026 bp in the 3'-untranslated region of the cDNA. To determine if both polyadenylation signals are utilized, Northern blots were hybridized to cDNA sequences upstream and downstream of the 5' polyadenylation signal (2034–2039). While Northern blots clearly show the presence of two transcripts of 2.2 and 3.1 kb in length when the upstream probe 1 is used, only a transcript of 3.1 kb is detected when the downstream probe 2 (Fig. 1) is hybridized to the same Northern blots (Fig. 6B). Thus these transcripts are most likely the result of alternate polyadenylation. In contrast, the murine band 7.2b gene does not apparently undergo alternative polyadenylation (25).

The Human Band 7.2b Gene Promoter Is Active in Erythroid and Nonerythroid Cells—The nucleotide sequence of the 5'-flanking genomic DNA upstream of the human band 7.2b cDNA transcription start site is shown in Fig. 7. Inspection of the sequences reveals features characteristic of a housekeeping gene promoter including lack of consensus TATA or CCAAT sequences and a high G + C content (68%, between positions 1 and 334). A recognition sequence for a transcription initiator (InR), CA, 3'TCC, (35) is double underlined. Consensus sequences for a number of potential DNA-binding proteins, including multiple potential Sp1 binding sites, are also present in the 5'-flanking sequences (Fig. 7).

To investigate if the region from −531 to +37 was capable of directing expression of a reporter gene, test plasmids pHB7-forward or pHB7-reverse (Fig. 8) were transiently transfected into erythroid (K562) or nonerythroid (NIH3T3) cells. The relative luciferase activity was determined 24 h after transfection and compared with the activity obtained with the parental promoterless vector. As shown in Fig. 8, the putative band 7.2b promoter plasmid, pHB7-forward, directed high level expression of the luciferase reporter gene in both erythroid and nonerythroid cells. In addition, the plasmid with the promoter in reverse orientation, pHB7-reverse, also directed expression in both cell lines but at a lower level, suggesting a bidirectional capability of the band 7.2b promoter.

Computer Analyses—When compared with sequences present in available data bases, there was no significant homology between the band 7.2b gene sequence and that of other genes or
-proteins (other than the mouse band 7.2b gene and protein). Searching using only the highly charged 25 amino acid NH2 terminus also failed to reveal any significant homologies.

**DISCUSSION**

The function of band 7.2b protein is unknown. Its importance, however, is underscored by its wide tissue and species distribution. In humans, Northern blot analysis detected message in essentially every tissue examined except brain, colon, and ovary. Middle, the same blots were stripped and rehybridized to probe 2. Bottom, the same blots were stripped and hybridized with a 2.0-kb human β-actin cDNA probe as a control for loading.

proteins (other than the mouse band 7.2b gene and protein). Searching using only the highly charged 25 amino acid NH2 terminus also failed to reveal any significant homologies.

**TABLE III**

| Allele | N + 4 | N + 2 | N   | N - 2 | N - 10 | Total |
|--------|-------|-------|------|-------|--------|-------|
| Caucasian | 0 | 5 (0.12) | 37 (0.88) | 0 | 0 | 42 |
| African-American | 2 (0.01) | 15 (0.12) | 105 (0.83) | 3 (0.02) | 1 (0.01) | 126 |
| Asian | 0 | 2 (0.09) | 20 (0.91) | 0 | 0 | 22 |
| Total | 2 (0.01) | 22 (0.12) | 162 (0.85) | 3 (0.02) | 1 | 190 |

**FIG. 5.** *An Imperfect SSR polymorphism in the human band 7.2b gene.* A, identification of polymorphic alleles using a PCR-based assay. A SSR was identified in the 5'-flanking DNA of the band 7.2b gene. Oligonucleotide primers corresponding to sequences flanking the SSR, one of which was end-labeled with [32P]ATP, were used to amplify genomic DNA in a PCR reaction. Amplification products were denatured and electrophoresed in an acrylamide gel, the gels were autoradiographed, and the results were analyzed. Five alleles of varying size were identified in individuals from different racial backgrounds using this PCR-based assay of genomic DNA, with the most common allele arbitrarily called N. B, sequence of the imperfect simple sequence repeat. Differences in the sequences of the five identified alleles are shown. The most common allele is denoted as N.

**FIG. 6.** *Northern blot Analysis.* Top, samples of 2 μg of poly(A)+ RNA from various human tissues were hybridized to a [32P]dCTP-labeled cDNA fragment (probe 1). Abundant message was detected in all tissues examined except brain, colon, and ovary. Middle, the same blots were stripped and rehybridized to probe 2. Bottom, the same blots were stripped and hybridized with a 2.0-kb human β-actin cDNA probe as a control for loading.

**FIG. 7.** *5'-Flanking genomic DNA sequence.* The nucleotide sequence of the 5'-flanking genomic DNA of the human band 7.2b gene is shown. Consensus sequences for potential DNA-protein binding sites are underlined. The locations of a recognition sequence for a transcription initiator (InR) site and the initiator methionine codon are double underlined. The junction between exon 1 and intron 1 is shown by the inverted triangle.

The Human Band 7.2b Gene
7.2b genes transcript that vary in their 3' determinants developmental or tissue-specific preferences for the highly conserved polyadenylation signal poly(A) to the newly formed 3' end of the transcript downstream. The genomic structure of the band 7.2b gene will allow structural studies of the band 7.2b gene in patients with hereditary xerocytosis. Band 7.2b protein does appear to form oligomers. Alterations in the polymerase that interacts with wild-type defective band 7.2b protein may interact with wild-type protein. Band 7.2b-deficient hydrocytosis patients apparently do not suffer any nonhematologic symptomatology (7).

A variety of mutations causing human disease have been described that affect RNA processing and translation (reviewed in Ref. 36). These mutations may be associated with dramatic decreases in steady state mRNA levels. This fact has important implications for mutation detection methods. Reverse transcriptase-PCR-based techniques are unlikely to detect mutations with markedly decreased mutant mRNA levels, necessitating study of these mutations at the level of genomic DNA (36). A recently described nonsense mutation in the band 7.2b gene promoter is similar to that observed in a wide variety of gene promoters, particularly housekeeping gene promoters such as those of the genes encoding dihydrofolate reductase, HMG CoA reductase, and hypoxanthine phosphoribosyltransferase (41-43). Studies are currently ongoing to further characterize the band 7.2b gene promoter and to determine if it truly has bidirectional activity.

Acknowledgments—We thank Dr. George Segal for providing blood samples from the family with hereditary xerocytosis and C. Wong and L. Lozovatsky for skilled technical assistance.

REFERENCES
1. Dacie, J. V. (1985) The Haemolytic Anaemias, 2nd Ed., pp. 259–281, Churchill Livingstone, Edinburgh, United Kingdom
2. Zarkowsky, H. S., Oski, F. A., Sha'afi, R., Shohet, S. B., and Nathan, D. G. (1968) N. Engl. J. Med. 278, 573–581
3. Clark, F. A., Naiman, J. L., Blum, S. F., Zarkowsky, H. S., Whaun, J., and Nathan, D. G. (1969) N. Engl. J. Med. 280, 909–916
4. Mentzer, W. C., Smith, W. B., Goldstone, J., and Shohet, S. B. (1975) Blood 46, 659–669
5. Kanazaki, A., and Yawata, Y. (1992) Br. J. Haematol. 82, 133–141
6. Lande, W. M., and Mentzer, W. C. (1985) Clin. Haematol. 14, 89–103
7. Stewart, G. W., Argent, A. C., and Dash, B. C. J. (1993) Biochim. Biophys. Acta 1125, 15–25
8. Lande, W. M., Thiemann, P. V. W., and Mentzer, W. C., Jr. (1982) J. Clin. Invest. 70, 1273–1280
9. Morle, L., Patther, B., Alloisio, N., Fees, C., Garay, R., Bost, M., and Duelaunay, J. (1989) Br. J. Haematol. 71, 141–146
10. Eber, S. W., Lande, W. M., Jarocco, T. A., Mentzer, W. C., Hohn, P., Wiley, J. S., and Schmor, W. (1989) Br. J. Haematol. 72, 452–455
11. Olivier, O., Girelli, D., Vettori, L., Baladra, G., and Corrocher, R. (1992) Br. J. Haematol. 80, 258–260
12. Wark, D., Mentzer, W. C., Cameron, T., and Johnson, R. M. (1991) J. Biol. Chem. 266, 17826–17831
13. Stewart, G. W., Hewearth, B. E., Keen, J. N., Argent, A. C., and Casimir, C. M. (1991) Blood 79, 1593–1601
14. Sanger, F., Nickslen, A., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
15. Tabor, S., and Richardson, C. C. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 4767–4771
16. Chegwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) Biochemistry 18, 5294–5299
17. Mason, P. J., Enver, T., Wilkinson, D., and Williams, J. G. (1993) in Genomics: A Practical Approach (Hames, B. D., and Higgins, S. J., eds.) pp. 47–54, IRL Press, Oxford
18. Edwards, J. B. D. M., Delort, J., and Mallet, J. (1991) J. Clin. Invest. 89, 2527–2532
19. Gallagher, P. G., Upender, M., Ward, D. C., and Forget, B. G. (1993) Genomics 18, 157–169
20. Lawson, R. W., Fritsch, E. F., Parker, R. C., Blake, G., and Maniatis, T. (1978) Cell 15, 1157–1174
21. Sanger, F., Nickslen, A., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 5463–5467
22. Tabor, S., and Richardson, C. C. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 4767–4771
23. Chegwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) Biochemistry 18, 5294–5299
24. Mason, P. J., Enver, T., Wilkinson, D., and Williams, J. G. (1993) in Genomics: A Practical Approach (Hames, B. D., and Higgins, S. J., eds.) pp. 47–54, IRL Press, Oxford
25. Edwards, J. B. D. M., Delort, J., and Mallet, J. (1991) J. Clin. Invest. 89, 2527–2532
26. Gallagher, P. G., Romana, M., Lieman, J. H., and Ward, D. C. (1993) Blood 86, 359–365
27. Ng, S-Y., Gunning, P., Eddy, R., Ponte, P., Leavitt, J., Shows, T., and Kedes, L. (1985) Mol. Cell. Biol. 5, 2720–2732
28. Genetics Computer Group (1994) Program Manual for the Wisconsin Package, version 8.0, Genetics Computer Group, Madison, WI
29. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
30. Kozak, M. (1984) Nucleic Acids Res. 12, 857–872
31. Wierenga, B., Hofer, E., and Weissmann, C. (1984) Cell 37, 915–925
32. Shapiro, M. B., and Senapathy, P. (1987) Nucleic Acids Res. 15, 7715–7717
33. Horowitz, D. S., and Krainer, A. R. (1994) Cell 74, 801–810
34. Labuda, D., and Striker, G. (1989) Nucleic Acids Res. 17, 2477–2491
35. Miller, D. R., Riddles, F. R., Lichtman, M. A., LaCelle, P. L., Bates, J., and Weed, R. I. (1971) Blood 30, 844–204
36. Javery, H., Khachi, A., Lo K., Zenele-Gregory, B., and Smale, S. T. (1994) Mol. Cell. Biol. 14, 116–127
37. Cooper, D. N. (1993) Ann. Med. 25, 11–17
38. Gallagher, P. G., Abou-Alfa, G. K., Floyd, P., Dhermy, D., Bursaun, E., Scarpa, A., Garbarz, M., and Forget, B. G. (1994) Blood 84, 1513–1527
39. Sach, A., and Wahle, E. (1993) J. Biol. Chem. 268, 22953–22958
40. Lin, B., Rommens, J. M., Graham, R. K., Kalchman, M., MacDonald, H., Nasir, L., Delaney, A., Goldberg, Y. P., and Hayden, M. R. (1993) Hum. Mol. Genet. 3, 1541–1545
41. Hor, J., Hsu, L.-J., Dougall, W. C., Visner, G. A., Burr, I. M., and Nich, H. S. (1992) Nucleic Acids Res. 20, 2985–2990
42. Farnham, P. J., and Schinke, R. T. (1986) Mol. Cell. Biol. 6, 365–371
43. Abrams, J. M., and Schinke, R. T. (1989) Mol. Cell. Biol. 9, 620–628
44. Ristow-Lim, D. E., Krueger, D., and Patel, P. I. (1991) Mol. Cell. Biol. 11, 4157–4164