Isothermal Multiple Displacement Amplification

A Highly Reliable Approach for Generating Unlimited High Molecular Weight Genomic DNA from Clinical Specimens

Rajyalakshmi Luthra and L. Jeffrey Medeiros

From the Department of Hematopathology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

Isothermal multiple strand displacement amplification (IMDA) of the whole human genome is a promising method for procuring abundant DNA from valuable and often limited clinical specimens. However, whether DNA generated by this method is of high quality and a faithful replication of the DNA in the original specimen, allowing for subsequent molecular diagnostic testing, requires verification. In this study, we evaluated the suitability of IMDA-generated DNA (IMDA-DNA) for detecting antigen receptor gene rearrangements, chromosomal translocations, and gene mutations using Southern blot analysis, polymerase chain reaction (PCR) methods, or sequencing methods in 28 lymphoma and leukemia clinical specimens. Molecular testing before and after whole genome amplification of these specimens using the IMDA technique showed concordance in 27 of 28 (96%) specimens. Analysis of IMDA-DNA by Southern blot analysis detected restriction fragments >12 kilobases long. No amplification bias was observed at all loci tested demonstrating that this method can be useful in generating large amounts of unbiased, high molecular weight DNA from limited clinical specimens. (*J Mol Diagn* 2004, 6:236–242)

Procuring sufficient DNA from clinical specimens to perform in-depth analysis at the molecular level is often hindered by the volume and cell count of a patient sample. For example, fine needle aspirates and small tissue biopsy specimens of lymphoproliferative disorders often yield inadequate amounts of DNA for detecting antigen receptor gene rearrangements using Southern blot (restriction fragment length) analysis. In addition, DNA obtained from small clinical specimens is frequently exhausted when multiple tests are performed, despite the use of techniques that require small amounts of DNA, such as the polymerase chain reaction (PCR). Hence, a method that representatively amplifies the entire genome with minimal bias would have a substantial impact on the

capability to perform comprehensive molecular analysis using small patient specimens.

Recently, a phi29 DNA polymerase-based amplification method that allows direct amplification of entire genomic DNA by isothermal multiple strand displacement (IMDA) has been described.^{1–3} Phi29 DNA polymerase is a highly processive enzyme that incorporates at least 70,000 nucleotides in one binding event and performs efficient strand displacement synthesis at a rate of 25 to 50 nucleotides/second with an error rate of 3×10^{-6} , which allows for high fidelity replication of input DNA template. These two properties, strand displacement and processivity, allow phi29 DNA polymerase to be used in novel isothermal amplification strategies to amplify nanograms of genomic DNA into several micrograms. This method holds promise for amplification of whole genomic DNA from samples as small as a single cell.

The objective of this study is to determine whether DNA generated by random hexamer-primed IMDA is uniformly amplified without bias. For this purpose, we evaluated the relative sequence representation of IMDA-DNA by comparing molecular test results obtained before and after IMDA using clinical specimens from patients with leukemia and lymphoma. The molecular tests assessed were antigen receptor gene rearrangement detection by Southern blot analysis or PCR, analysis of the t(14;18) and t(2;5) chromosomal translocations and assessment of *FLT3* gene mutations by PCR, and detection of point mutations in the *K-RAS* and *N-RAS* genes by sequencing.

Materials and Methods

Twenty-eight clinical specimens that were previously tested in our molecular diagnostic laboratory are included in this study. These specimens consisted of 2 peripheral blood samples, 20 bone marrow aspirates, 4 frozen tissue biopsy samples, 1 fine needle aspirate, and 1 formalin-fixed, paraffin-embedded tissue sample.

High molecular weight genomic DNA from peripheral blood, bone marrow, and fine needle aspirate specimens

Accepted for publication March 9, 2004.

Address reprint requests to Rajyalakshmi Luthra, Ph.D., The University of Texas M.D. Anderson Cancer Center, NA01.091, 8515 Fannin Street, Houston, TX 77054. E-mail: rluthra@mdanderson.org.

Table 1. Comparison of PCR Results Using Original and IMDA-Generated DNA

Sample	Type of test	Original DNA	IMDA-DNA
1	<i>IgH</i> -GR, FR 1	Monoclonal	Monoclonal
	<i>IgH</i> -GR, FR 2	Monoclonal	Monoclonal
	<i>IgH</i> -GR, FR 3	Polyclonal	Polyclonal
2	<i>IgH</i> -GR, FR 1	Monoclonal	Monoclonal
	<i>IgH</i> -GR, FR 2	Polyclonal	Polyclonal
	<i>IgH</i> -GR, FR 3	Monoclonal	Monoclonal
3	<i>IgH</i> -GR, FR 1	Polyclonal	Polyclonal
	<i>IgH</i> -GR, FR 2	Polyclonal	Polyclonal
	<i>IgH</i> -GR, FR 3	Polyclonal	Polyclonal
4	<i>FLT3</i> -ITD, D835	ITD of 21 bp (35%)	ITD of 21 bp (37%)
5	<i>FLT3</i> -ITD, D835	ITD of 51 bp (75%)	ITD of 51 bp (76%)
6	<i>FLT3</i> -ITD, D835	ITD of 93 bp (13%), D835 (18%)	ITD of 93 bp (13%), D835 (18%)
7	<i>FLT3</i> -ITD, D835	Mutation at D835 (58%)	Mutation at D835 (57%)
8	<i>FLT3</i> -ITD, D835	No mutation	No mutation
9	t(14;18)	Fusion involving <i>bcl-2</i> mbr	Negative for t(14;18)
10	t(14;18)	Fusion involving <i>bcl-2</i> mbr	Fusion involving <i>bcl-2</i> mbr
11	t(14;18)	Negative for t(14;18)	Negative for t(14;18)
12	t(14;18)	Fusion involving <i>bcl-2</i> mcr	Fusion involving <i>bcl-2</i> mbr
13	t(14;18)	Negative for t(14;18)	Negative for t(14;18)
14	Mutation in K- and N-RAS	Mutation in codon 12 of N-RAS, ggt to gat	Mutation in codon 12 of N-RAS, ggt to gat
15	Mutation in K- and N-RAS	No mutation	No mutation
16	Mutation in K- and N-RAS	Mutation in codon 12 of N-RAS, ggt to gat	Mutation in codon 12 of N-RAS, ggt to gat
17	Mutation in K- and N-RAS	No mutations	No mutations
18	t(2;5)	Positive for t(2;5)	Positive for t(2;5)
19	t(2;5)	Positive for t(2;5)	Positive for t(2;5)
20	t(2;5)	Negative for t(2;5)	Negative for t(2;5)

was extracted using the ABI 341 nucleic acid purification system (Applied Biosystems, Foster City, CA) or Auto-pure LS (Gentra Systems, Inc., Minneapolis, MN). Genomic DNA from frozen tissue was isolated using conventional organic-based methods. DNA from fixed-paraffin-embedded tissue was extracted using a kit from Qia-gen (Valencia, CA) according to the manufacturer's recommendations. The concentration of the DNA was determined by absorbance at 260 nm using a Beckman DU-7400 spectrophotometer (Fullerton, CA).

Isothermal Multiple Displacement Amplification

Isothermal multiple displacement amplification was performed using the REPLI-g whole genome amplification kit obtained from Molecular Staging (New Haven, CT). Briefly, 40 ng of DNA from each specimen was denatured at room temperature for 3 minutes and subjected to whole genome amplification in a 50- μ l reaction by random priming and strand displacement synthesis at 30°C according to the manufacturer's recommendations. As the amplification reaction reaches a plateau at approximately 8 hours, and no further change in the yield is observed after 8 hours, for convenience we incubated all specimens overnight. After overnight incubation, the reaction was terminated by incubation at 60°C for 10 minutes. The IMDA-generated DNA (IMDA-DNA) was then stored at -20°C for later use.

Molecular Tests

Eight specimens were tested for immunoglobulin (*Ig*) κ light chain and T-cell receptor (*TCR*) β chain gene rearrangements by Southern blot analysis. In addition, 20

specimens were assessed by PCR using a number of tests (Table 1). Three specimens were tested for immunoglobulin heavy (*IgH*) gene rearrangements by PCR. Five specimens from patients with acute myeloid leukemia were analyzed for *FLT3* gene mutations using a fluorescence-based PCR method, including two specimens with internal tandem duplications (ITD), one specimen with a D835 point mutation, one specimen with both ITD and D835 mutation, and one negative specimen. Five specimens were tested for the t(14;18) by a quantitative real-time PCR method, including three positive (two major breakpoint and one minor breakpoint cluster region) and two negative cases. Three specimens from patients with anaplastic large cell lymphoma (two positive and one negative) were tested for the t(2;5) by DNA-based nested long-range PCR. Four specimens from patients with myeloid neoplasms, three acute myeloid leukemias, and one myelodysplastic syndrome, were tested for K- and N-RAS mutations by sequencing (two positive and two negative). The original DNA was simultaneously tested along with IMDA-DNA for PCR-based analyses. Except for Southern blot analysis, all assays were performed in duplicate.

Antigen Receptor Gene Rearrangements by Southern Blot and PCR Analysis

Southern blot analyses for *Ig* κ and *TCR* β gene rearrangements were performed using DNA digested with the *EcoRI*, *HindIII*, and *BamHI* enzymes according to conventional procedures. Probes specific for the joining region of *Ig* κ (*J* κ) and the constant region of *TCR* β (*CT* β) were obtained from DAKO (Carpinteria, CA) and were labeled with ³²P. Blots hybridized initially with the *CT* β probe were then stripped and re-hybridized with the *J* κ probe.

Due to inadequate amounts of DNA, we were unable to analyze original DNA side-by-side with IMDA-DNA for Southern blot analysis. Instead, results obtained previously, during routine clinical testing, were compared with the results using IMDA-DNA. High molecular DNA extracted from the HL60 cell line using routine methods and not subjected to IMDA was analyzed as a control for restriction digestion and hybridization during Southern blot analysis. Presence of one or two non-germline bands in two or more restriction enzyme digests was considered evidence of monoclonal gene rearrangement.

In the PCR-based assay to assess for IgH gene rearrangement, we used variable region framework 1, 2, and 3 primers, each in combination with a mixture of joining region primers.^{4,5} The joining region primers were labeled with a fluorescent dye, 6-carboxyfluorescein (FAM), for the detection of PCR products by capillary electrophoresis and GeneScan (ABI 3700 Genetic Analyzer, Applied Biosystems) analysis. All PCR assays for gene rearrangements were performed in duplicate for each of the original and IMDA-DNA samples.

Detection of *FLT3* Gene Mutations

For detection of ITD and D835 mutation of the *FLT3* gene, PCR assays were performed using previously described primers.^{6,7} To facilitate the detection of PCR products by capillary electrophoresis, forward primers for ITD and D835 were labeled with a fluorescent dye, FAM. The presence of any PCR fragment larger than the wild-type allele of 328 bp was considered positive for ITD. The percentage of the mutated allele was calculated by dividing the area under the peak of the mutated fragment by the total area under the wild-type and mutated peaks in a sample. For D835 mutations, the PCR products were digested with the *EcoRV* restriction enzyme before capillary electrophoresis. The wild-type allele cut by this enzyme results in two fragments of 64 bp and 44 bp. In contrast, mutations at D835 that alter the *EcoRV* recognition site result in one 128-bp fragment. As only one of the primers was labeled with the fluorescent dye, only the 68-bp fragment of the two wild-type restriction fragments was detected during capillary electrophoresis. The percentage of mutated allele was calculated as described above.

Detection of *t*(14;18) and *t*(2;5)

To assess for the *t*(14;18)(q32;q21) involving either the major or minor breakpoints regions of the *bcl-2* gene, we used a quantitative real-time TaqMan PCR method coupled with capillary electrophoresis as described previously.^{8,9} To assess for the *t*(2;5)(p23;q35) involving the *ALK* and *NPM* genes, we performed nested, long-range PCR using a method described previously.¹⁰

Sequence-Based Mutation Analysis

Mutations in the K- and N-RAS (codons 12, 13, and 61) genes were assessed using primers described previous-

Figure 1. Ethidium bromide stained gels of restriction enzyme-digested IMDA-generated DNA (IMDA-DNA). Ten μg of DNA (per lane) was digested with *Bam*HI, *Hind*III, or *Eco*RI and electrophoresed through a 0.7% agarose gel containing 0.5 $\mu\text{g}/\text{ml}$ ethidium bromide. **Lane M**, molecular size marker; **lane C**, DNA from HL60 cell line; and **lanes 1 to 8**, IMDA-DNA from eight clinical specimens. **Lanes 1 to 8** show a typical smear of variously sized DNA fragments as expected from restricted genomic DNA indicating that IMDA-DNA is of high molecular weight and suitable for restriction digestion.

ly.¹¹ Sequencing was performed using Big Dye-terminator chemistry and the ABI 3700 sequencing instrument (Applied Biosystems).

Results

Beginning with 40 ng of DNA extracted from each clinical specimen, the final yield of DNA after IMDA ranged from 76 μg to 101 μg . The average yield of DNA after IMDA was $84.36 \mu\text{g} \pm 4.3 \text{ SD}$. The protein contamination of DNA as measured by absorbance readings at 230 nm and 280 nm showed small amounts of protein, possibly due to the polymerase used for amplification.

Antigen Receptor Gene Rearrangement Analysis by Southern Blot and PCR

Electrophoresis of restriction enzyme-digested IMDA-DNA by IMDA in all eight specimens showed that IMDA resulted in high molecular weight DNA. Furthermore, there were no inhibitors in these samples to prevent restriction enzyme digestion with *Eco*RI, *Hind*III, and *Bam*HI (Figure 1). The intensity of expected germline bands after digestion with *Eco*RI (9.3kb), *Hind*III (5.1 kb), and *Bam*HI (11.8 kb) in blots hybridized with the *J κ* probe was comparable to that of the control HL60 DNA (Figure 2). In Southern blots hybridized with the CT β probe, the intensity of *Hind*III (7.4 and 3.4 kb) and *Eco*RI germline bands (10.4 and 3.9 kb) in the IMDA-DNA were similar to that of the control HL60 DNA. The signal of the 10.4-kb *Eco*RI germline band, however, was weaker compared to the 3.9-kb *Eco*RI germline band in all amplified DNA samples. In addition, the 23.7-kb *Bam*HI germline band, although faintly visible, was less intense in IMDA-DNA (Figure 3). Comparison of Southern blot results obtained using the *J κ* probe and either original or IMDA-DNA showed complete concordance between the two sets of DNA. Using the CT β probe, the band patterns of *Eco*RI- and *Hind*III-digested DNA matched perfectly in the original and IMDA-DNA specimens in all eight specimens assessed. However, due to weak signal intensity of the high molecular *Bam*HI fragment in IMDA-DNA, it was

Figure 2. Southern blot analysis of *Ig* κ light chain gene rearrangement. Hybridization with joining region (*J* κ) probe shows that the intensity of expected germline bands with *EcoRI* (9.3 kb), *HindIII* (5.1 kb), and *BamHI* (11.8 kb) in IMDA-DNA specimens (1–8) was comparable to that of the control HL60 DNA (lane C). Lane M shows molecular size markers.

difficult to assess the rearrangement status with this restriction enzyme in three IMDA specimens. Overall, the Southern blot results for assessment of the *Ig* κ light chain and *TCR* β chain genes in IMDA-DNA, including the size and number of rearranged bands, matched the results obtained previously using original DNA. Similarly, PCR-based *IgH* gene rearrangement analysis showed complete concordance between IMDA-DNA and original DNA with the three framework region-specific primers (Figure 4 and Table 1).

FLT3 Gene Mutation Analysis

For the five samples tested for *FLT3* gene mutations, the results obtained using either IMDA-DNA or original DNA were completely concordant (Table 1). In ITD-positive cases, the ITD size and percentage of the ITD allele matched perfectly between the two DNA sources including detection of a minor clone (Figure 5). Similarly, in the D835-positive cases, the percentage of the mutant D835

Figure 3. Southern blot analysis of *TCR* β gene rearrangement. Hybridization with the *CT* β probe shows that the intensity of *HindIII* germline bands (7.4 and 3.4 kb) and *EcoRI* germline band (3.9 kb) in the IMDA-DNA was similar to that of the control HL60 DNA. The signal of the 10.4-kb *EcoRI* germline band, however, was weaker compared with the 3.9-kb *EcoRI* germline band and the 23.7-kb *BamHI* germline band is only faintly visible in IMDA-DNA. Lane M, molecular size markers; lane C, DNA from HL60 control; and lanes 1 to 8, IMDA-DNA from eight clinical specimens.

Figure 4. *IgH* gene rearrangement using PCR analysis. Original DNA and IMDA-DNA derived from a patient with chronic lymphocytic leukemia were subjected to analysis as described in the Materials and Methods section. PCR resulted in 404-bp and 143-bp amplification fragments (asterisk) using framework 1 and 3 primers, respectively, in both DNA samples. Glyceradehyde-3-phosphate dehydrogenase (GAPDH) served as the internal amplification control.

allele in the DNA generated by IMDA matched the amount in the original DNA.

t(14;18) and *t*(2;5) Analysis

The results of quantitative real-time PCR for detecting the *t*(14;18) matched in 4 of 5 samples. The amplification plots in Figure 6 show the relative levels of *bcl-2/JH* fusion sequences and cyclophilin (internal control) in the original DNA and IMDA-DNA in three patients: two with breaks in major breakpoint region (mbr) and one within a minor cluster region (mcr) breakpoint of *bcl-2*. When normalized to *cyclophilin*, a difference of less than onefold was observed in the levels of fusion sequences in the two specimen sources. In the one discordant case, the original DNA was positive for *t*(14;18) and IMDA-DNA was negative. In this case, the original DNA sample had very

Figure 5. PCR-based detection of *FLT3* gene mutations. Electropherograms generated by GeneScan software following capillary electrophoresis of fluorescent dye-labeled PCR fragments show the wild-type (WT) allele (328 bp) and identical internal tandem duplications (ITD) in original DNA (top) and the IMDA-DNA (bottom).

Figure 6. Quantitative real-time PCR-based detection of t(14;18). Real-time PCR amplification plots show the relative levels of *bcl-2/JH* fusion sequences and an internal control, cyclophilin, in original and IMDA-DNA (arrow) derived from a patient with a breakpoint in the minor cluster region (mcr) of *bcl-2* (left) and two patients with breakpoints in the major breakpoint region (mbr) (right).

low levels of the fusion sequence (*bcl-2/JH/cyclophilin* = 0.0058).

Long-range PCR results for t(2;5) matched perfectly in all three samples. As demonstrated in Figure 7, the fusion fragment sizes and band intensities were similar in both sample types.

RAS Gene Mutation Analysis

DNA generated by IMDA tested for mutations of the K- or N-RAS genes showed complete concordance with the original test samples in all four cases assessed (Table 1).

Discussion

Molecular testing, especially in leukemia and lymphoma patients for some tests, requires several micrograms of DNA depending on the method used for analysis. For instance, Southern blotting, the gold standard for determination of clonality and lineage in lymphomas and leu-

kemias¹² (although, to a great extent, being replaced by PCR), requires a minimum of 30 μ g. However, specimens obtained by fine needle aspiration and small needle biopsy specimens generally do not yield this amount of DNA. In these instances, methods such as IMDA that replicate specimen DNA without bias will have great utility. Since sequence representation bias is a potential problem for using IMDA-DNA, we subjected original DNA and DNA generated by IMDA to molecular analyses using identical conditions and compared the results.

The results of our preliminary study are very promising. Restriction enzyme digestion and Southern blot analysis in eight clinical specimens confirmed that IMDA-DNA is of high molecular weight. Using IMDA-DNA, results with the *J κ* and *CT β* probes to assess the *Ig κ* and *TCR β* chain genes, respectively, matched perfectly with the results using DNA of the original clinical specimens confirming that the IMDA generates DNA without bias. However, one of the limitations of IMDA-DNA for Southern blot analysis is the size of the amplified fragments. The average size produced by IMDA is around 12 kb in length. Hence, the larger fragments progressively produce weaker signal in Southern blot analysis as observed with the 23.7-kb germline band in *CT β* blots. Methods to improve this limitation are being addressed currently. PCR-based *IgH* gene rearrangement analysis of IMDA-DNA also showed complete correlation with original sample DNA.

PCR analysis of IMDA-DNA for the t(14;18) and t(2;5) chromosomal translocations, and assessment of mutations in the *FLT3* and *RAS* genes, correlated in 19 of 20 specimens. The one discrepant case was a post-treatment bone marrow sample with no morphological evidence of malignant lymphoma that showed a very low number of *bcl-2/JH* fusion sequences in the original sample DNA.

The high (96%) concordance rate between the results obtained using IMDA-DNA and original clinical specimen DNA demonstrates that the DNA generated by IMDA is of high quality and suitable for Southern blot analysis of restriction fragments and PCR analysis of altered gene sequences. Overall, our study provides evidence that nucleotide sequences of loci that are of interest in leukemia and lymphoma diagnosis are preserved and accurately replicated in amplified DNA by using the IMDA method.

The clinical utility of this IMDA method for amplifying DNA is illustrated by a recent cerebrospinal fluid (CSF) specimen sent to our laboratory that was obtained from a 27-year-old man with precursor T-cell lymphoblastic leukemia/lymphoma (Figures 8 and 9). This sample had a low cell count with immature cells suggestive of lymphoblasts. Thus, the patient's clinician was interested in molecular confirmation. As the amount of DNA obtained from the specimen was very limited (0.4 μ g), it was not feasible to assess for *TCR β* chain gene rearrangements by Southern blot analysis as had been requested. Hence, we performed family-specific four-color PCR analysis to assess for T-cell receptor γ chain gene rearrangements¹³ and we identified gene rearrangements involving *V γ 1* family in the DNA of the original clinical specimen (Figure 8). We also replicated 0.01 μ g of the purified DNA from

Figure 7. Long-range PCR detection of t(2;5). The ethidium bromide-stained gel shows the detection of *NPM-ALK* fusion sequences in two patients with anaplastic large cell lymphoma using original DNA (Lanes 1 and 3) and IMDA-DNA (Lanes 1A and 3A). A patient negative for t(2;5) in the original DNA (Lane 2) was also negative in IMDA-DNA (Lane 2A). Lane M, molecular size markers; lane NC, negative control.

Figure 8. TCR γ gene rearrangement assessment of cerebrospinal fluid (CSF) specimen by PCR. Two dominant TCR γ rearrangements of 247 and 255 bp (asterisks), both of which used the V γ family 1, were detected in original (top) and IMDA-DNA (middle, from purified DNA; bottom, from cells). β -globin was co-amplified as an internal amplification control. The peaks without asterisks correspond to internal size standards.

this specimen using this IMDA method and obtained 90 μ g of high molecular weight DNA, sufficient for assessment of TCR β chain gene rearrangement by Southern blot analysis. The yield of DNA from this sample was similar to other clinical samples even though the initial starting DNA concentration was fourfold lower in this specimen (10 ng *versus* 40 ng). This observation is similar to data obtained by Dean et al¹ who demonstrated that the yield of IMDA-amplified DNA is consistent within a

TCR β -Southern Blot

Figure 9. TCR β -gene rearrangement assessment of cerebrospinal fluid (CSF) specimen by Southern blot analysis. IMDA-DNA using cells (lane 1) or purified DNA (lane 2) as starting material show identical band patterns with *Eco*RI, *Hind*III, and *Bam*HI restriction enzymes. Lane M, molecular size markers; lane C, DNA from HL60 control.

range regardless of the amount of starting material over a 5-log range. Thus, with this limited clinical specimen, following IMDA we were able to perform Southern blot analysis and demonstrate TCR β chain gene rearrangement (Figure 9). Using the replicated DNA, we also repeated the PCR analysis of the T-cell receptor γ chain gene rearrangement and obtained results identical to those in the original DNA (Figure 8).

Another advantage of the IMDA method is that DNA does not have to be purified for replication. The IMDA method allows replication of DNA directly from biological specimens. To evaluate the utility of this approach, we subjected 300 cells from the CSF specimen described above to IMDA according to the manufacturer's instructions and generated 85 μ g of DNA. The PCR results for the TCR γ chain gene rearrangements were identical to those in the original DNA of this specimen (Figure 9). Southern blot results for the TCR β chain gene using IMDA-DNA derived from CSF cells were also identical to the results obtained with IMDA-DNA derived from original DNA sample (Figure 8), confirming that the quality of DNA generated by this method was similar, whether cells or purified DNA were used as the starting material for replication. The ability to generate large amounts of high quality DNA from a limited number of cells is highly attractive as it eliminates DNA isolation steps and reduces manual errors. In summary, we believe that the results we present show that this IMDA method faithfully replicates whole genome DNA and will find great utility in the clinical laboratory setting.

Acknowledgments

We thank Dr. Seiyu Hosono of Whole Genome Amplification Technology (Molecular Staging Inc., New Haven, CT) for suggestions and critical reading of the manuscript. We also thank Susan Biscanin, Seema Hai, Pramod Mehta, and Nubia Reeves in the Molecular Diagnostic Laboratory for analysis of the samples, and David Gallo-way of the Department of Scientific Publications for editorial suggestions.

References

1. Dean FB, Hosono S, Fang L, Wu X, Faruqi AF, Bray-Ward P, Sun Z, Zong Q, Du Y, Du J, Driscoll M, Song W, Kingsmore SF, Egholm M, Lasken RS: Comprehensive human genome amplification using multiple displacement amplification. *Proc Natl Acad Sci USA* 2002, 99: 5261-5266
2. Lage JM, Leamon JH, Pejovic T, Hamann S, Lacey M, Dillon D, Segraves R, Vossbrinck B, Gonzalez A, Pinkel D, Albertson DG, Costa J, Lizardi PM: Whole genome analysis of genetic alterations in small DNA samples using hyperbranched strand displacement amplification and array-CGH. *Genome Res* 2003, 13:294-307
3. Dettler JC, Jett JM, Lucas SM, Dalin E, Arellano AR, Wang M, Nelson JR, Chapman J, Lou Y, Rokhsar D, Hawkins TL, Richardson PM: Isothermal strand-displacement amplification applications for high-throughput genomics. *Genomics* 2002, 80:691-698
4. Derksen PW, Langerak AW, Kerkhof E, Wolvers-Tettero IL, Boor PP, Mulder AH, Vrints LW, Coebergh JW, van Krieken JH, Schuurin E, Kluin PM, van Dongen JJ: Comparison of different polymerase chain reaction-based approaches for clonality assessment of immunoglob-

- ulin heavy-chain gene rearrangements in B-cell neoplasia. *Mod Pathol* 1999, 8:794–805
5. Theriault C, Galoin S, Valmary S, Selves J, Lamant L, Roda D, Rigal-Huguet F, Brousset P, Delsol G, Al Saati T: PCR analysis of immunoglobulin heavy chain (IgH) and TcR- γ chain gene rearrangements in the diagnosis of lymphoproliferative disorders: results of a study of 525 cases. *Mod Pathol* 2000, 13:1269–1279
6. Kiyoi H, Naoe T, Nakano Y, Yokota S, Minami S, Miyawaki S, Asou N, Kuriyama K, Jinnai I, Shimazaki C, Akiyama H, Saito K, Oh H, Motoji T, Omoto E, Saito H, Ueda R: Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. *Blood* 1999, 93:3074–3080
7. Yamamoto Y, Kiyoi H, Nakano Y, Suzuki R, Kidera Y, Miyawaki S, Asou N, Kuriyama K, Yagasaki F, Shimazaki C, Akiyama H, Saito K, Nishimura M, Motoji T, Shinagawa K, Takeshita A, Saito H, Ueda R, Ohno R, Naoe T: Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. *Blood* 2001, 97:2434–2439
8. Sanchez-Vega B, Vega F, Medeiros LJ, Lee MS, Luthra R: Quantification of bcl-2/JH fusion sequences and a control gene by multiplex real-time PCR coupled with automated amplicon sizing by capillary electrophoresis. *J Mol Diagn* 2002, 4:223–239
9. Luthra R, McBride JA, Cabanillas F, Sarris A: Novel 5' exonuclease-based real-time PCR assay for the detection of t(14;18)(q32;q21) in patients with follicular lymphoma. *Am J Pathol* 1998, 153:63–68
10. Sarris AH, Luthra R, Cabanillas F, Morris SW, Pugh WC: Genomic DNA amplification and the detection of t(2;5)(p23;q35) in lymphoid neoplasms. *Leuk Lymphoma* 1998, 29:507–514
11. Hirsch-Ginsberg C, LeMaistre AC, Kantarjian H, Talpaz M, Cork A, Freireich EJ, Trujillo JM, Lee MS, Stass SA: RAS mutations are rare events in Philadelphia chromosome-negative/bcr gene rearrangement-negative chronic myelogenous leukemia, but are prevalent in chronic myelomonocytic leukemia. *Blood* 1990, 76:1214–1219
12. Medeiros LJ, Carr J: Overview of the role of molecular methods in the diagnosis of malignant lymphomas. *Arch Pathol Lab Med* 1999, 123:1189–1207
13. Vega F, Medeiros LJ, Jones D, Abruzzo LV, Lai R, Manning J, Dunmire V, Luthra R: A novel four-color assay to assess for T-cell receptor gamma gene rearrangements in lymphoproliferative lesions. *Am J Clin Pathol* 2001, 116:17–24