ABSTRACT

The advantages of using 1, 96, or 384 precision glass syringes in automated high-throughput microdispensers in creating highly uniform and reproducible DNA, protein, and organic compound array filters and slides are described. Using the Hydra® Microdispenser and Tango™ Liquid Handling system, 0.1–5 ng (in 50–300 nL) PCR-amplified, human cancer-related genes and housekeeping genes were spotted onto nylon membranes and coated slides. Protein solutions of 50 μg/mL to 1 mg/mL were spotted onto coated slides or onto MaxiSorp™ 96-well plates. Up to 6144 spots/membrane and up to 1000 spots/slide were printed. The size of the spots created by glass syringes was uniform and reproducible (precision variation of less than 5%) from spot to spot and membrane to membrane. Using a Tango 384 system, a total of ten 6144-spot filters can be produced in approximately 25 min, translating into a spotting speed of 2.5 min/membrane.

INTRODUCTION

Arrays have become the accepted molecular biology tool for the simultaneous analysis of thousands of genes, proteins, and compounds (1–4). DNA and protein arrays have widespread use in functional genomics research and in clinical prognostics, diagnostics, pathology, and toxicology. Compound arrays are used for drug discovery and determination of drug efficacy.

A number of printing technologies are now being widely used for the creation of arrays (6,7). The most popular ones are non-contact printing with inkjet and contact printing with pins. Even though these two technologies permit the successful creation of arrays, some difficulties in implementing printing have been observed (6). Some of the complexities typically encountered are variation in spot size (due to inconsistency in the volumes printed or presence of air bubbles), splashing, evaporation of the samples during the process of delivery, clogging of the pins, and difficulties in thoroughly cleaning the spotting devices. Many of these difficulties could be circumvented by using nondisposable precision glass syringes for the printing process.

This study shows the advantages of using precision glass syringes in high-throughput liquid handling systems to create arrays of up to 6144 spots/membrane and up to 1000 spots/slide.

MATERIALS AND METHODS

Fluorescein (Molecular Probes, Eugene, OR, USA) was dissolved to a concentration of 10 μg/mL solution using a 0.1 M Tris-HCl (pH 8.0) buffer. A Tango™ Liquid Handling System and a Hydra®-PP Microdispenser (Robbins Scientific, Sunnyvale, CA, USA) equipped with 1, 96, or 384 100-μL stainless-steel syringes (with Teflon® coating) or syringes with DuraFlex™ (titanium alloy) needles were used for arraying purposes. Fluorescence detection was performed using a Nucleo-VISION™ Imaging Workstation from Nucleotech™ (San Mateo, CA, USA). The PCR-amplified DNA samples, which possess an M13 sequence in each fragment, were provided by Genetic-Lab (Sapporo, Japan). The PCR core kit was purchased from Roche Applied Science (Mannheim, Germany). The
amplified samples were purified using a QIAquick® PCR purification kit (Qiagen, Valencia, CA, USA). A 10 mM Tris-HCl (pH 7.5) buffer was used as blank in contamination studies. The Biodyne™ Plus membrane, 0.45 μM (8 × 12 cm), was purchased from Pall (East Hills, NY, USA). FAST™ and CAST™ microarray slides were purchased from Schleicher & Schuell BioScience (Keene, NH, USA). Array-Slides (silanated) and MaxiSorp™ 96-plates were provided by Nalge-Nunc International (Roskilde, Denmark).

### The Tango Liquid Handling System

The Tango system incorporates 1, 96, or 384 nondisposable precision glass syringes arrayed in standard Society for Biomolecular Screening (SBS) microplate spacing. Its stage is composed of 12 nests. During all analyses for this study, one nest was dedicated to the wash module, one nest to a 96- or 384-well PCR plate (called the source plate), and 10 nests to nylon membranes, which were placed on a vacuum manifold. The vacuum manifold holds the membrane in position and reduces diffusion, thus concentrating each sample and reducing its diameter. The source plates and the membranes (placed in a vacuum manifold) could be stacked in the Tango Plate Stacker and be removed or placed onto the stage by the plate stacker’s rotational gripper.

Because nondisposable glass syringes are used for all spotting, it is crucial to follow an efficient syringe-cleaning procedure for the inhibition of carryover contamination (Robbins Application Note 10; http://www.robsci.com/hug.html). After each spotting operation, the syringes were washed in the Tango wash module with 20 μL of water for six wash cycles (one wash cycle is defined as an aspiration and a dispensing; in this instance, the wash volume was set at 20 μL).

**The Hydra Automated Dispensing System**

The Hydra-PP Microdispenser is equipped with nondisposable syringes, a computer-controlled plate-positioning stage, and an automated syringe washing system (8). The stage is composed of two nests. In this study, one nest was dedicated to the source plate and the other nest was dedicated to a vacuum manifold containing a nylon filter. After every spotting operation with the Hydra-PP system, the syringes were washed in three wash steps (a wash step is defined as a predetermined number of wash cycles that is performed before the wash solution is replaced with fresh solution) of three wash cycles each, with each cycle having a wash volume of 20 μL water (Robbins Application Note 5; http://www.robsci.com/hug.html).

### Supplemental Wash Procedures for Tango and Hydra Systems

When the Tango or Hydra systems were used for dispensing higher concentrations of DNA (≥100 ng/μL), the washing procedure was modified to include a one-time rinse with 2% bleach, followed by multiple rinses in water before performing blotting. After the last use of the microdispenser for the day, in addition to the above washes, the syringes were washed in Coulter® Clenz (Beckman Coulter, Fullerton, CA, USA) for three cycles, soaked in it for 30 min, and then washed with water for three cycles. Finally, the head was washed with 100% ethanol for two cycles, and the syringes were emptied and dried overnight.

### Isolation of RNA, Preparation of Probes, and Detection

For nylon filters, PolyA-RNA isolation and biotin-labeled probe preparation were performed using a Toyobo Array Kit (Toyobo, Tsuruga, Japan). Hybridization (using a GeneNavigator cDNA array system from Toyobo) was performed following the manufacturer’s instructions. Signals were detected using a chemiluminescence kit (Imaging High™; Toyobo) and a Fluor-S® MAX Multilmage System (Nippon Bio-Rad® Laboratories, Tokyo, Japan) (5).

For slides, all spotting, probe preparation, and hybridization procedures were performed according to the manufacturer’s instructions.

### RESULTS

#### Arraying Speed

Using a Tango or Hydra system equipped with 1, 96, or 384 precision glass syringes arrayed in microplate spacing, 50–300 nL of a 10 μg/mL fluorescein solution or 0.1–5 ng PCR-amplified human housekeeping and cancer-related genes were spotted onto nylon membranes. These compact DNA filters contain 96, 384, 1536, or 6144 spots, with each spot corresponding to a different gene. The speeds of the arraying process for DNA membranes were determined and are reported in Table 1. The arraying times include the time required for the processes of priming (trial dispensing), spotting, and washing. To produce a 384-spot membrane, for example, using a Hydra-PP equipped with 96 syringes and a 96-well source plate, the source plate had to be changed four times. To prevent carryover contamination.

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### Table 1. Arraying Speed for Nylon Filters Using the Hydra and Tango Systems Equipped with 96 or 384 Precision Glass Syringes

<table>
<thead>
<tr>
<th>Number of spots</th>
<th>Arraying speed using Hydra-PP 96 (min) ± SD</th>
<th>Arraying speed using Hydra-PP 384 (min) ± SD</th>
<th>Arraying speed using Tango 384 (min) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td>3.0 ± 0.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>384</td>
<td>11.0 ± 0.8</td>
<td>3.0 ± 0.3</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>1536</td>
<td>40.0 ± 2.2</td>
<td>11.0 ± 0.5</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td>6144</td>
<td>150.0 ± 4.2</td>
<td>40.0 ± 1.5</td>
<td>13.0 ± 1.0</td>
</tr>
</tbody>
</table>

The times indicated include priming, sample spotting, and the washing periods for the preparation of a single membrane. Each spot corresponds to a different gene. Standard deviations are shown for each speed.
The syringes had to be washed four times. This process takes a total of 11 min. To produce a similar membrane (with 384 spots) using a Hydra-PP equipped with 384 syringes, the same procedure takes approximately 3 min because only one wash step is required. To produce higher-density membranes of 6144 spots, it is much more efficient to use the fully automated high-throughput Tango system. With the Tango system, it takes only 13 min to spot one membrane with 6144 DNA spots. As the Tango stage is composed of 12 nests, with one nest dedicated to the wash module, one nest to the source plate, and 10 nests to nylon membranes, a total of ten 6144-spot membranes can be produced in 25 min (2.5 min/6144-spot membrane). Furthermore, because generally genes are spotted in duplicates on arrays (to validate reproducibility), these spotting times can be reduced by half since the number of washes required is cut by half. Therefore, a 6144-spot membrane (3072 genes in duplicate) can be produced in approximately 1 min on the Tango system.

**Spot Uniformity and Reproducibility**

Before using the Hydra and Tango systems for spotting, the uniformity and consistency of the volumes dispensed across the arrays of syringes on the systems were determined by the CV for specific dispensing volumes (Robbins Application Note 6; http://www.robsci.com/hug.html). A high uniformity for dispensing volumes of 50–300 nL used in the arraying assays was evident, with CVs of less than 5%. Next, various DNA-, fluorescein-, and protein-spotted membranes and slides were prepared with these precision glass syringes. The uniformity in spot size and the consistency in the quantity of the samples dispensed are evident from the chemiluminescence and fluorescence images in Figure 1, A–C. The size of the spots created by precision glass syringes was highly uniform and reproducible from spot to spot and membrane to membrane. This is shown in Figure 1D, in which two identical membranes containing 560 different genes in duplicate were created using a Hydra system. Using either system, up to 6144 spots could be spotted onto membranes and up to 1000 spots onto slides, with the spot diameter as small as 580 μm and a spot-to-spot distance of 406 μm.

**Cross-Contamination**

Following a proper washing procedure ensured the elimination of sample-to-sample carryover contamination. This is shown in Figure 2, in which a membrane containing an alternate of PCR-amplified genes (β-actin and
G3PDH) and 10 mM Tris-HCl buffer as blank (containing no DNA sample) spotted in 20 rows was produced. As indicated, no carryover contamination is observed between spotted DNA samples and buffer spots.

**Dispense Performance with Protein Arrays**

Protein arrays were prepared using a Tango system equipped with 1, 96, or 384 syringes. BSA-fluorescein conjugate solutions from 50 μg/mL to 1 mg/mL were spotted onto coated slides or onto Nunc-Immuno™ MaxiSorp 96-well plates (the plates have a charged polystyrene surface with high binding capacity for proteins; Nalge-Nunc International) in a 3 × 3 pattern (Figure 3). When spotting BSA solutions, a dis-
pensing performance equal to those achieved when spotting nucleic acids or organic compounds (such as fluorescein) was observed.

**DISCUSSION**

The Tango and Hydra systems incorporate nondisposable precision glass syringes that offer a new type of printing technology free of disposable tips, pins, or inkjets. These syringes offer a reliable and accurate method of creating highly reproducible and uniform DNA and protein arrays. Glass syringes can be used to dispense volumes as low as 50 nL with very high precision (in the order of 95%). As a consequence, the size of the spots created by precision glass syringes is very consistent and reproducible from spot to spot and membrane to membrane.

Precision glass syringes are inexpensive, simple to operate, robust, and easily replaceable. Since samples do not evaporate or dry once inside the syringes, there is no need for humidification. Consequently, the printed samples dry quickly and sufficiently without spreading or dispersing onto the printing surfaces.

The inside diameter of syringes used in this study was approximately 230 μm. Following a proper syringe washing procedure, no clogging of syringes or sample cross-contaminations were observed. Furthermore, the syringes can be siliconized to prevent the buildup of air bubbles. With inkjet and pin technologies, bubbles have been reported to create inconsistencies in the volumes printed, causing spot variations. In this study, the sizes of the spots printed by syringes varied from 400 to 600 μm. With these spot sizes, it was observed that the quantity and the quality of the printed spots were not affected by dust. Therefore, printings were performed in a typical laboratory room instead of a clean room. As a consequence, a significant reduction in operation expenses was achieved.

Other difficulties typically encountered in implementing non-contact printing with inkjet or contact printing with pin technologies—such as difficulties in thoroughly cleaning the spotting devices, the drying of samples during the process of delivery, shearing of DNA samples, variation in spot size (due to inconsistencies in the volumes printed), and the so-called “donut-shaped” effects—were not observed when precision glass syringes were used for arraying.

**CONCLUSION**

With a dispensing precision variation of less than 5% and a spotting speed of less than 25 min/ten 6144-spot filters and with no sign of cross-contamination, precision glass syringes in high-throughput liquid dispensers such as the Hydra and Tango systems offer an accurate, fast, reliable, and cost-effective means of creating DNA, protein, and compound arrays.

**REFERENCES**


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