High-throughput electrophoresis using the Tango™ automated liquid handling system and the E-Gel® 96 system

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Abstract

Automating electrophoresis significantly reduces the time required for loading a large number of samples, increasing the speed and throughput of electrophoretic analysis. In addition, the reliability and consistency of automation maximizes resolving power and increases the precision of fragment separation. Here we demonstrate an automated, high-throughput method for simultaneously loading 96 samples onto an electrophoresis gel, using the Tango™ automated liquid handling system and the E-Gel® 96 system.

Introduction

Electrophoresis is a fundamental technique used to identify and separate DNA, RNA, and protein molecules. It is routinely used to quantify and determine the quality of samples prior to performing downstream applications. Here we demonstrate an automated, high-throughput method of simultaneously loading 96 samples onto an electrophoresis gel using the Tango™ system (Apogent Discoveries) and the Invitrogen E-Gel® 96 system. This procedure significantly reduces the time required for loading a large number of samples. Consequently, an increase in the overall speed of electrophoresis analysis is achieved. Furthermore, automation reduces sample-to-sample variability caused by errors in manually dispensing samples, resulting in an increase in the precision of electrophoresis analysis.

Materials and Methods

A Tango™ system (Apogent Discoveries, Sunnyvale, CA) equipped with 96, 100 µl standard syringes with DuraFlex™ needles was used for liquid handling. The E-Gel® 96 2% agarose gel (Cat. no. G7008-02), E-Gel® 96 holder (Cat. no. G7300-01), E-Gel® 96 mother base (Cat. no. G7100-01), 10X BlueJuice™ Gel Loading Buffer (Cat. no. 10816-015), 0.2 µ-9.5 kb RNA Ladder (Cat. no. 15620-016) and E-Gel® 96 Low Range DNA Marker (Cat. no. 12369-013) were provided by Invitrogen Corporation, Carlsbad, CA. PCR core kit (Cat. no. 1 578 553) and human genomic DNA (Cat. no. 1 691 112) were purchased from Roche, Mannheim, Germany. A Human β-actin amplimer set (Cat. no. 5402-1) was purchased from Clontech Laboratories, Palo Alto, CA. Samples were prepared in a skirted cycleplate-96 (Cat. no. 1047-20-0, Apogent Discoveries/Robbins Scientific, Sunnyvale, CA) and loaded following the procedures outlined in the E-Gel® 96 High-Throughput Agarose Electrophoresis System manual.

Dispensing precision of the Tango™ liquid handling system. Prior to use of the Tango™ system, the uniformity and consistency of sample volumes dispensed across the 96 syringes used in this study were determined by measuring the coefficient of variance, C.V. (http://www.robsci.com/hug.html). A high uniformity for dispensing volumes equal to, and higher than, 100 nl was evident with C.V.s less than 5%.

Operating the Tango™ system for loading samples. The Tango™ system incorporates precision glass syringes (96 or 384) arrayed in standard SBS microplate spacing. The stage of the Tango™ System is composed of 12 nests. For this protocol, one nest was dedicated to the wash module, one to a reservoir containing 2% bleach, one to a reservoir containing deionized water, one to a skirted cycleplate-96 containing DNA or RNA samples (called the source plate), and one to an E-Gel® 96 gel (placed on the E-Gel® 96 holder). As many as four E-Gel® 96 holders can fit on the Tango™'s stage. In order to clean the syringes and prevent carryover contamination, a Tango™ protocol was created that incorporated three water wash cycles and one wash cycle with 2% bleach, followed by an additional three water wash cycles, before and after load-

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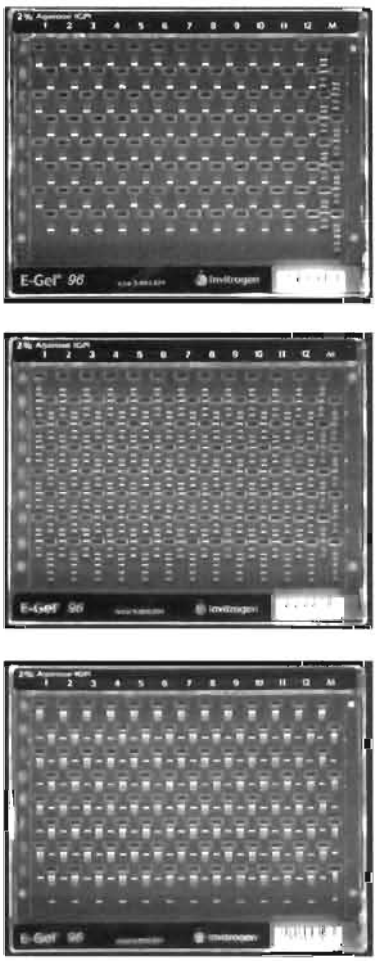
ing the E-Gel® 96 gel (one "wash cycle" is defined as an aspiration and a dispense; in this instance, the wash volume was set at 20 µl). To load the DNA/RNA samples, the Tango™ protocol called for pre-loading the 96 wells in the E-Gel® 96 gel with 10 µl of water (a 5 µl air gap, followed by 10 µl of water was aspirated into the syringes and then emptied into the wells of the gel). Next, the samples (from the skirted cycleplate-96) were loaded onto the E-Gel® 96 gel (a 5 µl air gap, followed by 10 µl of sample was aspirated into the syringes and then emptied into the wells of the gel). Once the samples were loaded, the E-Gel® 96 gel was transferred onto the E-Gel® 96 mother base to begin electrophoresis. Electrophoresis was complete in 12 minutes. Gel results were visualized and photographed under ultraviolet light.

Results and Discussion

Using the Tango™ system, DNA and RNA samples were loaded onto E-Gel® 96 gels. The loading time, including the time required for the process of priming (trial dispensing required higher dispensing precision) was approximately 15 seconds. Figure 1A shows that DNA samples loaded resolved as a single, sharp, high-quality band. Figure 1B demonstrates the exceptional quality of separation between different lengths of DNA fragments. No electrophoresis flaws such as diffusion of the sample, smearing, or tailing were detected. In addition to the loading of DNA samples, polyA-tailed RNA samples were also successfully loaded onto the E-Gel® 96 gel using the Tango™ system. As indicated in Figure 1C, no RNA degradation was observed when samples were loaded with the Tango™ system. These results demonstrate a simple, fast, and precise method for automating electrophoresis using the E-Gel® 96 system on a robotic platform.

Figure 1 – Electrophoresis results using the Tango™ automated liquid handling system and the Invitrogen™ E-Gel® 96 system



A: Sharp resolution
Sample: 1 µl (50 ng) of β-actin PCR product (length: 838 bp), DNA marker (M): E-Gel® 96 Low Range DNA Marker.

B: Clear separation
Sample: E-Gel® 96 Low Range DNA Marker (90 ng).

C: RNA remains intact
Sample: 150 ng of 0.24-9.5 Kb RNA ladder.

Conclusion

The Tango™ system and the Invitrogen™ E-Gel® 96 system work together to provide a fast, simple, precise, and automated method for simultaneously loading and analyzing a large number of samples for high-throughput electrophoresis. ■