Analysis of Rearranged T-Cell Receptor β-Chain Genes by Polymerase Chain Reaction (PCR) DNA Sequencing and Automated High Resolution PCR Fragment Analysis

By M. Kneba, I. Bolz, B. Linke, and W. Hiddemann

Polymerase chain reaction (PCR)-directed amplification and sequencing of rearranged immune genes for identification of clone-specific markers are increasingly being used in acute lymphoblastic leukemia (ALL) and non-Hodgkin’s lymphoma (NHL) patients instead of the time consuming and labor intensive Southern analysis. In previous reports, no single region elements that are joined together by a complex rearrangement are conserved sequences of the JP genes. In combination with a previously published consensus Vβ primer, these Jβ primers specifically amplify TCR-β V-N(D)N-J junctions from genomic DNA. Using this approach we studied DNA extracted from biopsy material of nine patients with T-cell lymphoproliferative disorders, one c-ALL patient, and five patients with nonmalignant diseases. T-cell lines Molt 3, Jurkat, and HM 2 served as monoclonal controls. Individual PCR products were sequenced after cloning. The nucleotide sequences of 96 randomly chosen recombinant vectors were determined. In the polyclonal controls all analyzed clones differed in their TCR-β V-N(D)N-J junctions. In the T-cell lines, in all of the T-cell malignancies, and in the c-ALL, monoclonal PCR products could be identified by demonstration of clonally restricted V-N(D)N-J junctions. The PCR results were confirmed by automated fluorescence quantification and size determination of PCR products after separation in a high-resolution polyacrylamide gel. The procedure allows rapid and specific characterization of clonal TCR-β rearrangements from genomic DNA and will significantly simplify current experimental approaches to identify and to quantitate malignant T cells during initial staging and follow-up of T-lineage NHL and ALL patients.

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ers directed against conserved sequences of the Jβ genes. In combination with a previously published consensus Vβ primer these Jβ primers amplify specific TCR-β V-N(D)N-J junctions from genomic DNA. The PCR results were confirmed by automated fluorescence quantification and size determination of PCR products after separation on a high-resolution polyacrylamide gel.36-39

MATERIALS AND METHODS

Clinical samples and cell lines. Nine patients with T-cell lymphoproliferative disorders, one patient with c-ALL, and five patients with nonmalignant diseases were selected for this study (Table 1). All patients were admitted to the Department of Internal Medicine of the University of Göttingen between 1986 and 1991 (6 T-ALL, 1 case with Philadelphia chromosome positive c-ALL, 4 peripheral T-NHL, and 1 case with large granular CD8 lymphocytosis [LGL]). The lymphomas were categorized according to the updated Kiel classification,40 using conventional morphologic and immunohistologic techniques. The T-cell lines Jurkat, Molt 3, and HM 2 (obtained from ATCC [Rockville, MD]) served as monoclonal controls. Four cases with unspacific lymphadenitis and one case with toxoplasmosis were used as polyclonal samples.

Table 1. Clonality of TCR-β Gene Rearrangements Detected by Southern Blotting, PCR-Directed Sequencing, or Genescan Analysis

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>Southern Blotting</th>
<th>Sequencing*</th>
<th>Genescan Analysis†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reactive lymphadenitis</td>
<td>G</td>
<td>0/5</td>
<td>p</td>
</tr>
<tr>
<td>2</td>
<td>Reactive lymphadenitis</td>
<td>G</td>
<td>0/8</td>
<td>p</td>
</tr>
<tr>
<td>3</td>
<td>Toxoplasmosis</td>
<td>G</td>
<td>ND</td>
<td>p</td>
</tr>
<tr>
<td>4</td>
<td>Reactive lymphadenitis</td>
<td>G</td>
<td>ND</td>
<td>p</td>
</tr>
<tr>
<td>5</td>
<td>Reactive lymphadenitis</td>
<td>G</td>
<td>ND</td>
<td>p</td>
</tr>
<tr>
<td>6</td>
<td>Jurkat</td>
<td>R</td>
<td>10/10</td>
<td>m</td>
</tr>
<tr>
<td>7</td>
<td>Molt 3</td>
<td>ND</td>
<td>2/2</td>
<td>m</td>
</tr>
<tr>
<td>8</td>
<td>HM 2</td>
<td>ND</td>
<td>4/4</td>
<td>m</td>
</tr>
<tr>
<td>9</td>
<td>T-ALL</td>
<td>2R</td>
<td>10/10</td>
<td>m (oligo)</td>
</tr>
<tr>
<td>10</td>
<td>T-ALL</td>
<td>2R</td>
<td>2/7</td>
<td>m (biallelic)</td>
</tr>
<tr>
<td>11</td>
<td>T-ALL</td>
<td>ND</td>
<td>6/6; 5/5</td>
<td>m (biallelic)</td>
</tr>
<tr>
<td>12</td>
<td>T-ALL</td>
<td>1 R</td>
<td>3/4</td>
<td>m</td>
</tr>
<tr>
<td>13</td>
<td>c-ALL</td>
<td>ND</td>
<td>5/5</td>
<td>m</td>
</tr>
<tr>
<td>14</td>
<td>T-NHL</td>
<td>1 R</td>
<td>2/8; 4/8</td>
<td>m (p)</td>
</tr>
<tr>
<td>15</td>
<td>T-NHL</td>
<td>1 R</td>
<td>3/4</td>
<td>m</td>
</tr>
<tr>
<td>16</td>
<td>T-NHL</td>
<td>2-3 R</td>
<td>5/7; 2/7</td>
<td>m (p)</td>
</tr>
<tr>
<td>17</td>
<td>T-NHL</td>
<td>1 R</td>
<td>2/9; 2/9</td>
<td>2/9 oligo</td>
</tr>
<tr>
<td>18</td>
<td>CD8 Thy-lymphocytosis</td>
<td>1 R</td>
<td>2/4</td>
<td>oligo</td>
</tr>
</tbody>
</table>

Abbreviations: R, rearrangement; G, germline configuration; ND, not determined; m, monoclonal; p, polyclonal.

* Oligo, oligoclonal PCR product as indicated by the electrophoretic fluorescence-intensity/PCR product-size pattern on gene scanning. m (oligo) denotes a dominant monoclonal product peak and in addition a few minor side bands; m (biallelic) signifies the presence of two distinct monoclonal PCR products in one DNA sample indicating the presence of two rearranged TCR-β alleles in the neoplastic T-cell population. m (p) denotes a monoclonal product peak in a background of polyclonal bands.

† In case 11, the 284-bp and the 249-bp DNA fragments were cloned separately.

Table 2. Oligonucleotide Primers Used for TCR-β PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vβ Con</td>
<td>5' CTCGAATCT (T/G) T (A/G) CTGTTA (C/T) C (G/A) (T/A) CA 3'</td>
</tr>
<tr>
<td>Jβ I (1)</td>
<td>5' CTGGATCC (T/A) GAG (T/C) C I (G/A) GT (C/T) CC I I I CCAA 3'</td>
</tr>
<tr>
<td>Jβ I (2)</td>
<td>5' CTGGATCCAC I (T/A) GAG (T/C) C I (G/A) GT (C/T) CC 3'</td>
</tr>
<tr>
<td>Jβ II (1)</td>
<td>5' CTGGATCC (G/C) AGCC (T/G) I GTGCC I G (G/C) I CCGAA 3'</td>
</tr>
<tr>
<td>Jβ II (2)</td>
<td>5' CTGGATCCAC I GT (G/C) AGCC (T/G) I GTGCC 3'</td>
</tr>
</tbody>
</table>

Sequences are adopted from published germline sequences.41-48

Joining region (downstream) primers are complementary inverse to the germline sequences. Artificial restriction enzyme recognition sequences (EcoRI, BamHI) at the 5' ends are underlined.

Genomic Southern blot analysis. Southern analysis of genomic DNA for identification of TCR-β rearrangements was performed as previously described49,50 with a radiolabeled TCR-β constant-region probe after EcoRI and BamHI restriction enzyme digestion.

PCR conditions. The oligonucleotide primers used in this study are shown in Table 2. The Vβ consensus primer (Vβ con) was adopted from the published sequence of Lessin et al.51 This consensus primer was designed to anneal to a region of 88% to 100% homology among the different Vβ gene families. PCR was performed on an automated thermocycler (Model 480; Perkin-Elmer, Cetus, Foster City, CA) in 50 μL of 1X PCR buffer. Standard reaction buffer, [50 μm/mL Tris-HCl pH 9.0, 20 μm/mL (NH4)2SO4, 3 μm/mL MgCl2, 200 μm/mL of dATP, dGTP, dCTP, and dTTP] containing 500 ng DNA template and 1 U Tfi DNA Polymerase (Biozym, Hameln, Germany) was used. Primers were used at a concentration of 10 μm/mL. For the automated fluorescent fragment analysis, PCR was performed under the same conditions except with a lower MgCl2 (1.5 μm/mL) and primer concentration (3 μm/mL). Incorporation of EcoRI and BamHI restriction sites at the 5' ends of the primers facilitated the insertion of PCR products into the cloning site of the vector pUC19. For fluorescent PCR fragment analysis primer Vβcon was labeled at its 5' end with a chlorinated equivalent to 6-carboxyfluorescein (HEX) and high performance liquid chromatography (HPLC) purified (Applied Biosystems, Weiterstadt, Germany). For TCR-β PCR two rounds of amplification were performed in which first primer set Vβ Con/βII (1) and Jβ II (1). Cycle conditions with primers Vβ Con/Jβ II (1) and Jβ II (1) were 1 minute at 92°C (denaturing), 40 seconds at 50°C (annealing), and 30 seconds at 72°C (strand elongation) for 40 cycles. The first denaturation and last primer extension step were extended to 5 minutes. In a second round of 30 cycles with 1 minute at 92°C, 40 seconds at 55°C, and 30 seconds at 72°C and primer set Vβ Con/βII (1) and Jβ II (1) PCR products of around 255 bp (range ~240 to 290 bp) were generated. Amplification products were analyzed by electrophoresis in an ethidium bromide containing 2.5% agarose gel (Sigma, Deisenhofen, Germany).

Cloning and DNA sequencing. Cloning of PCR products was performed as previously described.52 Recombinant plasmids were sequenced by the Taq-cycle-sequencing method involving the universal forward sequencing primers for PUC 19 and the Ready Reaction Dye Deoxy Terminator Cycle Sequencing kit (Applied Biosystems, Weiterstadt, Germany). For each DNA sample 2 to 11 randomly chosen clones were sequenced. Sequencing reaction prod-
Fig 1. Ethidium bromide stained 2.5% agarose gel of PCR products synthesized with the TCR Vβ and Jβ consensus primers and DNA from polyclonal controls (1-5), cell lines Jurkat (6), Molt 3 (7), and HM 2 (8), 9 cases with T-cell lymphoproliferations (cases 9-12 and 14-18), and one c-ALL (case 13). Cases are numbered according to Tables 1 and 3 and are identical to those in Fig 2. PCR size marker (Promega, Madison, WI; Cat. No. 631611) is in the right lane. Note that intense and relatively sharp bands are seen with polyclonal as well as clonal PCR products.

RESULTS

Southern blot analysis of TCR-β rearrangements. Thirteen DNA samples were studied by Southern analysis for TCR-β rearrangements. The results are shown in Table 1. Whereas Southern blotting demonstrated monoclonal TCR-β rearrangements in all T lymphoproliferations tested, only germline bands were obtained in the four cases with unspecified lymphadenitis and in the case with toxoplasmosis (polyclonal controls), as expected. In cases 17 and 18 relatively weak rearranged TCR-β bands were observed obviously as a result of a low lymphoma T cell content in these diagnostic samples.

TCR-β PCR. After two rounds of amplification all 18 DNA samples generated a PCR product in the expected size range between 240 and 290 bp for TCR-β V-D-J recombinations as directly visualized after electrophoresis in an ethidium bromide containing agarose gel (Fig 1). Under standard PCR conditions (see Materials and Methods) in the polyclonal controls and the T-NHL samples (cases 1 to 5 and 14 to 18, respectively) several bands of unknown origin were seen above and below the dominant specific TCR-β PCR product (data not shown). However, use of GENESCAN fluorescent-primer reaction buffer containing HPLC-purified primers and in addition a much lower primer (3 μmol/L) and MgCl₂ (1.5 mmol/L) concentration markedly improved the specificity of the reaction (Fig 1). Case 11 was exceptional as it produced two clearly separated bands of 249 bp and 284 bp in the absence of other background bands. As could be shown by sequencing, both bands represent the two clonally rearranged TCR-β alleles in this particular case (Table 3).

Sequencing of cloned PCR products. As previously seen with amplified TCR-γ genes, visual analysis after electrophoresis in agarose gels alone did not allow us to distinguish exactly between monoclonal and polyclonal TCR-β PCR products. For verification of the specificity of the TCR-β PCR and to demonstrate the polyclonality or monoclonality of the PCR fragments, we therefore excised the PCR products that made up the dominant band of around 255 bp from the gel and cloned them after ligation into the polylinker site of the pUC19 sequencing vector. In case 11 the 284 bp and the 249 bp DNA fragments were cloned separately. The TCR Vβ-N(Dβ)N-Jβ sequences of the cloned inserts were determined by isolation of recombinant clones from 2 to 11 randomly chosen separate bacterial colonies for each patient or cell line. The sequencing approach confirmed the specificity of the PCR demonstrating TCR Vβ-N(Dβ)N-Jβ junctions in all cases. The results are shown in Table 3. By sequencing a total of 96 TCR Vβ-N(Dβ)N-Jβ junctions in 15 samples (samples 1, 2, and 6 through 18), clonality could be demonstrated exclusively in the T-cell lines, in 5 of 5 of the ALLs, in 4 of 4 of the lymphomas, and in the case with T lymphocytosis. In one case (no. 17) nine clones were sequenced and three rearrangements appeared twice, and three only once, suggesting that three clonally restricted junctions and at least three background rearrangements were present in this case (Table 3). However, GENESCAN analysis showed an oligoclonal pattern with at least eight distinct product peaks in this case (Fig 2). Given the peak heights in the Genescan analysis, it is likely that more than three nonrandom rearrangements exist in the T-cell population, which probably would have been discovered if additional clones had been analyzed. A similar situation exists for case no. 18, in which four clones were sequenced and one was found twice. In all sequenced clones a distinct Vβ and Jβ gene segment could be identified. A Dβ element was found in all except four clones. The junctional regions showed an extensive diversity due to the addition of N-region nucleotides between the Vβ-Dβ and Dβ-Jβ junctions and seem
**Table 3. TCR-β V-(N)(D)N-J Junctional Sequences**

<table>
<thead>
<tr>
<th>Case (n)</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vβ</td>
<td>5.4</td>
<td>1.1</td>
<td>2.3</td>
<td>1.1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>DJβ</td>
<td>1.1</td>
<td>2.3</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Jβ</td>
<td>2.3</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>V Region</td>
<td>TACTTTGTCGCGACCAGCTGT</td>
<td>TACTTTGTCGCGACCAGCTGT</td>
<td>TCTATCTCTCTGTGCCAGTAG</td>
<td>TCTATCTCTCTGTGCCAGTAG</td>
<td>TCTATCTCTCTGTGCCAGTAG</td>
<td>TCTATCTCTCTGTGCCAGTAG</td>
<td>TCTATCTCTCTGTGCCAGTAG</td>
<td>TCTATCTCTCTGTGCCAGTAG</td>
<td>TCTATCTCTCTGTGCCAGTAG</td>
</tr>
<tr>
<td>DJ Region</td>
<td>GAGGGCTCGAGG</td>
<td>GAGGGCTCGAGG</td>
<td>GAGGGCTCGAGG</td>
<td>GAGGGCTCGAGG</td>
<td>GAGGGCTCGAGG</td>
<td>GAGGGCTCGAGG</td>
<td>GAGGGCTCGAGG</td>
<td>GAGGGCTCGAGG</td>
<td>GAGGGCTCGAGG</td>
</tr>
<tr>
<td>J Region</td>
<td>GCTTCTACATCTGCAGTGC</td>
<td>GCTTCTACATCTGCAGTGC</td>
<td>GCTTCTACATCTGCAGTGC</td>
<td>GCTTCTACATCTGCAGTGC</td>
<td>GCTTCTACATCTGCAGTGC</td>
<td>GCTTCTACATCTGCAGTGC</td>
<td>GCTTCTACATCTGCAGTGC</td>
<td>GCTTCTACATCTGCAGTGC</td>
<td>GCTTCTACATCTGCAGTGC</td>
</tr>
</tbody>
</table>

**TCR-β V-(N)(D)N-J junctional sequences are shown.** The PCR-derived sequences are aligned to the known germ line Vβ and Jβ sequences. Underlined nucleotides flank the template independent (N) sequences. The PCR products were generated from genomic DNA under the described PCR conditions with standard probe. The scanning results are shown in Fig 2. Because of which had been previously analyzed by Southern blotting and hybridization with a radioactively labeled TCR-β gene probe. The scanning results are shown in Fig 2. Because of the hypervariable character of rearranged TCR-β V-(N)(D)N-J junctions, the size distribution pattern of a given PCR product characterizes the corresponding T-cell population. Whereas DNA extracts from polyclonal lymphoid-cells (samples 1 to 5) yielded a "fluorescence spectrum" of DNA bands composed of polyclonal PCR fragments of different sizes, products derived from homogeneous clonal cell populations—the cell lines, the acute leukemias, and the T-NHL cases 13 and 15—produced one or two sharp and dominant peaks of fluorescence corresponding to the PCR-amplified clonally rearranged alleles. Case 9, which showed a biallelic Southern rearrangement pattern, yielded only one sharp and prominent peak in addition to four clearly separated small peaks. In two leukemias (cases 10 and 11) the TCR-β PCR demonstrated a clear biallelic rearrangement in agreement with the Southern blot data. The T-NHL samples 14, 16, 17, and 18, however, showed one or two prominent peaks of fluorescence over a background of polyclonal PCR products. As could be demonstrated by sequencing of cloned PCR products.
Fig 2. Electrophoretic profiles of fluorescent TCR-β PCR products from five polyclonal controls (cases 1-5), one c-ALL, and nine patients with malignant T-cell proliferations analyzed with the GENESCAN 672 software on the automated sequencer. Relative fluorescence intensities (y-axis) are plotted as a function of PCR fragment size (x-axis) for each PCR product. Cases are numbered according to Tables 1 and 3. The x-axis reflects the PCR product size in base pairs.
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products (Table 3) and by the weak rearranged bands on Southern analysis in cases 17 and 18, this fluorescence pattern is most likely caused by coamplification of monoclonal as well as polyclonal TCR- V-N(D)N-J junctions of neoplastic and admixed "contaminating" nonmalignant T cells, which can make up a substantial cell fraction in some of the nodal T-cell lymphomas.

To confirm the specificity of the gene scanning results we reamplified plasmid DNA extracted from randomly selected recombinant clones of PCR products from which the DNA sequences shown in Table 3 had been derived. Gene scanning of the fluorescent PCR products of the cloned plasmids clearly demonstrated that their sizes were in complete agreement with the most prominent PCR fragments generated in separate PCR runs with the corresponding diagnostic DNA samples (data not shown).

DISCUSSION

In the present study we characterized the TCR- V- N(D)N-J junctions from a variety of hematologic malignancies including not only T-ALL but also peripheral T-cell NHL by PCR amplification of genomic DNA and sequencing. We previously described a technique that allows accurate distinction between monoclonal and polyclonal TCR- V-N-J junctions by temperature gradient gel electrophoresis (TGGE) of TCR- V PCR products. However, our attempts to analyze the TCR- β PCR products by TGGE gave unsatisfactory results (not shown). The most likely explanation for this observation is the use of highly degenerate TCR- β PCR primers, which also contained inosine residues. This leads to double stranded TCR- β PCR products that are thermally unstable during TGGE resulting in relatively broad bands or a diffuse smear. In search for a more robust procedure that is not influenced by thermal instability of PCR fragments we analyzed the PCR products after fluorescent labeling by a new method, automated fluorescence quantification and size determination after separation on a high-resolution polyacrylamide gel in an automated DNA sequencer. As we have shown here, complete agreement with respect to clonality and size of the predominant PCR fragments was obtained between this new method and PCR-directed sequencing. However, it should be noted that with both methods unequivocal results were obtained only with the polyclonal control samples and in cases in which leukemia/lymphoma cells were in high abundance (the cell lines, the leukemias, and the T-NHL cases no. 14 to 16). In cases in which the neoplastic cells were in low abundance or probably truly oligoclonal (cases 17 and 18), the interpretation of the data was much less clear-cut. A similar approach, for which the term "spectratyping" was proposed, has been described for the analysis of circulating T-cell repertoire complexity in normal individuals and bone marrow recipients or the Vβ-specificity of superantigen activation. In this procedure, the TCR- β repertoire complexity is measured based on CDR3 size-heterogeneity within Vβ families and was used as an indicator for immunocompetence in individuals or of reconstitution of the immune system after bone marrow transplantation. As we have shown here, the TCR- β spectratype can also be useful as marker for clonality in ALL and T-NHL and for discrimination between monoclonal and polyclonal T-cell populations. A similar technique has been described for detection of immunoglobulin gene rearrangement in one case with B-ALL.  

Despite the complexity of the TCR- β locus, several techniques using the PCR amplification of TCR- β regions have been developed. The published strategies for TCR- β DNA PCR are rather complex and impractical for routine diagnostics. In addition, most of the previous studies using PCR techniques to investigate the use of TCR repertoires in the clinical setting rely strongly on strategies that target TCR- β RNA transcripts. The analysis of mRNA reverse transcribed cDNA sequences places certain constraints on the minimal quality of the genetic material necessary to perform PCR analysis. Unfortunately, fresh or snap frozen diagnostic material from ALL or T-NHL patients for extraction of RNA is often not available in clinical practice. However, DNA suitable for PCR is more easy to isolate, even from paraffin embedded material and is more stable than RNA and there is no need for reverse transcription before performing PCR. The described convenient and nonradioactive strategy that targets genomic DNA for analysis of rearranged TCR- β chain genes by PCR in combination with computer assisted size determination and nonradioactive DNA sequencing of clonal TCR- β V-D-J joinings circumvents a number of the problems encountered with RT-PCR. In addition, in dilution experiments it should be possible to get quantitative information on the amount of distinct T-cell clones in diagnostic samples from the intensity of the fluorescence signal if the PCR is carried out with a clone-specific primer. This would provide the means for the broad application of analyzing disease-related clonal T cells in DNA samples from patients with T-lymphoproliferative diseases by sensitive PCR-based techniques. In mixing experiments the lower detection limit for the detection of a fluorescent clone-specific PCR product was as low as 1 Jurkat cell diluted in 10 5 peripheral blood mononuclear cells in a single 35 cycle allele-specific PCR run with a Jurkat-specific Dβ- N-Jβ region primer and a Jurkat-specific Vβ (8.1)-primer (data not shown). The extensive junctional and combinatorial diversity favor the TCR- β gene as a target for detection of MRD especially in T-NHL using PCR-mediated amplification techniques.

This technique has several advantages compared with conventional Southern analysis such as speed, safety, comfort, low cost, and it requires no radionucleotides. Since fluorescent TCR- β-PCR products appearing as a relatively pure monoallelic peak of fluorescence in the absence of significant background side bands can be sequenced by direct cycle sequencing without cloning, the method can also be used as a first step in a sequencing strategy. The procedure will significantly simplify current experimental approaches to detect and to quantitate malignant T cells during initial staging and follow-up of NHL and ALL patients. It is anticipated that this efficient and simple method of detecting monoclonal rearranged TCR- β genes and clonotypic TCR- β sequences from DNA could have vast application in the study of T-
NHL and ALL patients. Furthermore, this technique should also be applicable for the analysis of PCR amplified TCR-\(\gamma\), TCR-\(\delta\), and IgH-CDR 3 regions.

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Analysis of rearranged T-cell receptor beta-chain genes by polymerase chain reaction (PCR) DNA sequencing and automated high resolution PCR fragment analysis

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