High-Throughput Electrophoresis Using the Robbins® Tango™ Liquid Handling System and the Invitrogen™ 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 of electrophoresis analysis, and maximizing the resolution power (clear separation of fragments) of this technique. In addition, automation increases the precision of electrophoresis analysis. Here we demonstrate an automated, high-throughput method of simultaneously loading 96 samples onto an electrophoresis gel, using the Robbins Tango system and the Invitrogen E-Gel 96 system.

Introduction

Electrophoresis is a fundamental technique used to identify and separate DNA, RNA and protein molecules. It is routinely used for quantification and determination of 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 Robbins Tango systems and the Invitrogen E-Gel 96 system. This procedure significantly reduces the time requirement 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 and as a result increases the precision of electrophoresis analysis.

Materials and Methods

A Tango system (Apogent Discoveries/Robbins Scientific, Sunnyvale, CA) equipped with 96, 100 µL standard syringes with DuraFlex™ needles was used for liquid handling. The E-Gel® 96 pre-cast 2% agarose gel (Cat. #G7008-02), E-Gel® 96 holder (Cat. # G7300-01), E-Gel® 96 mother base (Cat. # G7100-01), BlueJuice™ gel loading buffer (Cat. # 10816-015), 0.2-9.5 Kb RNA Ladder (Cat. no. 15620-016) and E-Gel® 96 Low Range DNA Marker (Cat. # 12369-013) were provided by Invitrogen Corporation, Carlsbad, CA. PCR core kit (Cat. # 1 578 553) and human genomic DNA (Cat. # 1 691 112) were purchased from Roche, Mannheim, Germany. A Human b-actin amplimer set (Cat. # 5402-1) was purchased from Clonetech Laboratories, Palo Alto, CA. Samples were prepared in a skirted cycleplate-96 (Cat. # 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.

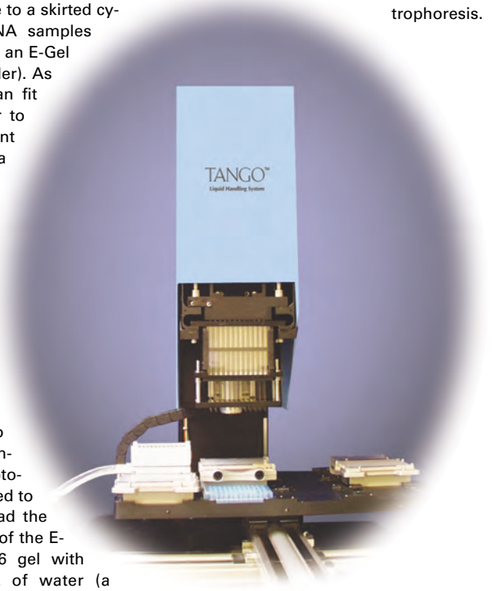
Protocol

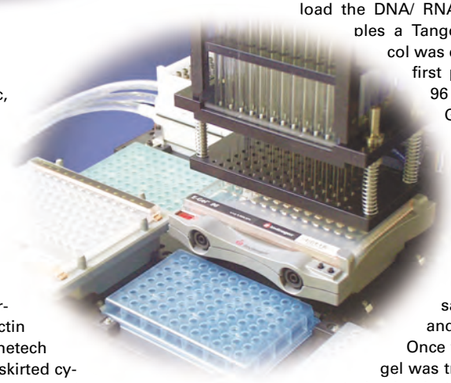
Dispensing Precision of the Tango liquid Handling System

Prior to the use of the Tango system, the uniformity and consistency of the sample volumes dispensed across the arrays of syringes (96 syringes in this study) were determined by the coefficient of variance (CV) (http://www.robsci.com/hug.html). A high uniformity for dispensing volumes equal to and higher than 100nL was evident with CVs of 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. It's stage 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 onto the Tango's stage. In order to clean the syringes and prevent carry-over contamination, a Tango protocol was created that incorporated three water wash cycles in the Tango wash module and one wash cycle with 2% bleach (from the bleach reservoir), followed by an additional three water wash cycles in the wash module, before and after loading 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 20µL). To load the DNA/ RNA samples a Tango protocol was created to first preload the 96 well of the E-Gel 96 gel with 10µL of water (a 5µL of air gap followed by 10µL 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 into the E-Gel 96 gel (a 5µL of air gap followed by 10µL 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 to the E-Gel 96 mother base to begin electrophoresis. When electrophoresis was complete, the 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 for a higher dispensing precision) was approximately 15 seconds. Figure 1A shows that DNA samples loaded traveled as a single high-resolution, high-quality band. Figure 1B shows 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.

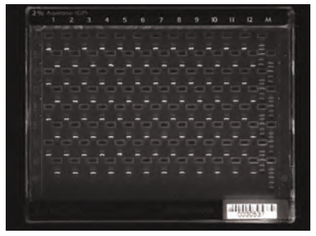


Figure 1A. Electrophoresis result using the Tango Automated Liquid Handling System and the Invitrogen E-Gel 96 system. Sample: 1ml (50ng) of b-actin PCR product (length: 838bp), DNA marker: E-Gel 96 Low Range DNA Marker.

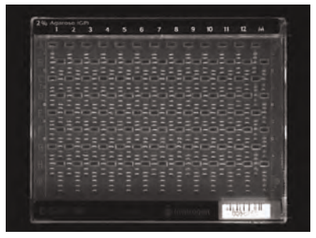


Figure 1B. Electrophoresis result using the Tango Automated Liquid Handling System and the Invitrogen E-Gel 96 system. Sample: E-Gel 96 Low Range DNA Marker (90ng).

Figure 1C. Electrophoresis result using the Tango Automated Liquid Handling System and the Invitrogen E-Gel 96 system. Sample: 150ng of 0.24-9.5 Kb RNA ladder

Conclusion

The Robbins 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.