The asymmetric-PCR SSCP modifications described here consistently and reproducibly result in improved amplification of DNA segments with much improved resolution of products in the absence of artifacts. This technique allows unambiguous assignment of genotypes and can clarify or confirm findings of other techniques of DNA polymorphism analysis.

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Richard M. Pokorny, Allan B. Dietz1, Susan Galandiuk and Holly L. Neibergs

University of Louisville School of Medicine
Louisville, KY

The Human Gene Therapy Research Institute
Des Moines, IA, USA

DNA Template as a Source of Artifact in the Detection of p53 Gene Mutations Using Archived Tissue

Determination of the spectra of mutations in the p53 gene may provide clues regarding environmental carcinogens involved in the etiology of human cancers (3). Exons 5–8 of the p53 gene are usually amplified by the polymerase chain reaction (PCR) before further analysis for mutations by techniques such as single-strand conformation polymorphism (SSCP) analysis and DNA sequencing. Detection of gene mutations using archived tissue in paraffin blocks provides an advantage in that tumor characteristics are readily retrieved for comparison with the corresponding mutation spectrum. Since fixation might be expected to affect the quality of DNA for genetic study, conditions for fixation, DNA extraction and PCR amplification have been optimized to provide sufficient amplified product for further mutation analysis (4,10). However, base mis-incorporation resulting from PCR amplification of damaged DNA from formalin-fixed, paraffin-embedded tissues has not been carefully assessed.

Two human colon cancer (A and B) and two breast cancer (C and D) specimens had been fixed in 10% buffered formalin and embedded in paraffin by conventional procedures. The ages of the tissue blocks ranged from 2 to 8 years. The procedure for DNA extraction was modified from Wright and Manos (11). In summary, two 10-µm-thick sections of formalin-fixed, paraffin-embedded tissue were scraped into a 0.5-mL tube for deparaffinization, rehydration and proteinase K digestion. Tissue sections were digested in 500 µL of proteinase K solution [50 mM Tris base, 100 mM NaCl, 1 mM Na2EDTA, 0.5% (vol/vol) Tween® 20, 0.5 mg/mL proteinase K, pH 8.2] at 55°C. Aliquots of 100 µL were removed sequentially after 8, 16, 24, 32 and 48 h of digestion. After heat treat-
ment at 95°C for 10 min, DNA was extracted by adding 1/3 vol of saturated NaCl and precipitated by 2 vol of absolute ethanol, as described previously (8). The amount of DNA was quantified fluorometrically using the Hoechst 33258 dye procedure (7).

Exon 5 of the p53 gene was amplified with two sets of primers, E5: 5′-TCAACTCTGTCTCTCTCTTT CCT-3′ and 5′-CTGGGCAACCCAGC CCTGTCTGT-3′ and E5A: 5′-TGCCCTGACTTTCAACTCTGT-3′ and 5′-CATGTGCTGTGACTGCTTGTA-3′. The E5 primer set amplified a 256-bp product containing the entire exon 5. E5A primers generated a partial exon 5 fragment of 172 bp.

Triplicate PCRs were carried out for each sample after successive periods of DNA extraction. Fifty nanograms of DNA template were used for each PCR amplification (40 cycles). PCR conditions were as published (6) except that annealing temperatures of 62°C and 55°C were selected for the E5 and E5A primer sets, respectively.

Before SSCP analysis, PCR products were analyzed on 10% polyacrylamide TBE gels (Novex, San Diego, CA, USA) to ensure no nonspecific amplification. Products from triplicate PCRs were run side by side in the “Cold SSCP” analysis, which was performed with 1.25× TBE running buffer (111 mM Tris base, 111 mM boric acid, 2.5 mM Na₂EDTA, pH 8.4) and 20% polyacrylamide TBE gels (Novex) at 25°C buffer temperature and a constant 300 V for 2.5 h, as described previously (5). Constant inner and outer buffer temperatures were carefully controlled throughout the SSCP analysis to obtain a maximum sensitivity. Gels were then stained with SYBR™ Green II (Molecular Probes, Eugene, OR, USA) at a 1:10000 dilution with deionized water for 20 min and photographed with the IS-1000 Imaging System (Alpha Innotech, San Leandro, CA, USA).

Triplicate PCRs and SSCP analyses for the 172- and 256-bp products are illustrated in Figures 1 and 2, respectively. SSCP patterns were dissimilar among the triplicate PCR products of samples resulting from short periods of proteinase K digestion, indicating artifactualy introduced mutations. For the 172-bp fragment, the error frequency was estimated to be approximately one mistake per 172 nucleotides (nt), since only one or one pair of extra band(s) was seen in SSCP analysis. Multiple extra bands in the 256-bp PCR product indicate that the error frequency per nucleotide is more than 2/256. Both these error frequencies are much higher than reported in the literature for DNA from unfixed cells, in which the estimates of Taq DNA polymerase infidelity are one mistake per 363–5411 nt (1). The higher error rate of PCR in our study suggests that a poor DNA template can further enhance the infidelity of DNA polymerase.

Table 1 displays the minimum digestion time required for each study specimen to obtain consistent SSCP patterns among triplicate PCRs. All the study cases were free from misincorporation artifacts in the amplification of the 172-bp fragment after 48 h of proteinase K treatment. This time-dependent effect suggests that longer proteinase K treatment in DNA extraction may further remove components contributing to the polymerase error. These are most likely to be nucleoproteins, which are cross-linked among each other during formalin fixation (2).

For the 256-bp PCR product, a consistent SSCP pattern was observed only...
The fidelity can be markedly improved by longer proteinase K treatment in DNA extraction and by designing a study to minimize the size of the fragment to be amplified.

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Yih-Horng Shiao, Gregory S. Buzard1, Christopher M. Weghorst2 and Jerry M. Rice

1NCI-FCRDC

2Ohio State University

Columbus, OH, USA

Easy Method for the Cloning of Mammalian Cell Colonies


Recent advances in immunology have led to a renewed interest in the development of tumor vaccines. Frequently, the patient’s own tumor cells are considered a source of antigens for the vaccine. One approach for obtaining sufficient tumor cells from limited clinical material is culturing cells from individual patients. In addition, the test-

Figure 1. Phase-contrast photomicrograph of B16 colony after isolation. A monolayer of B16 melanoma cells was irradiated by UV light for 1 min. A small area of cells was protected from the UV light by the black ink dot in the picture. After 48 h, there is some outgrowth of the “colony” past the confines of the spot.