derived vectors may also be affected depending on whether these regions contribute to this activity (14). Interestingly, although pGL3-basic (Promega, Madison, WI, USA) is derived from pUC vectors, it lacks many of the AP1 motifs found in pBLCAT3; however, it is still transactivated in experiments where other transcription factors such as NF-Y, c-Ets-2, PEA3, Sp1, or GATA-4 are overexpressed (D.A. Young, unpublished observations).

This research suggests that caution is necessary when overexpressing transcription factors with reporter plasmids during promoter analysis. Furthermore, it underlines the necessity of including a vector-only control in such studies. Several groups have designed new CAT vectors by deleting prokaryotic sequences immediately upstream of the CAT gene and replacing them with fragments containing a tandem dimer of the SV40 early polyadenylation signal (1,3,11,14). This prevents the processing of mature CAT mRNA from the vector and subsequent translation. Indeed, this can reduce pBLCAT3 vector read-through by up to 8-fold (3). CAT vectors such as pBLCAT5 (ATCC reference no. 77412) and pBLCAT6 (ATCC reference no. 77413) (1), which are derived from pBLCAT2 and pBLCAT3 or other modified vectors such as pJFCAT1 (3), have these modifications. However, these vectors may also need to be checked for transcription factor inductibility before being used in promoter analysis.

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Received 28 May 2002; accepted 2 August 2002.

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Automated Agarose Gel Electrophoresis of dsDNA Fragments on a Commercial DNA Sequencer

BioTechniques 33:1008-1014 (November 2002)

Electrophoretic separation of dsDNA molecules, such as PCR products and restriction digest fragments, is usually accomplished in agarose and sometimes in composite agarose-polyacrylamide gels (3). Under nondenaturing conditions, the electrophoretic mobility of dsDNA fragments is primarily determined by their size but also influenced by the sequence-dependent secondary structure (9). Conventional analysis of dsDNA fragments ranging in size from hundreds to thousands of base pairs have regularly been accomplished on thick slab gels (1) and involved labor-intensive manual processes. Sample loading onto the gels is also tedious and ergonomically challenging. Recently developed membrane-mediated loading techniques successfully addressed this latter issue (4). Electric field-mediated separation technologies significantly advanced during the last two decades, primarily because of the better understanding of the electrophoretic separation process (8) and the recent development of micro-separation methods such as ultra-thin-layer gel electrophoresis (10,11), capillary gel electrophoresis (5), and microchip electrophoresis (6).
These new techniques have emerged in recent years to address rapid DNA sequence analysis, featuring automation and very high resolving power. On the other hand, the development of automated high-throughput devices for rapid, large-scale PCR product analysis, mapping, and expression profiling using minute quantities of reagents is still in great demand.

Here we report on a novel agarose gel-based approach using a commercially available DNA sequencer (ABI PRISM® 377; Applied Biosystems, Foster City, CA, USA) for the rapid analysis of dsDNA fragments. This system is capable of automatic analysis of multiple fluorescent dye-labeled DNA molecules in a wide size range (2). After the samples are loaded onto the system’s vertical ultra-thin gel, they undergo electric field-mediated separation with simultaneous detection and computer analysis of the signal. Electrophoretic separation can be viewed on-screen in real time, and final data can be generated in a variety of formats. The system accepts gel plates in different lengths, including the shortest plate having 12-cm effective separation distance (sample well to detection scan line). The injected DNA fragments are size-separated by electrophoresis through a gel matrix. At the lower portion of the gel, they pass through a region where a laser beam continuously scans the gel. The laser beam excites the fluorescent dyes attached to the DNA fragments. The emitted light is then detected at the corresponding wavelength by a cooled charge-coupled device, so the different fluorescent dyes/labels can be detected with one pass of the laser beam. The data collection software evaluates the signal intensities using appropriate filtering systems for consecutive processing. The fluorescent illumination/detection system can detect up to five different dyes (multicolor imaging), providing increased throughput. Slab gel electrophoresis-based DNA sequencing systems almost exclusively employ cross-linked polyacrylamide gels. However, non-cross-linked gels, such as ultra-thin layer format agarose and agarose-based composite matrices, can also be applied for faster and less demanding separation challenges, such as PCR product analysis, where usually only one or just a few fragments are present in a wider fragment length range (7). Visualization of the dsDNA molecules can be easily accomplished by non-covalent complexation with ultra-sensitive fluorescent agents, enabling real-time imaging and data analysis.

In our new approach, we introduced regular agarose and/or composite agarose-linear polyacrylamide matrices into a short separation cassette. For pure agarose gels, the agarose powder was dissolved in the gel-buffer solution by boiling in a microwave oven. For composite gels, an appropriate amount of 10% linear polyacrylamide solution was added to the melted agarose solution and kept at 60°C for at least 10 min before use (7). Since for the analysis of PCR products sequencing-grade separation was not required, the shortest possible separation setting was applied (12 cm from injection to detection, 16 cm total length). Following the conventional cassette filling methodology (2), the melted agarose or composite agarose-linear polyacrylamide gel was poured onto preheated glass plates of the separation cassette to form the separation matrix. After several minutes of cooling, the gel solidified between the glass plates and the platform was ready to be used in the automated DNA sequencing system (ABI PRISM 377). Agarose III and SFR agarose were from Amresco (Solon, OH, USA), and the 10% linear polyacrylamide (700–1000 kDa) was from Polysciences (Warrington, PA, USA). The dsDNA fragments were robotically loaded onto the tips of a 64-tab membrane loader (The Gel Company, San Francisco, CA, USA) that was placed into the gel-filled vertical separation assembly, and the samples were injected by the application of the electric field. The 100-bp DNA ladder (Invitrogen, Carlsbad, CA, USA) was diluted with double deionized water (18 mΩ/cm) to a working concentration of 2.5 ng/µL and stored at -20°C until use. To assure rapid separation, 0.5× TBE buffer (45 mM Tris, pH 8.3, 45 mM boric acid, 1 mM EDTA; Sigma, St Louis, MO, USA) was used both in the gel and in the buffer reservoirs. This allowed the application of high separation voltages (1000 V or 60 V/cm) without significant Joule heat development. While automated DNA sequencers are originally designed for the analysis of covalently labeled DNA molecules, we attempted to use the system for the analysis of both non-covalently stained and covalently labeled dsDNA fragments. The SYBR Gold® non-covalent staining dye was purchased from Molecular Probes (Eugene, OR, USA), and the Texas Red® (covalent dye)-labeled 100-bp DNA ladder and the FITC-labeled primer were from Sigma. All buffer solutions were filtered through a 0.2-µm nylon membrane syringe filter (Fisher Scientific, Pittsburgh, PA, USA). The high-temperature module of the system was switched off during the experiments (room temperature analysis).

Figure 1 shows the picture of the raw data screen of composite agarose gel-based separation of dsDNA molecules using the automated DNA sequencing instrument. Figure 1 (top panel) depicts the separation of a 100-bp DNA ladder standard stained with SYBR Gold dye before the analysis. The particular composition of agarose-linear polyacrylamide gel (1% agarose III and 2% linear polyacrylamide) used as separation matrix in this instance provided a wide separation range of 100–1000 bp. As one can observe, the agarose-based gel matrix in conjunction with membrane-mediated injection enabled good performance and reproducible separation of the test mixture components for all 64 lanes in less than 23 min. In spite of the fact that we did not have the 96-lane upgrade kit for our instrument, we assume with confidence that our approach would also work properly in a 96-lane upgraded format. Large-scale analysis of PCR products with simultaneous separation of 58 samples is shown in Figure 1 (bottom panel). The first and last three lanes in this case are SYBR Gold-labeled 100-bp DNA ladder standards for appropriate sizing. A different agarose (2% SFR)-linear polyacrylamide (1%) gel composition was used in this instance, which was optimized to attain the highest resolving power in the PCR product size range of 50–250 bp; thus, the longer fragments of the ladder standard...
Figure 1. Picture of the raw data screen of agarose gel-based separation of 100-bp DNA ladder standard samples and various size PCR fragments using the ABI PRISM 377 automated DNA sequencing device. All samples were stained by SYBR Gold (0.25×) before the analysis. Top, 1% agarose III and 2% linear polyacrylamide, 0.5× TBE; 64 lanes; sample, 100-bp DNA Ladder (2.5 µg/mL). Bottom, 2% SFR agarose and 1% linear polyacrylamide, 0.5× TBE; samples, various size PCR product samples (lanes 4–61) and 100-bp DNA Ladder (lanes 1–3 and 62–64). Sample introduction: 64-tab membrane loader, 0.2 µL each sample. Electrophoresis conditions: 1000 V, 12 cm separation distance (16 cm total gel length), room temperature.

Figure 2. Typical two-color electropherograms using different type (covalent and non-covalent) and color (green and red dye) labeling. (A) Covalently (Texas Red) labeled 100-bp DNA ladder (red detection channel of the system). (B) Mixture of a Texas Red-labeled 100-bp DNA ladder and SYBR Gold (1×)-labeled PCR product (shown by the arrow) (blue detection channel of the system). (C) Simultaneous multicolor detection of a Texas Red-labeled 100-bp DNA ladder and a FITC-labeled DNA primer (red and blue detection channels of the system). Sample introduction: 64-tab membrane, 0.2 µL each sample. Electrophoresis conditions are the same as in Figure 1. Gel: 1% agarose III and 2% linear polyacrylamide gel in 0.5× TBE.
B depicts the electropherogram of the detection channel of the system. Trace Red labeled 100-bp DNA ladder (red) shows the separation of the covalently (Texas Red and blue dyes) and non-covalent labels using different labeling modes (covalent and non-covalent) and colors (green and red dyes). Trace A shows sequentially spotted samples of 100-bp Texas Red-labeled DNA ladder and PCR sample on the membrane tabs, masking the fluorescence of the covalent Texas Red label of the ladder. Trace C delineates simultaneous multicolor detection of the Texas Red-labeled 100-bp DNA ladder and a FITC-labeled DNA primer (red and blue detection channels of the system), proving the feasibility of multicolor/multispectral imaging in conjunction with agarose-based separation matrices.

In conclusion, a simple, automated, rapid, and economical dsDNA analysis method was developed using agarose-based separation matrices and common reagents on a commercially available DNA sequencing device. The system described in this paper greatly enhances the productivity of DNA fragment analysis by automating the current manual procedure and reducing the separation time and human intervention from sample injection to data analysis. Today, most high-throughput laboratories could afford the purchase of a used ABI PRISM 377 system (less than $8000) that can make our method widely available. Membrane-mediated sample loading in conjunction with robotic sample spotting supports high-throughput and large-scale analysis of PCR products. Please note that spotted loading membranes can be stored for several days until analyzed, and their bar coding may facilitate easy identification and cataloging. Visualization of the dsDNA fragments was accomplished by non-covalent labeling with the fluorescent SYBR Gold dye just before the electrophoresis process, enabling real-time laser-induced fluorescent imaging and data analysis.

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Received 19 June 2002; accepted 2 August 2002.

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