Benchmarks

staining was indistinguishable from that obtained without prior incubation with the ABTS reagent (Figure 2). Consequently, both quantitative levels and cellular distribution data can be readily obtained from the same cells in this assay.

Presumably, this assay can be extended to monitor the expression, conformational change, or posttranslational modification of any protein for which an appropriate antiserum exists. Obviously, it is necessary to optimize the assay with each different antiserum employed. It is worth varying antiserum concentration over a broad range to enhance the signal-to-noise ratio. A separate but important issue is normalizing the measured signals to the number of cells in the assay. One option is to employ a duplicate column of cells within each plate for separate staining with an antibody against a constitutively expressed "housekeeping" protein. Alternatively, if the plate reader can detect fluorescent probes, then immunofluorescent staining can be normalized to the DNA content of the same set of wells using a DNA intercalating dye such as DAPI or propidium iodide.

In summary, the methods described here are simple, sensitive, and quantitative, and they can be readily adapted for high-throughput assays to monitor a range of appropriate targets.

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**Laurence Shumway and
Lawrence M. Schwartz**
*University of Massachusetts
Amherst, MA, USA*

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Partial Restoration of Degraded DNA from Archival Paraffin-Embedded Tissues

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The development of PCR methods for the analysis of archival paraffin-embedded tissues (PET) has significantly improved the efficiency of medical research and diagnostics. However, the utilization of PET has some limitations related to the partial degradation of DNA during the fixation procedure (7,10). Although several studies have reported the successful amplification of the DNA sequences up to 1 kbp long, it is generally agreed that the fragments below 180 bp seem to be the most suitable for routine, highly reproducible PCR analysis of PET (9,10). The investigations aimed to improve the yield and quality of PET-derived DNA have drawn a high level of attention. The proposed technical tips include the right choice of the fixative protocol (4), prolonged proteinase K digestion (5), use of Chelex® X-100 ion exchange resin (2), etc. Unfortunately, the actual efficiency of these modifications varies between different laboratories; therefore, the domestic optimization of DNA isolation may require an experimental comparison of the published approaches. Here, we present a novel supplementary manipulation that leads to a noticeable facilitation of PCR analysis of archival tissues.

We assumed that the poor quality of PET-derived DNA is at least to some extent attributed to the single-strand breaks (1). Therefore, the non-denatured DNA template can be partially restored by filling of the nicks in the DNA polymerase reaction (8). This would eventually result in better suitability of archival DNA for genetic investigations and would loosen the limit for the maximal size of the PCR fragments. To achieve the DNA restoration, we have developed the following protocol.

First, the 5 µm-thick, formalin-fixed, paraffin-embedded tissue sections are subjected to the standard deparaffinization procedure (incubation in xylene and ethanol for 30 min each).

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Second, to achieve the availability of tissue DNA for DNA polymerase, partial digestion of the sections by proteinase K is performed. The specimens are placed into the PCR tubes containing 500 μ L TE solution (10 mM Tris-HCl, pH 8.0, 1 mM EDTA), 20 μ g/mL proteinase K (20 min at room temperature), then the proteolytic enzyme is carefully washed away by three changes of TE. This step has been adapted from the TUNEL assay (3) and turned out to be absolutely necessary for subsequent DNA restoration (data not shown). Note that excessive exposure to proteinase K could result in tissue disintegration, which will decrease the DNA yield and complicate the washing procedure.

Third, further pretreatment of the archival sections includes gentle lysis and partial solubilization in 100 μ L of the PCR-like mixture containing 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 2% Triton[®] X-100, 200 μ M each dNTP (55°C for 60 min). The *Taq* DNA polymerase (Applied Biosystems, Foster City, CA, USA) is then

added for the final concentration of 0.1 U/ μ L, and the DNA-polymerase reaction is carried out for 20 min at 72°C.

Fourth, the restored DNA is isolated according to the standard protocol (6). Briefly, intensive protein digestion is achieved by the addition of proteinase K up to 500 μ g/mL and subsequent incubation of the sections at 55°C for 16 h. Finally, the enzyme is inactivated by heating of the samples at 95°C for 10 min, and the obtained DNA lysates are suitable for subsequent PCR.

As shown in Figure 1, the *Taq* DNA polymerase-driven reconstruction of PET-derived DNA increases the success rate of PCR and allows one to amplify larger DNA fragments. Of note, the enzymatic restoration of highly purified, phenol/chloroform-extracted DNA did not bear any advantage in our hands when compared to the described quick protocol (data not shown). In conclusion, the proposed method has potential significance for all applications related to the genetic analysis of formalin-fixed, paraffin-embedded archival tissues.

Figure 1. The effect of *Taq* DNA polymerase-driven restoration of PET-derived DNA. Six randomly selected archival breast cancer tissue sections (lanes 1–6) were subjected to DNA isolation in duplicate. The processing of the samples had been done as described in the text, without (left) or with (right) the addition of *Taq* DNA polymerase. PCR was performed under standard conditions. The 10- μ L reactions included 1 μ L DNA-containing tissue lysate, 0.5 U heat-activated *Taq* DNA polymerase, 1 $\times$ PCR buffer, 1.5 mM $MgCl_2$, 200 μ M dNTP, 1 μ M each primer, and were carried out for 40 cycles of 95°C for 35 s, 57°C for 45 s, and 72°C for 45 s after an initial activation of the polymerase at 95°C for 10 min. The products were separated in the polyacrylamide gel. The primers were 5'-AGGCTGGTTGCAACTGGG-3' and 5'-GAGGTCAAATAAGCAGCAGG-3' for p53 gene, 5'-TGAAGGAGAAGGTGTCTGCGGA-3' and 5'-TGGCCAGCGCGGTGAGAGTG-3' for MTHFR gene, and 5'-CAACTTCATCCACGTTCACC-3' and 5'-GAAGAGCCAAGGACAGGTAC-3' for β -globin gene.

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This work is supported by INTAS grant no. 96-1551 and RFBR grant no. 99-04-48043. Address correspondence to Dr. Evgeny Imyaninov, Group of Molecular Diagnostics, N.N. Petrov Institute of Oncology, St.-Petersburg 197758, Russia. e-mail: evgeny@imyaninov.spb.ru

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E.N. Imyaninov, M.Yu. Grigoriev, V.M. Gorodinskaya, E.Sh. Kuligina, K.M. Pozhariski, A.V. Togo, and K.P. Hanson
N.N. Petrov Institute of
Oncology
St.-Petersburg, Russia