Comprehensive, High Throughput Workflow for Automated gDNA Isolation from iSWAB Oral Samples

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Introduction

High quality DNA is a critical factor in determining success of many molecular biology applications, such as next-generation sequencing (NGS), qPCR, microarrays, etc. Other potential applications include clinical molecular diagnostics, genetic analyses, pharmacogenomics, animal speciation studies, and forensics. Buccal swabs or saliva are the most commonly used sample types for these types of analyses because of their non-invasiveness and ease of collection. However, these existing non-invasive technologies suffer from low genomic DNA recovery along with high bacterial contamination. Current swab-based products require manual swab removal and are not automation-friendly. MAWI DNA Technologies' iSWAB collection device addresses these issues with its innovative tube insert design that maximizes the release of cells collected on swabs into an existing proprietary selective mammalian cell lysis buffer and allows for long-term, room temperature storage of the sample while preventing the release of bacterial DNA (Figure 1). The design is particularly attractive because it eliminates the need for swab retention and is compatible with a variety of commercially available swabs (Figure 1). LIMS-compatible barcodes are also included on each iSWAB device for efficient sample traceability and storage purposes.

Omega Bio-tek offers the Mag-BIND® Blood & Tissue DNA HDQ 96 Kit (M6399) for rapid and reliable isolation of high quality DNA from blood samples, saliva, swabs, mouse tails, dried blood spots, and cultured cells, which is capable of being automated on an open-ended liquid handling platform such as the Hamilton Microlab® STAR™. The Omega/Hamilton automated protocol is capable of extracting DNA from 96 samples in under 1 hour. Here, we provide a comprehensive, automated workflow for the extraction of gDNA from iSWAB oral samples utilizing Omega Bio-tek's extraction chemistry on the Hamilton STAR™.

Materials & Methods

Twenty-one independent samples were collected in iSWAB collection devices, 9 of which were collected with Copan FLOQSwabs™ and the rest with Puritan cotton swabs. The samples were sent to our laboratory by 2-day ground shipping and stored at room temperature for 14 days from the time of collection to the time of DNA purification. The automation protocol began with 250 µL of the samples from the iSWAB collection devices being rapidly arrayed into 96 wells using 300 µL Hamilton Slim CO-RE® tips (Figure 2). The Hamilton STAR™ was programmed to perform various liquid handling and magnetic bead-based tasks as demanded by the Mag-BIND® Blood & Tissue DNA HDQ 96 protocol for the extraction of genomic DNA. The extracted DNA was quantified using Promega's QuantiFluor® dsDNA system.

Performance Evaluation of the Purified DNA

Purified DNA from 5 samples collected using Puritan cotton swabs were selected for this proof-of-concept study. The quality of the purified DNA for downstream applications was assessed by real-time PCR performed on triplicates of 1X (~12 ng), 10X, 100X, 1,000X, and 10,000X serial dilutions. Agilent's Brilliant III 2X SYBR® mix and universal human primers were used following a standard amplification protocol on the ABI 7900. The real-time PCR performance was evaluated based on qPCR reaction efficiency and R^2 value.

Bacterial DNA Estimation

The assay as described in [1] was performed to quantify the precent of bacterial contamination in the samples. We chose 5 of Copan FLOQ™ collected samples as our data set for this assay.

Figure 1. a) MAWI's iSWAB collection device; b) iSWAB-ID rack with 50 devices; c) Commercially available swabs compatible with the device (L to R): Copan FLOQ swab, Puritan cotton swab on a polyester stick, Puritan cotton swab on a wooden stick, rayon swab.

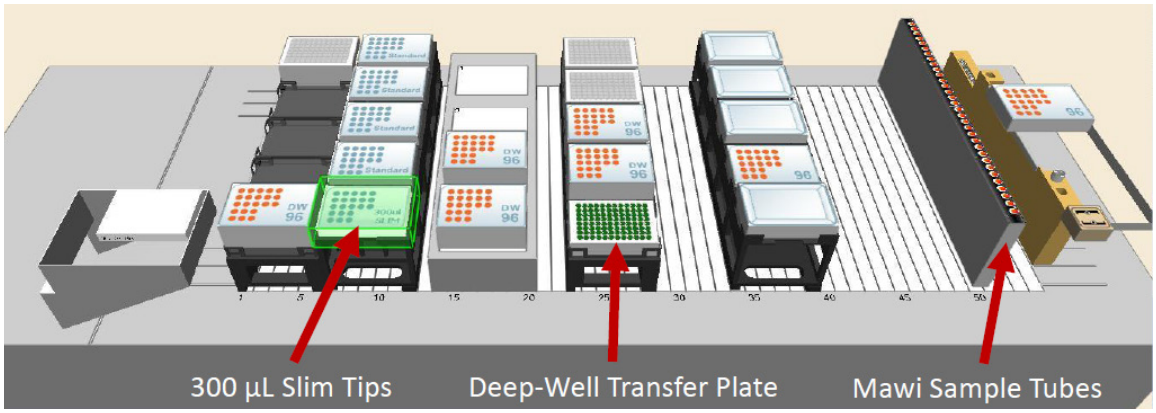

Figure 2. Hamilton STAR™ deck layout for automated gDNA isolation from iSWAB samples.

Briefly, real-time PCR was performed with the PCR primers from the 16S rRNA gene that is known to be conserved across a wide variety of prokaryotic microorganisms. Serial dilutions of pure bacterial DNA (here, *E. coli*) was used to construct a standard curve, which was later used to estimate the amount of bacterial DNA in the iSWAB samples. A known quantity of *E. coli* (5 ng) was included as our internal standard (IS) to more accurately calculate the bacterial DNA in the sample. Each PCR reaction used 14 ng of DNA sample (TD). To compensate for variations in amplification efficiency between the runs, 2 aliquots of the same sample was run in parallel -- 1 spiked with IS (S) and the other unspiked (US). A correction factor (CF) was calculated as the ratio of IS to the difference between the spiked and unspiked. The percent bacterial DNA contamination in the sample compensating for the differences in PCR reaction efficiency was estimated as below:

$$\% \text{ Bacterial DNA} = CF \times {}^{US}/_{TD} \times 100$$

Results

The purified DNA yield from the iSWAB samples are shown in Figure 3. Standard curves were generated by 5-fold serial dilutions of purified DNA in the range of 0.0012 ng to 12 ng. Figure 3 depicts a representative image of the standard curve. 10,000X dilution was omitted from the curve as the C_t values were close to that of the no template controls and no longer accurate. Linear regression was performed on the data points to arrive at the slope and regression co-efficient (R^2). The slope of the standard curve was employed to determine the qPCR efficiency ($E = 10^{-1/slope} - 1) \times 100$).

Table 1 lists the real-time PCR efficiencies and R^2 values for each of the 5 samples tested. A robust linear correlation was observed ($R^2 > 0.99$) for all the 5 samples tested. qPCR efficiency ranged from 86.56% to 96.16%. We observed good fidelity in the C_t values among the 4 replicates tested at each dilution with the average standard deviation ranging from 0.17 to 0.55 depending on the sample (Table 1). The above discussed parameters suggest that the isolated DNA was of high quality and suitable for downstream applications.

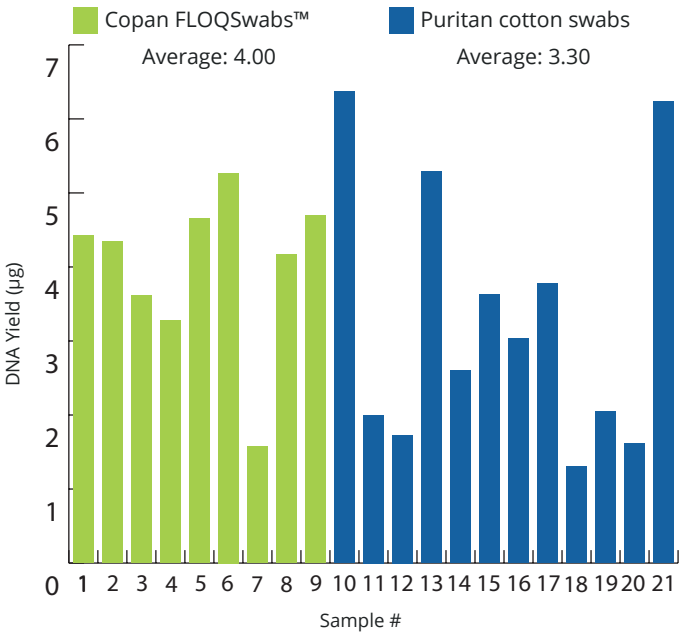

Figure 3. DNA yields from the iSWAB collection devices collected using either Copan FLOQSwabs™ or Puritan cotton swabs.

DNA from oral samples typically contain bacterial DNA in addition to human genomic DNA. This bacterial DNA has no practical relevance and hinders performance of applications downstream. Hence, we estimated the bacterial DNA as a percent of total DNA in the iSWAB samples as described [1]. Our findings indicate bacterial DNA ranged from 0.15 to 4.50% of the total DNA yield among the samples tested (Table 2). A median of 2.34% of the total oral DNA collected with MAWI's iSWAB collection device was found to be bacterial. In comparison, bacterial DNA content with the iSWAB device is much lower than various existing oral collection techniques (Table 3).

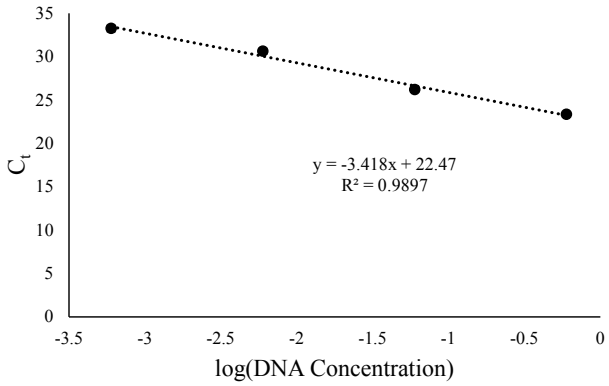

Figure 4. Representative image of the standard curve from serial dilutions of the iSWAB sample.

Table 1. Quality assessment of purified DNA.

Puritan Cotton Sample	qPCR Efficiency	R^2	Average Standard Deviation
10	96.16	0.99	0.27
11	88.81	0.99	0.55
12	86.56	1	0.33
13	96.04	1	0.47
14	89.14	0.99	0.17

Conclusions

An automated, high throughput protocol was developed for rapidly extracting DNA from MAWI's iSWAB collection devices utilizing Omega Bio-tek's extraction chemistry on the Hamilton STAR™. The process yielded high quality, amplifiable DNA suitable for a variety of downstream applications. MAWI's iSWAB collection device offers a reliable option for oral sampling with significantly less bacterial contamination when compared with other techniques. It has potential to make significant contributions, especially in the areas of human genome analyses, forensics, and applications such as NGS where bacterial contamination poses a serious problem.

Table 2. Percentage of bacterial DNA in the iSWAB samples.

Copan FLOQ™ Sample #	% Bacterial Contamination
1	4.50
2	3.56
3	1.36
4	2.34
5	0.15
Median:	**2.34**

Table 3. Comparison of the median percentage of bacterial DNA contamination in various oral collection techniques.

Collection Method	Median % of Bacterial DNA	Reference
Mouthwash	66.0	[2]
Mouthwash	50.5	[2]
Cytobrush	88.5	[2]
Oragene®/saliva	11.8	[2]
iSWAB	2.34	This study

Product Information

Description	Product No.	Preps
Mag-BIND® Blood & Tissue DNA HDQ 96 Kit	M6399-00	1 x 96
	M6399-01	4 x 96
iSWAB DNA Collection kit	iSWAB-DNA-120	-

References

[1]. DNA Genotek, "Bacterial DNA Assay," PD-PR-065 Issue 4, 2012.

[2]. DNA Genotek, "Human Genomuic DNA Content of Saliva Samples Collected in Oragene® DNA," PD-WP-011 Issue 4, 2012.

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