A Simple, User-Friendly, High Throughput DNA Extraction Workflow Using 2-D Barcoded Buccal Swabs on Hamilton Firefly NIMBUS® 96

Kiranmai Durvasula[1], Jeff Roeder[1], Travis Butts[1]
[1]Omega Bio-tek, Inc.

Introduction

Buccal swabs are one of the most commonly used tools for collecting DNA samples because of their non-invasiveness, ease of collection and transportation. Compared to other sampling alternatives such as blood, buccal sampling is cost-effective, pain-free and can be easily done by the subject with no special training. Omega Bio-tek has developed LIMS-compatible, 2-D barcoded swabs for efficient sample collection and full sample traceability throughout the DNA extraction process. When integrated with Omega Bio-tek's E-Z 96® Spin-Out Device, these 2-D Spin-Out Swabs offer a rapid, high yielding, automation-friendly solution for DNA isolation. In this study, we provide an automated DNA extraction solution using 2-D Spin-Out Swabs on the Hamilton Firefly NIMBUS® 96 utilizing Omega Bio-tek's extraction chemistries that is capable of processing 96 samples in under 2 hours with sample tracking.

Materials and Methods

Omega Bio-tek's 2-D Spin-Out swab is a unique collection device that consists of a basket with a swab securely attached to it's cap, which has a barcode printed on it. To the outside end of the cap is a detachable flag with a barcode that matches the barcode on the cap. The basket also has the matching numbers of the barcode printed on the side that can be noted manually, if required.

Sixteen independent oral samples were collected using Omega Bio-tek's 2-D Spin-Out Swabs. After sample collection, each 2-D Spin-Out Swab was re-inserted back into the basket; the flag detached from the cap and saved for record keeping. The 2-D Spin-Out Swab with the basket was placed in an individual well of the E-Z 96® Spin-Out Plate Adaptor in a 96-well deep well plate prefilled with lysis buffer. The integrated 2-D barcode on the cap can then be scanned using a top-down 2-D barcode reader, such as the FluidX Cabriolet. This allows for the identification of the sample location within the 96-well plate. The need to manually input a well location and sample ID into your laboratory information management system is completely eliminated.

The E-Z 96® Spin-Out Plate Adaptor's unique design

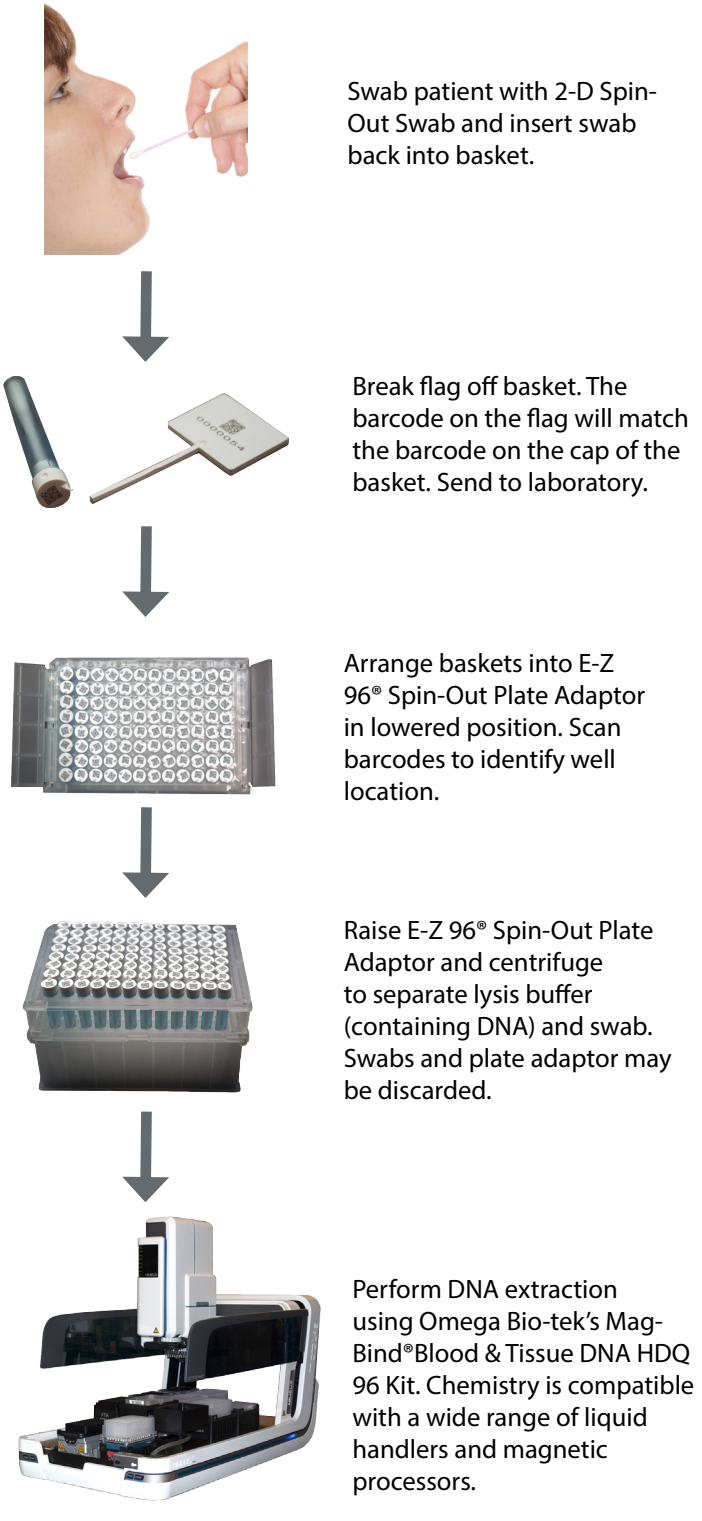

Figure 1. Complete extraction workflow using 2-D Spin-Out Swabs.

allows for samples to be raised and lowered into the lysis buffer. The samples were submerged in the lysis buffer with the adaptor in the lowered position, allowing for the incubation and lysis of the sample. The adaptors were then raised to lift the baskets with the swabs above the liquid level and centrifuged to recover all the residual lysate from the swabs. After centrifugation, 2-D Spin-Out Swabs along with the Spin-Out Plate Adaptor was discarded. The 96-well deep well plate containing the lysate was moved to the Hamilton Firefly NIMBUS® 96 for further purification using Omega Bio-tek's Mag-Bind® Blood & Tissue DNA HDQ 96 Kit (M6399). The workflow of the extraction process using 2-D Spin-Out Swabs is outlined in Figure 1.

The extracted DNA was quantified using Promega's QuantiFluor® dsDNA system. Eight samples were randomly selected and real-time PCR was performed on triplicates of undiluted, 10-fold and 100-fold diluted DNA extracts to tes for the suitability of DNA for downstream applications. Agilent's Brilliant III 2X SYBR® mix and universal human primers were used following a standard amplification protocol on the ABI 7900.

Results

The DNA yield from the oral samples collected using the 2-D Spin-Out Swabs is as shown in Figure 2. The average DNA yield was approximately 2.48 µg. Table 1 lists the average C_t values from triplicate experiments on the undiluted, 10X and 100X dilution of the purified DNA. There was no detectable fluorescence in the no template controls. The average ΔC_t between the 10-fold and undiluted samples was lower than 3.3, indicative of the presence of inhibitors. As expected, the average ΔC_t between the 100-fold and 10-fold diluted sample was approximately ~3.3, bringing the amplification efficiency close to 100%.

Table 1. Average C_t values from real-time PCR performed using universal human primers on triplicates of 1X, 10X and 100X dilutions of DNA isolated from 2-D Spin-Out Swabs.

Sample Number	Average C_t		
	1X	10X	100X
4	22.42	25.05	28.77
7	22.11	24.63	27.93
9	22.18	24.96	28.36
10	22.24	24.67	28.28
11	22.55	25.47	28.81
12	22.77	25.24	28.64
13	23.29	26.10	29.46
14	23.27	26.30	29.22

Conclusion

DNA obtained from the 2-D Spin-Out Swab samples was of sufficiently high quality and suitable for a variety of downstream applications, such as real-time PCR. The 2-D Spin-Out Swabs offer a reliable, non-invasive approach to sample collection and tracking, and along with the E-Z 96® Spin-Out Device can be easily incorporated into most high throughput automation workflows.

Ordering Information

Description	Product No.	Preps
Mag-Bind® Blood & Tissue DNA HDQ 96 Kit	M6399-00	1 x 96
	M6399-01	4 x 96
E-Z 96® Spin-Out Plate Adaptors	AC7088-01	10 x 96
2-D Spin-Out Swabs	AC7076-00	100 preps

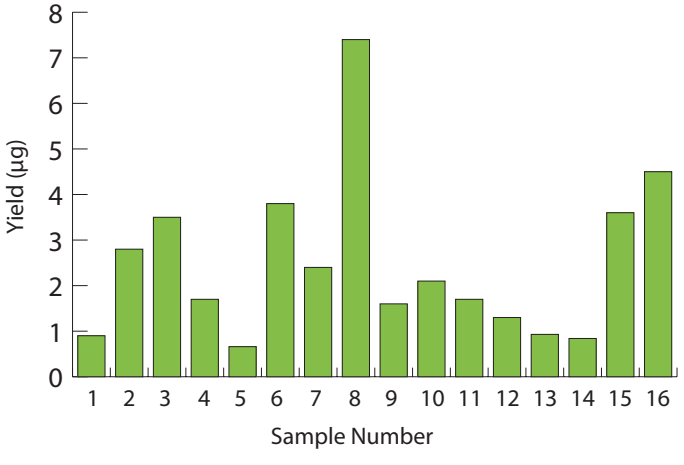

Figure 2. DNA yield from oral samples collected using 2-D Spin-Out Swabs.

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OMEGA bio-tek

400 Pinnacle Way, Suite 450
Norcross, GA 30071

www.omegabiotek.com
info@omegabiotek.com
(800) 832-8896