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The DNA IQ™ System on the Tecan Freedom EVO® 100

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These new automated scripts incorporate the ability to process aqueous and swab-type samples, start with samples in microcentrifuge tubes or deep-well plates, and process any number of samples between 1 and 96 to minimize reagent waste.

INTRODUCTION

Since its introduction, the automated DNA IQ™ System[a] for isolating genomic DNA has become a workhorse for the forensic community. Promega now introduces scripts for the use of this valuable chemistry on the Tecan Freedom EVO® 100 automated liquid handler (Figure 1). The Tecan instrument boasts a number of advantages, including independent liquid-handling and gripping arms, adjustable tip spacing, independent volume control on each tip, and disposable-tip volumes of up to one milliliter. In addition, the Tecan Freedom EVO® 100 for the DNA IQ™ System is configurable as either a lower cost 4-tip instrument or a higher throughput 8-tip instrument. The user can select the instrument configuration based on the need for lower cost or higher throughput.

Promega has developed and optimized scripts for DNA isolation on both the 4-tip and 8-tip Tecan Freedom EVO® 100 instruments using the DNA IQ™ System for casework and databasing samples. These scripts are able to process liquid samples preprocessed with a proteinase K incubation (designated "aqueous" samples) and samples on solid supports preprocessed by incubation with DNA IQ™ Lysis Buffer (designated "swab" samples). For "swab" samples, the scripts are compatible with the Slicprep™ 96 Device (Cat.# V1391), which allows removal of the incubation liquid and solubilized material from the solid support without having to transfer material to another tube or plate. In addition, both scripts are able to process 1–96 samples, and samples can be placed directly in a deep-well plate to start or can be transferred from microcentrifuge tubes.

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The DNA IQ™ System scripts take advantage of the many unique features of the Tecan Freedom EVO® 100.

- **Number of Tips:** One of the most basic options on the instrument is the number of tips on the liquid-handling arm. We have created DNA IQ™ System scripts for both the lower cost 4-tip option and the higher throughput 8-tip option. While the process remains the same for both numbers of tips, the 4-tip script requires 2 hours and 20 minutes, while the 8-tip script is complete in 1 hour and 30 minutes. (Times are based on processing 96 swab samples starting in a deep-well plate. Starting with samples in microcentrifuge tubes will increase the time.)

- **Tip Sizes:** Disposable tips are used for all experiments and are adequate in all liquid-handling steps. We used two different types of tips depending on what type of pipetting is being done. For all reagent transfers, we used 1ml filter, disposable tips, which allow for time and cost savings through multipipetting. These tips are also conductive, so the instrument can sense liquid levels in the reagent troughs, ensuring enough reagent for each step in the process. Two sets of 200µl filter, disposable tips are used, one for all sample transfer and waste removal steps and a second set for all elution steps. Here we use the less expensive, nonconductive tips to reduce the overall costs of the script.

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- **Adjustable Tip Spacing:** The liquid-handling arm of the Tecan Freedom EVO® 100 has the ability to adjust individual tip spacing. We have taken advantage of this feature by allowing users to present samples either in a 2.2ml deep-well plate or individual microcentrifuge tubes.

- **Independent Tip Control:** Since each tip on the Freedom EVO® is controlled independently in terms of height and volume, we can process 1–96 samples without wasting reagents on empty wells in a column. Using the Tecan Freedom EVO® 100 allowed us to process individual samples rather than an entire column.

- **Powerful Software:** EVOware® is the powerful software that controls the Tecan Freedom EVO® 100. This software allows a single script to have many customizable features and allows rapid customization for the chosen hardware. The single scripts for the 4- and 8-tip instruments query the user for information about sample type (aqueous or swab), starting sample vessel (plate or tubes) and sample number (1–96). With this information, the script is customized to meet the user's needs. In addition to the core EVOware® functionality, users can take advantage of new options that enable effective tracking of all sample-handling steps and simple programming of normalization tasks.

RESULTS

Aside from the special features of the Tecan Freedom EVO® 100 scripts, sample processing and results from the DNA IQ™ System remain unchanged. Here we report results from the DNA IQ™ System scripts run on the Tecan Freedom EVO® 100.

CONSISTENT CONCENTRATION

To examine consistency of DNA yield, we purified DNA from 48 × 20µl liquid whole blood samples using the 4-tip Tecan Freedom EVO® 100 DNA IQ™ script. To minimize variability in the starting material, all blood used in these experiments was from the same individual. Prior to extraction the samples were preprocessed by incubating at 56°C for 1 hour with 80µl of proteinase K solution [as described in the *Tissue and Hair Extraction Kit (for use with DNA IQ™) Technical Bulletin* #TB307]. Samples were processed using the "aqueous" DNA IQ™ System script on the Tecan Freedom EVO® 100. Samples were eluted with 100µl of DNA IQ™ Elution Buffer and quantitated using the Quant-iT™ PicoGreen® dsDNA reagent. The average DNA concentration was found to be 1.76ng/µl with a standard deviation of 0.38ng/µl.

SAMPLE SIZE VERSUS DNA YIELD

The DNA IQ™ System allows DNA purification in two formats. When samples have limited amounts of DNA, the DNA IQ™ System provides a consistent amount of DNA; when samples have DNA in excess of the

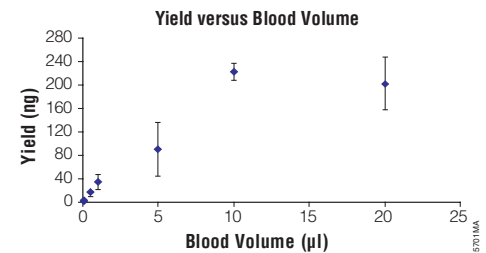**Figure 1. The Tecan Freedom EVO® 100 instrument.**

Figure 2. A plot of DNA yield versus input blood volume. DNA was purified from blood samples (0.05–20µl) in triplicate using the DNA IQ™ System method on the Tecan Freedom EVO® 100. Quantitation of the eluates was performed using the Quant-iT™ PicoGreen® dsDNA reagent.

binding capacity of the DNA IQ™ Resin, a fixed yield is provided. To illustrate this using the DNA IQ™ script on the Tecan Freedom EVO® 100, purification was performed from a dilution series of liquid whole blood samples. Triplicate samples of 0.05–20µl of whole blood were preprocessed by incubating at 56°C with 80µl of proteinase K solution. As shown in Figure 2, DNA yield increased with sample amount up to approximately 10µl of blood, where the resin becomes saturated.

CROSS-CONTAMINATION

To assess cross-contamination in the DNA IQ™ script, we used the Tecan Freedom EVO® 100 to purify DNA from a checkerboard pattern of buccal and blank swabs. Samples were preprocessed using the Slicprep™ 96 Device. Four hundred microliters of DNA IQ™ Lysis Buffer was added to each well of the Slicprep™ 96 Device containing either a buccal or blank swab. The plate was sealed, incubated at 70°C for 1 hour and centrifuged at 1,500 × *g* for 5 minutes. Samples were processed using the "swab" DNA IQ™ script on the Tecan Freedom EVO® 100. One hundred microliters of DNA IQ™ Elution Buffer was used to elute the samples, and DNA from buccal swabs was diluted to a

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concentration of approximately 1ng/µl. One microliter of each buccal swab sample and 10µl of each undiluted blank was amplified in a 25µl PowerPlex® 16 System[b–d] reaction. Electropherograms from representative buccal swab and blank samples are shown in Figure 3. DNA from buccal swabs gave robust amplification, whereas there were no detectable amplification products in reactions with blank swabs.

CONCLUSIONS

The Tecan Freedom EVO® 100 automated liquid handler is the newest and most flexible instrument available to automate the DNA IQ™ System. These new automated Tecan scripts incorporate the ability to process "aqueous" and "swab" samples, start with samples in microcentrifuge tubes or deep-well plates, and process any number of samples between 1 and 96 to minimize reagent waste.

Using these scripts, we observed the expected, linear DNA yields with limiting samples and consistent, fixed DNA concentrations when samples contained DNA in excess of the binding capacity of the resin. We have not observed any cross-contamination attributable to this script. Based on the data presented here, the 4-tip and 8-tip DNA IQ™ automated scripts provide the quality performance you would expect from the DNA IQ™ System while taking advantage of the strengths of the Tecan Freedom EVO® 100.

For more information about the instrumentation necessary to run these scripts, contact Tecan. Additional hardware and plates are available from Promega.
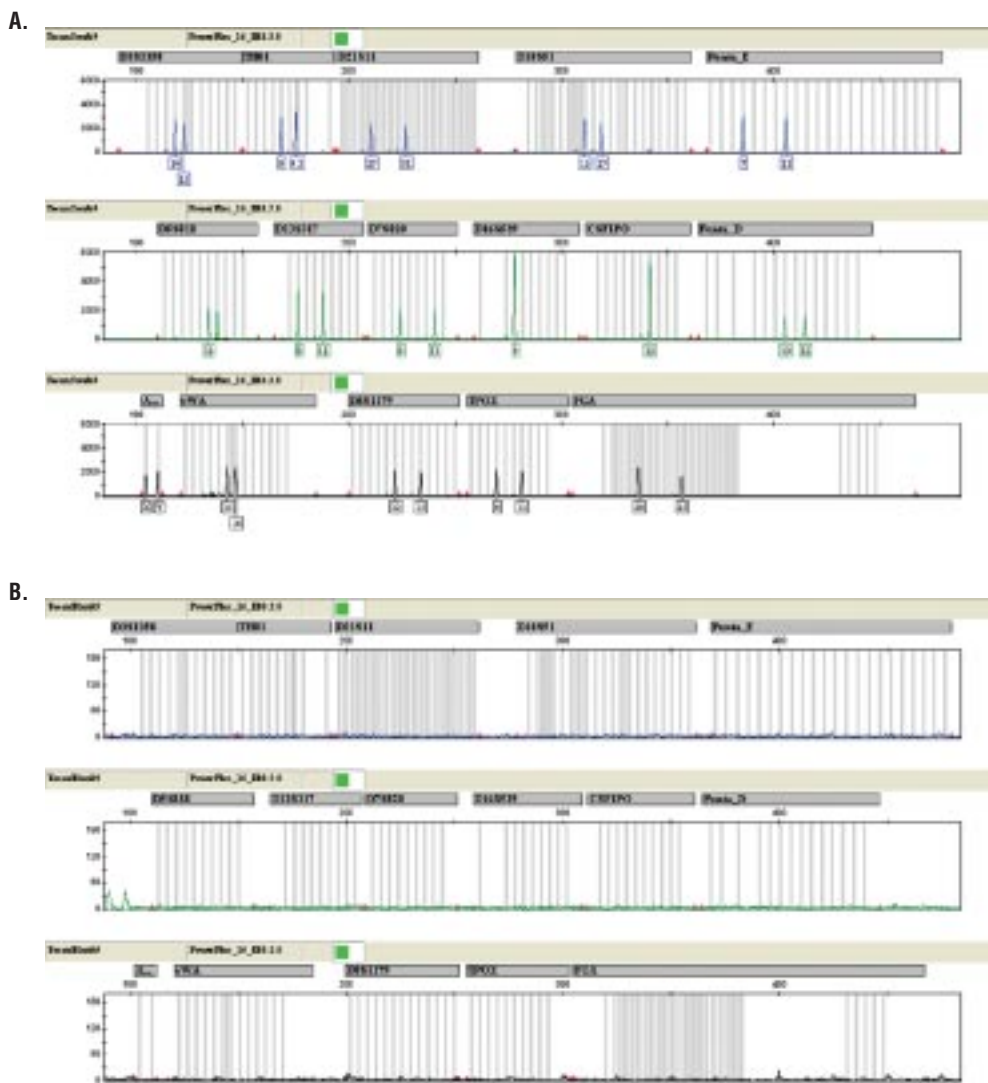
A.

B.

Figure 3. Cross-contamination results. Electropherograms showing representative traces from buccal swab (**Panel A**) and blank samples (**Panel B**). Samples were placed in a 96-well, deep-well plate in a checkerboard pattern and preprocessed by incubating with DNA IQ™ Lysis Buffer in the Slicprep™ 96 Device. DNA from buccal swab samples was adjusted to a final concentration of 1ng/µl. One microliter of swab samples or 10µl of blanks were amplified using the PowerPlex® 16 System with 10/22 cycling on the GeneAmp® 9700 PCR System. Amplification products were detected with the ABI PRISM® 3100 Genetic Analyzer.

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