A New System For Automated Agarose Gel Electrophoresis

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Abstract

Many high-throughput (HTP) processes would benefit from the ability to integrate an automated agarose gel electrophoresis step. The E-Gel® 96 system resolves DNA or RNA bands over a 16-mm run length in a standard 96-well gel cassette with a running time of just 12 minutes. Fully automating the E-Gel 96 system allows throughput to exceed 20,000 samples per day. Here we demonstrate the use of the system to analyze a variety of DNA and RNA samples, including PCR products and plasmid restriction digests, over a range of electrophoretic conditions.

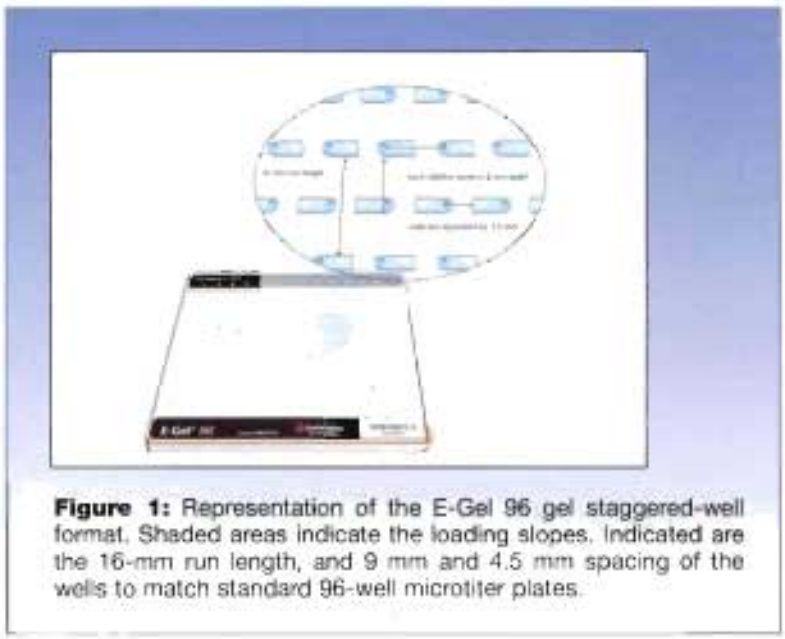

Figure 1: Representation of the E-Gel 96 gel staggered-well format. Shaded areas indicate the loading slopes. Indicated are the 16-mm run length, and 9 mm and 4.5 mm spacing of the wells to match standard 96-well microtiter plates.

Introduction

Agarose gel electrophoresis of DNA and RNA samples plays a critical role in the analysis and quality control of scientific experiments. Unfortunately, as modern experimental methods have moved towards robot-compatible HTP formats (e.g., 96-well plates), innovations in agarose gel electrophoresis technology have severely lagged behind. This is primarily due to the fact that neither the process of pouring a gel nor the equipment used to run the gels are particularly robot compatible. In some specific work applications, such as HTP sequencing, genotyping, microarray, and SNP projects, the lack of a suitable agarose gel electrophoresis system has created intense process bottlenecks or caused the elimination of useful quality control steps for the sake of throughput.

The gels offers a 16 mm run length in a 96-well structure.

The recent availability of pre-cast gels with appropriate 8-, 12- and 96-well spacing have improved the situation somewhat. However, these pre-cast gels are suboptimal for many HTP laboratories due to running distances of only 0.8 cm. The running distance can be increased by diagonally offsetting the entire array of wells, but the complexity of this setup can make results difficult to read and interpret. In addition, these electrophoresis systems still require liquid buffer, and thus are not truly automation friendly.

To overcome these difficulties and meet the goals of high-throughput electrophoresis, the E-Gel concept of dry, enclosed, disposable agarose gel cassettes that can be run without liquid buffers and with no staining/ destaining procedures were reconfigured to enable simultaneous analysis of 96 samples. In addition to eliminating the need to pour gels and prepare liquid buffers, the new staggered-well format provides a 16 mm run length -- twice the distance dictated by the column structure of a 96-well microtiter plate. sample wells of the E-Gel 96 gels are spaced at standard intervals that conform to those on standard multi-channel pipettes (8, 12 and 96) for high-throughput, robot-mediated loading. E-Gel 96 cassettes run in bases that can be interlocked together, enabling simultaneous running of multiple gels. For HTP purposes, the array of bases can be integrated onto a robotic system. Here we demonstrate the use of the system to run different types of DNA and RNA samples under various experimental conditions. The 16-mm run length improves band resolution and the 96-well structure allows analysis of a large number of samples. With

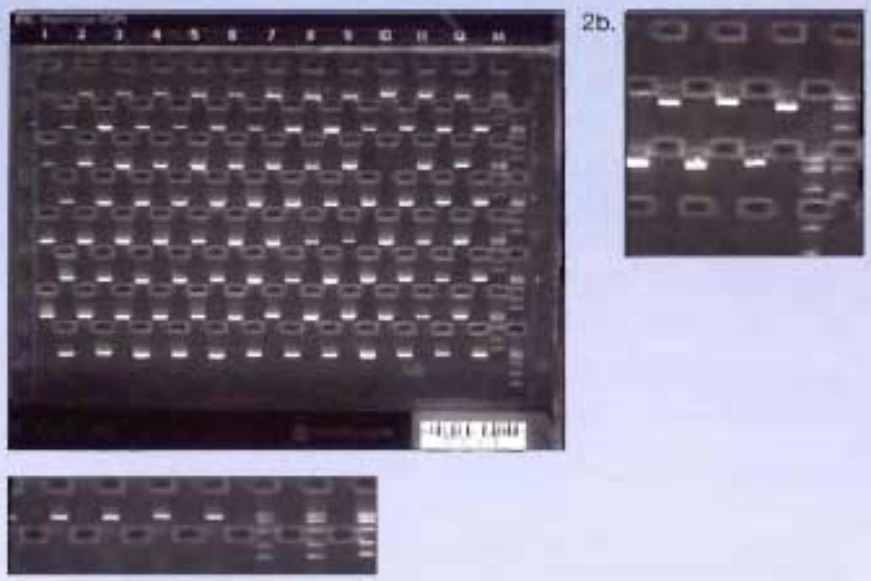

Figure 2: Gel images of plasmid DNA on an E-Gel 96 1% agarose gel. Plasmid DNA was isolated from 96 random clones from a HeLa cDNA library. Panel A. Photographic image of an E-Gel 96 agarose gel electrophoresed for 12 minutes. Lanes 1-12, rows A-H: 0.5 µl of each DNA. Lane M: E-Gel 96 High Range DNA Marker.
Panel B. Close-up image of plasmid DNA electrophoresed for 20 minutes. DNA was electrophoresed for an extended length of time to increase separation. Lanes 1-3: 0.5 µl of plasmid DNA. Lane M: E-Gel 96 High Range DNA Marker. Panel C. Image of random plasmid clones and dilutions of the E-Gel 96 High Range DNA Marker electrophoresed for 12 minutes. Lanes 1-5: 0.5 µl of plasmid DNA. Lane 6: 0.5X E-Gel 96 High Range DNA Marker (5 µl, 10 ng per band). Lane 7: 1X E-Gel 96 High Range DNA Marker (10 µl, 20 ng per band). Lane 8: 2X E-Gel 96 High Range DNA Marker (20 µl, 40 ng per band).

Figure 3: Comparison of the DNA band resolution on the pre-cast staggered-well E-Gel 96 and non-stagger well gel formats. PcDNA3.1(+) was digested with *Nco* I. Expected band sizes are 3342 bp, 1351 bp, and 735 bp. Panel A. Restriction digest resolved on an E-Gel 96 staggered-well gel. Gel was electrophoresed for 12 minutes. Lanes 1-4: 300 ng *Nco* I-cut pcDNA3.1(+). Lane M: E-Gel 96 Low Range DNA Marker. Panel B. Restriction digest resolved on a 1.2% non-staggered pattern pre-cast gel system. All samples were loaded in a total volume of 10 µl. This gel was electrophoresed for 6 min. at 120 V. Lanes 1-4: 300 ng *Nco* I-cut pcDNA3.1(+). Lane M: 6.5 µl of the ladder designed for this gel system. The ladder contains bands of 100 bp, 250 bp, 500 bp, 1000 bp, 2500 bp and 5000 bp.

a 12-minute running time, throughput can exceed 20,000 samples per day.

Materials and methods

All materials were supplied by Invitrogen Corporation (Carlsbad, California) unless otherwise indicated.

Electrophoresis

The E-Gel 96 gel contains agarose and ethidium bromide (for visualizing results) in a closed UV-transparent, disposable cassette (130 mm × 100 mm × 6 mm). Each gel is designed for a single electrophoresis run. Loading sites consist of openings in the upper lid of the cassette and sample wells within the agarose positioned beneath the openings. A slope in the structure of each opening draws the loaded sample into the wells. To increase the run length from 8 mm in a standard column structure, well positions are staggered (Figure 1). Wells are staggered in such a way that gels can be loaded with standard 8-, 12-, or 96-tip pipettes and robot loading pins. Alternating rows position the loading slopes to the left or right of the well. Lower edges of the slopes, where the sample well begins, are 9 mm apart. This matches the separation of wells in standard 96-well microtiter plates. The horizontal distance from the end edge of one well to the starting edge of the next well is 4.5 mm. This staggered-well format provides a running distance of 16 mm.

DNA and RNA samples were electrophoresed using E-Gel 96 1% and 2% agarose gels, E-Gel 96 mother and daughter bases, E-Gel 96 holders, the E-Gel 96 High Range DNA Marker, and the E-Gel Low DNA Mass Ladder.

Loading samples

Four different types of samples: plasmids, digested plasmids, PCR products, and RNA were loaded onto the gels. Plasmids were obtained from a plated HeLa DNA library. Ninety-six individual colonies were inoculated into Terrific Broth containing 100 µg/ml ampicillin. Cultures were grown and processed according to the direct load method of the Concert™ 96 Plasmid Purification System. The pcDNA™3.1(+) plasmid was restriction digested with 2 U/µg *Nco* I at 37 C for 2 hours using the buffer supplied by the manufacturer. PCR products of different sizes were kindly provided by Knut Madden. Total cell RNA was purified from $1 \times 10^{(6)}$ HeLa cells using Invitrogen's Micro-to-Midi Total RNA Purification System. RNA was quantitated by spectrophotometry.

Prior to loading, DNA samples were diluted into either deionized (DI) water or 10 mM Tris, pH 7.5, 1 mM EDTA. RNA samples were diluted in RNase-free DI water. Both the High Range DNA Marker and the Low DNA Mass Ladder were diluted 1:1 with DI water. Total sample and marker volume loaded into the wells was 20 µl. Samples were loaded using the Robbins® Tango™ Automated Liquid Handling System (Robbins Scientific, Apogent, Sunyvale, California). A 5 µl air gap was drawn up into the tips of the Robbins Tango prior to aspirating sample. The entire 25 µl volume was dispensed into the wells.

Electrophoresis time was 12 minutes unless otherwise indicated. Electronic gel images were produced and analyzed using a Kodak 120 system (Rochester, New York) and the E-Gel™ 96 Editor software.

Results

Analysis and quantitation of plasmid DNA

Many high-throughput studies, such as plasmid-based sequencing projects, microarray experiments, and contract cDNA library construction, require the preparation of large numbers of individual plasmid templates. As a quality control step, laboratories routinely test their DNA templates after isolation and prior to costly downstream processes using agarose gel electrophoresis. Figure2A shows the unedited electrophoresis results of plasmids isolated from a HeLa cell cDNA library. To further increase separation, the electrophoresis time can be increased to 20 minutes from the default 12 minutes (Figure 2B). Due to the supercoiled nature of the DNA plasmids, only relative sizing estimates can be made when compared to a linear marker. A quantitative titration of the E-Gel 96 High Range DNA Marker can be used to estimate mass of plasmid DNA (Figure 2C).

Analysis of plasmid digestion

The staggered-well format provides a 16 mm run length that results in superior band resolution. This feature is important when separating multiple bands as with DNA digested with restriction enzymes. A restriction digest containing fragments of 3342 bp, 1351 bp and 735 bp is very well resolved during a 12-minute run (the default run time) on the staggered-well E-Gel 96 gel (Figure 3A). The same restriction digest does not adequately resolve on a non-staggered well gel format (Figure 3B).

Analysis, sizing, and quantitation of PCR products

PCR was performed on a variety of templates and the resulting products analyzed on an E-Gel 96 2% agarose gel (Figure 4A). Results were reconfigured using E-Gel 96 Editor software. Lane positions are highlighted by drawing a border around the running area of each lane (Figure 4B). The Editor program then rearranges the lanes into a linear format (Figure 4C) that can be imported into the various gel analysis software programs.

To analyze the band quality of different DNA amounts, we ran the E-Gel Low DNA Mass Ladder on an E-Gel 96 2% agarose gel. The results of this analysis showed that a band of 5 ng DNA (100 bp) is clearly visible (Figure 5).

Electrophoresis of RNA samples

Gel analysis with RNA samples is generally more troublesome than with DNA samples due to the added care needed working with RNA. Using a disposable E-Gel 96 gel eliminates many of the opportunities for RNase contamination. To run RNA samples on the E-Gel 96 gel, we have found it best to load the RNA samples in RNase-free water. In the absence of denaturing agents to remove secondary structure, most RNA samples do not resolve as well as in a standard denaturing RNA gel. These limits aside, the system can be a very convenient and useful method for basic analysis of RNA samples as shown in Figures 6A and 6B. The 18S and 28S bands present in HeLa RNA (Figure 6A) are clearly resolved and show no signs of degradation. The RNA ladder run in all lanes in Figure 6B also shows no signs of degradation. In addition, only minimal amounts of RNA are required to give ample signal. Figure 6 shows that 150 ng of RNA can be efficiently resolved, allowing conservation of precious samples.

Robot compatibility and high-throughput use

The E-Gel 96 system was designed to meet the needs of HTP labs requiring automation of gel electrophoresis. The gels run in a series of mother and daughter bases that consist of a base and power supply. The mother base plugs directly into an electrical outlet (110 or 220V). The daughter bases can be interlocked to the mother base and/or to each other. Each base has the same footprint as a standard SBS 96-well plate, allowing it to fit on a robotic loading platform. When an E-Gel 96 gel cassette is placed into a base, the sample wells are positioned for loading just as a standard 96-well plate would be. This makes programming robots very straightforward (specific information pertaining to robotic programming can be found on the Invitrogen web site at www.invitrogen.com/egels). Gels have been successfully loaded with either disposable or fixed tips, depending on the robotic platform and customer needs. As seen in Figure 6B, the precision and reproducibility of samples loaded by automation is excellent. Loading time for this gel was 15 seconds.

Tracking of samples in HTP labs is often accomplished through the use of barcodes. Each E-Gel 96 cassette is labeled with a unique barcode for tracking purposes. The barcode is fluorescent and shows up in gel images of the cassette (Figures 2A, 4A,

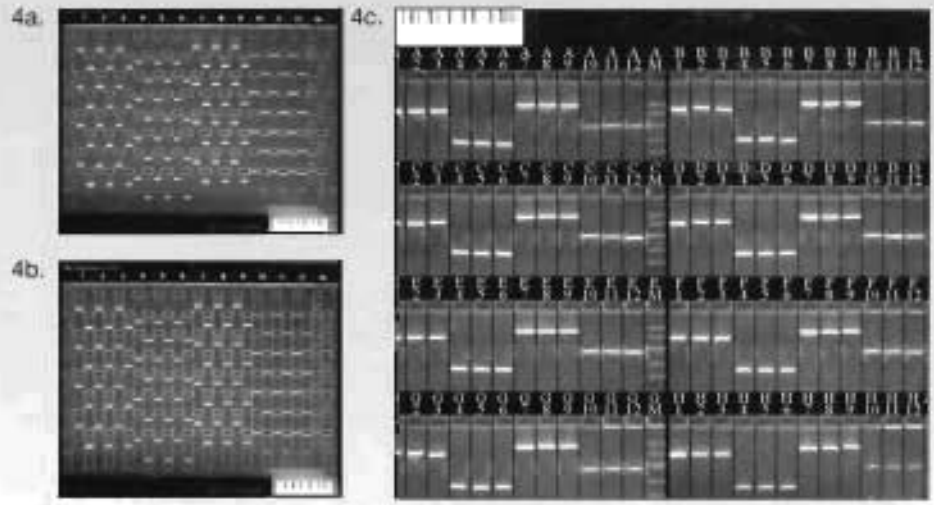

Figure 4: Gel images of PCR products electrophoresed on an E-Gel 96 2% agarose gel.
Panel A. Photographic image of an E-Gel 96 2% agarose gel. Lanes 1-3: 800 bp PCR product (2 µl). Lanes 4-6: 150 bp PCR product (8 µl). Lanes 7-9: 1494 bp PCR product (5 µl). Lanes 10-12: 353 bp PCR product (1 µl). Lane M: E-Gel 96 Low Range DNA Marker. The Marker is composed of 100 bp, 200 bp, 400 bp, 800 bp and 2000 bp DNA bands of equal mass (~ 20 ng each).
Panel B. Image of the gel in Panel A undergoing editing to reconfigure the staggered-well pattern. The E-Gel 96 Editor software algorithms define lane positions (red and green rectangles) based on the three crosshairs in the gel cassette. Fine tuning, if needed, can be done using additional features of the software.
Panel C. Reconfigured E-Gel 96 gel ready for use in gel analysis programs. Lane and row numbers have been assigned to aid in visual interpretation of the data and the barcode image is transposed to the file for gel/sample verification.

5.

Figure 5: Gel image of PCR products on an E-Gel 96 2% agarose gel with a marker for quantitation.
Lane 1: 30 ng of a 400 bp PCR product. Lane 2: 60 ng of a 400 bp PCR product. Lane 3: 40 ng of an 800 bp PCR product. Lane 4: 80 ng of an 800 bp PCR product. Lane 5: 90 ng of a 2000 bp PCR product. Lane 6: 180 ng of a 2000 bp PCR product. Lane M: E-Gel 96 Low DNA Mass Ladder. The marker contains the following band sizes and DNA amounts: 100 bp - 5 ng, 200 bp - 10 ng, 400 bp - 20 ng, 800 bp - 40 ng and 2000 bp - 100 ng.

6a.

6b.

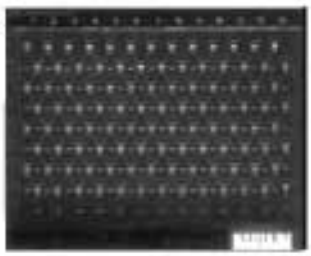

Figure 6: Gel images of RNA electrophoresed on an E-Gel 96 2% agarose gel.
Panel A. The gel was electrophoresed for 14 minutes. Lanes 9-12, both rows: 150 ng of HeLa RNA loaded in DI water.<p>
Panel B. A Robbins Tango robot was programmed to dilute and dispense 150 ng of 0.24 - 9.5 kb RNA Ladder in DI water and load the samples into all lanes of an E-Gel 96 2% agarose gel.</cap>

and 6B). It is also transferred to files of edited gel images by E-Gel 96 Editor software (Figure 4C).

Discussion

This report demonstrated the utility of the E-Gel 96 system for electrophoresis in a robot-compatible format, making it convenient for HTP use. Using the system, many routine gel electrophoresis steps such as gel casting, buffer preparation, and gel staining/destaining are eliminated. DNA and RNA samples can be analyzed within 12 minutes. As little as 5 ng of DNA is clearly visible and the 18S and 28S bands in 150 ng of total RNA are evident on a gel.

The E-Gel 96 system combines two seemingly contradicting features. The loading sites are placed at standard intervals to conform with the spacing between tips on standard multi-channel pipettes (96-well microplate format) and, at the same time, the wells are arranged in a staggered format that provides a run length twice the distance of the column-format 96-well gels. Additional features of the E-Gel 96 cassettes stem from their being essentially fully contained, UV-transparent, single-use gels. Minimal handling reduces the chances of introducing external contaminants. By including ethidium in the gel, there is no exposure to ethidium bromide.

The E-Gel 96 System addresses virtually all of the issues required for moderate HTP users and for full automation of the electrophoresis process when a large number of samples is to be analyzed. The system contains a mother base that can be plugged directly into an electrical outlet and daughter bases that can be interlocked to the mother base and to each other. Each base contains its own built-in power supply. An array of gels can be run simultaneously with the potential throughput using one robot exceeding 20,000 samples per day. Users can incorporate an analysis or quality control step into their HTP processes without creating a bottleneck.

Acknowledgements

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