the desired fragment after one round of bandstab. If the desired level of purity had not been achieved from the first round of bandstab, it was possible to re-amplify the target fragment from the gel used to check the yield and purity of the initial bandstab products.

The bandstab approach can bypass the need for cloning PCR products and all associated work required to generate recombinants, check the insert size and prepare recombinant DNA. The bandstab technique may be used to obtain a pure template ready for direct DNA sequencing within hours of first visualizing the band on a gel, with considerable savings in time, labor and reagents.

REFERENCES


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Sequential Extraction of DNA and DNA-Binding Proteins from Low Cell Numbers


It is possible to purify nuclear-binding proteins from a limited supply of cells with relative ease and convenience. More recently, these protocols have improved, resulting in less manual labor and greater flexibility, thereby allowing the analysis of multiple samples (1,2). However, when using these techniques, it is difficult to efficiently purify nuclear proteins and extract genomic DNA from low cell numbers, particularly when dealing with clinical samples. We regularly deal with low lymphocyte numbers from patients with B-cell chronic lymphocytic leukemia (B-CLL) and often do not have enough material to accommodate or provide reasonable yields for nuclear protein and genomic DNA. We now describe a simple protocol that allows for the isolation of genomic DNA following the extraction of DNA-binding proteins. This technique complements existing protocol by Andrews and Faller.
Benchmarks (1), which involves the extraction of nuclear-binding proteins from a limited supply of cells. Following the isolation of nuclear proteins, the chromatin pellet is left intact after high salt extraction, and DNA is simply extracted from the original source with minimal waste. It is recommended that the salt buffers be freshly prepared just before protein isolation. In contrast, the DNA lysis solution can be prepared beforehand and will remain stable at room temperature for up to 3 months. To prepare for extraction, up to $1 \times 10^6$ cells are collected and spun down to a cell pellet. The cells are washed twice in 2 mL ice-cold phosphate-buffered saline (PBS) and spun down again to a cell pellet. All manipulations can now be carried out in a 1.5-mL microcentrifuge tube.

The preparation of nuclear extracts was described originally by Andrews and Faller (1) and is used with no alterations. The cells are resuspended in 0.8 mL ice-cold low-salt buffer (10 mM HEPES-KOH, pH 7.9, 1.5 mM MgCl$_2$, 10 mM KCl, 0.5 mM dithiothreitol [DTT] and 0.2 mM phenylmethyl sulfonyl fluoride [PMSF]). They are kept on ice for 10 min with regular gentle mixing, vortex mixed for 10 s and then centrifuged for 10 s at 14,000× g at room temperature. Without disrupting the pellet, the supernatant is gently decanted. The pellet is resuspended dropwise with gentle agitation in high-salt buffer (20 mM HEPES-KOH, pH 7.9, 25% glycerol, 420 mM NaCl, 1.5 mM MgCl$_2$, 0.2 mM EDTA, 0.5 mM DTT and 0.2 mM PMSF). It is incubated on ice for 20 min and then centrifuged for 2 min at 14,000× g at 4°C. The supernatant is divided into aliquots without disturbing the pellet, and the nuclear extracts are stored at -70°C until use. The nuclear pellet is not discarded because it contains the DNA.

To prepare genomic DNA, the pellet is resuspended in 0.8 mL lysis solution (0.1 M Tris-HCl, pH 8.3, 5 mM EDTA, 1% sodium dodecyl sulfate [SDS] and 0.2 M NaCl). Once the nuclear pellet is redissolved, 0.5 mL chloroform is added and mixed for 15 min. Best results are obtained by using a rotary carousel at room temperature. Simple chloroform extraction is usually sufficient in removing the remaining

![Figure 1. EtdBr-stained agarose gel of purified DNA.](image-url)
proteins. It is spun at 10,000×g for 10 min to separate the DNA from the organic phase. The proteins should be visible as a tight interface separating the two phases. Then 0.7 mL of DNA is removed to ensure that the white protein interface is left intact. Following extraction, the DNA is precipitated using one volume of absolute ethanol and gently agitated until the viscosity is eliminated. The DNA should now be visible as a white cotton-like clump. Vortex mixing must be avoided because it can partially shear the DNA. The DNA is spun down for 5 min at 11,000×g. The supernatant is gently decanted, removing remaining traces of ethanol with a yellow pipet tip. The DNA pellet is redissolved in TE buffer (10 mM Tris, pH 7.4, 0.1 mM EDTA, pH 8.0) or double-distilled water and incubated at room temperature. A carousel has been most effective in redissolving precipitated DNA pellets. DNA quality can be checked on an ethidium bromide (EtBr)-stained 0.8% agarose gel. The DNA is quantitated using a spectrophotometer by checking for quantity and purity at 260 and 280 nm, respectively. DNA yields of at least 56 μg (n = 14) have been obtained with a ratio of 1.86 per 1 × 10⁶ cells for the human T-cell leukemia cell line (CEM-CCRF).

This method has been used successfully with lymphocytes isolated from patients with B-CLL and various human cell lines including the CEM cell line, Daudi and the HL-60 line. Figure 1 is an example of the DNA isolated using this protocol. The DNA typically migrates at greater than 20 kb on a 0.8% agarose gel and is suitable for Southern and PCR analyses. The protocol is rapid and cost-effective, requires little manual labor and is ideal when working with multiple samples. The flexibility to isolate nuclear-binding proteins and genomic DNA from the same biological sample is a clear advantage when dealing with finite biological material. The DNA is routinely used in restriction digests and Southern blotting in analyzing mdr-1 gene polymorphism’s in B-CLL patients.

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Non-Isotopic Technique for the Identification of Endonucleases Involved in Apoptosis


A characteristic feature of apoptosis is the degradation of genomic DNA into discrete oligonucleosome fragments by the action of a Ca^{2+}/Mg^{2+}-dependent endonuclease. The endonuclease relevant to apoptosis has not been identified, but several possible enzymes have been described (2,4–6). One technique to identify potential nuclease activities is zymography, where protein extracts are resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in gels containing DNA (7). After electrophoresis, proteins are allowed to renature by washing the gel with buffer and then activated by addition of cations. Nuclease activity is then determined by staining the gel with ethidium bromide (EtBr) and visualizing the DNA with UV transillumination. Nuclease activity of a given molecular weight is identified as cleared areas of enzyme digestion in the gel not stained by EtBr. One potential problem with this approach is that histones bind to the DNA in the gel and produce false-positive nuclease activity by interfering with binding of EtBr to DNA (1,3). To circumvent this problem, the use of radiolabeled DNA in the gel and visualization by autoradiography has been reported (5).

Here we describe a simple non-isotopic technique where the gel is stained with silver to visualize DNA.

Protein extracts containing histones were prepared by extraction of rat liver nuclei with high salt TE buffer (1 M NaCl, 50 mM Tris-HCl, pH 7.5, 1 mM EDTA) and analyzed for nuclease activity by the zymographic method of Rosenthal and Lacks (7) using the Mini-PROTEAN® Gel System (Bio-Rad, Hercules, CA, USA) with a 12% polyacrylamide gel containing 250 µg/mL calf thymus DNA. Bovine pancreatic DNase I was used as a standard. After separation by SDS-PAGE, gels were washed overnight in several changes of TE buffer. Gels were then soaked in TE buffer containing Ca^{2+} (2 mM) and Mg^{2+} (5 mM) for 24 h at 37°C, stained with EtBr (0.5 µg/mL TE) and photographed under UV transillumination (Figure 1A). Before silver staining, the gel was soaked in 5% SDS on a rotary shaker (for 2 h) to remove protein. The gel was then silver stained according to the Silver Stain Kit modified protocol (Bio-Rad), omitting the acetic acid in the fixation step, photographed on a light box (Figure 1B) and dried onto filter paper. The silver stains the DNA incorporated in the gel, and nuclease activities are identified as clear spots on a dark brown background.

The nuclease activity of DNase I (lane 2) and the false-positive histone staining of histone H1 (lane 1) appear as cleared areas in the gel when visualized by EtBr staining (Figure 1A). In the silver-stained gel, however (Figure 1B), DNase I nuclease activity (lane 2)