A High-Yield, High-Performance Automated gDNA Isolation System Utilizing the iSWAB-DNA Oral Sample Collection Device

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Introduction

It is important to obtain high-quality genomic DNA for either simple or complex genomics applications such as genotyping or next generation sequencing. Obtaining high quality gDNA depends mainly on two factors: 1) Quality of sample, and 2) DNA extraction chemistry. Using the automated chemagic M-PVA magnetic bead technology from PerkinElmer, it is possible to obtain high yields of genomic DNA from a number of biological samples. The PerkinElmer chemagic automated extraction system is the only platform capable of extracting samples of varying volume from 10 µl to 10 ml. Although buccal swab collection has been widely adopted for its simplicity, non-invasive nature, and primary method for infant and child collections, it remains a challenging sample type to process for obtaining high quality human gDNA with long fragment sizes. Some of the challenges facing buccal swab extractions are low yield, instability of the sample especially during long transit times, and high levels of bacterial genomic DNA contamination which can significantly affect DNA sequencing coverage. MAWI DNA Technologies' iSWAB collection devices address these issues. Stability of samples is achieved with pre-filled proprietary selective mammalian cell lysis buffer, which allows long term storage at room temperature. The collection device enables and maximizes the release of cells collected on one or more swabs into the buffer, thus concentrating the content from several swabs to a single device while minimizing the release of bacterial DNA. In this report, we describe for the first time the ability to extract the entire volume of the iSWAB sample (up to 1 mL) in a single gDNA extraction method/iSWAB device, resulting in high-yield, high purity genomic DNA. This provides facilities such as biobanks and biorepositories that receive iSWAB-DNA samples with a high throughput, automated workflow to generate large quantities of nucleic acids for their operations.

Materials and Methods

One hundred and fourteen (114) independent buccal swabs were collected with iSWAB devices (Figure 1), consisting of 57 Adults and 57 children. After collection, 7 µl of Proteinase K

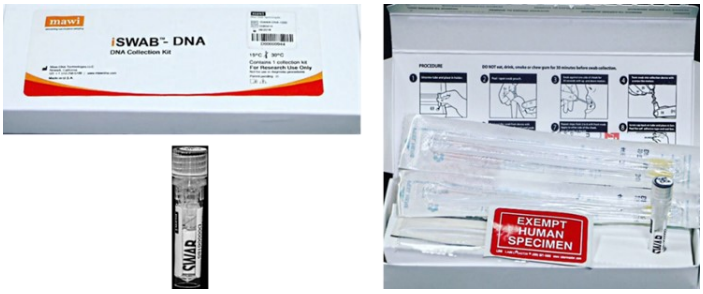

Figure 1. MAWI's iSWAB-DNA-1200 Collection Kit used in this study: The kit is designed to be a two way shipping package which includes sterile swabs, biospecimen bag, extra bar-code label that matches the bar-code on the device, and iSWAB-DNA sample collection device.

was added to each sample and the devices were incubated overnight at 37°C in order to achieve maximum lysis. The 1 mL lysate was transferred into a chemagic 24 Deep Well Plate-XL with 1 mL chemagic Blood Lysis Buffer. The plate was transferred to the PerkinElmer chemagic MSM I (Magnetic Separation Module) instrument using the chemagic 24 Rod Head (Figure 2) and was operated using a saliva extraction protocol (CMG-1081 using the 2 mL input) . This protocol can accommodate up to 24 samples at a processing time of approximately 45 minutes. Eluates were then quantified by Nanodrop and PicoGreen analyses. Quality and size determinations were made looking at the 260/280 and 260/230 ratios and Agilent Tape station electropherogram results.

Results and Discussion

In order to quantify double stranded genomic DNA, it is often necessary to use quantification methods that detect dsDNA using fluorescence-based systems such as PicoGreen. This typically accounts for the double stranded Human genomic DNA. A UV-based quantification system such as Nanodrop accounts for total nucleic acid content, but also gives a good indication of purity in the sample. For these reasons, we used these quantifi-

cation methods to determine the total yield (µg) and purity (A260/280). The average gDNA yield of the samples was 15.85 µg by Nanodrop and 6.85 µg for ds gDNA as determined by PicoGreen, while the average purity was 1.82 (Table 1). In general the adults had higher yields compared to the children, which is expected. Most importantly, the data clearly displays the versatility of iSWAB-DNA for obtaining high quality human gDNA with fragment sizes >50 kb from both adults and children (Figure 3), which is a testament of its universal applicability for non-invasive sample collection regardless of the population segment.

In conclusion, the chemagic gDNA automated extraction process has shown to be a reliable and efficient extraction method to obtain high yields of high quality DNA from iSWAB samples by processing the full content of each iSWAB-DNA collection device in a single preparation, thus reducing cost and turnaround time.

All SAMPLES (N=114)	NANODROP YIELDS (µg/Sample)	260/280	260/230	PICOGREEN YIELDS (µg/Sample)
Mean	15.85	1.82	1.61	6.85
Median	14.51	1.83	1.7	4.96

ADULTS (N=57)	NANODROP YIELDS (µg/Sample)	260/280	260/230	PICOGREEN YIELDS (µg/Sample)
Mean	18.39	1.79	1.67	9.82
Median	17.08	1.83	1.78	9.04

CHILDREN (N=57)	NANODROP YIELDS (µg/Sample)	260/280	260/230	PICOGREEN YIELDS (µg/Sample)
Mean	13.31	1.84	1.55	3.88
Median	12.54	1.86	1.57	3.14

Table 1. Average yields and quality of Human gDNA from the universal sample collection system, iSWAB-DNA, for (Top) All Samples, (Middle) Adults and (Bottom) Children as measured by Nanodrop and PicoGreen.

Figure 2. (A) PerkinElmer chemagic MSM I instrument with a close up image of the chemagic 24 Rod Head. (B) DeckLayout for the Processing of the iSWAB-DNA Samples on the chemagic MSM I.

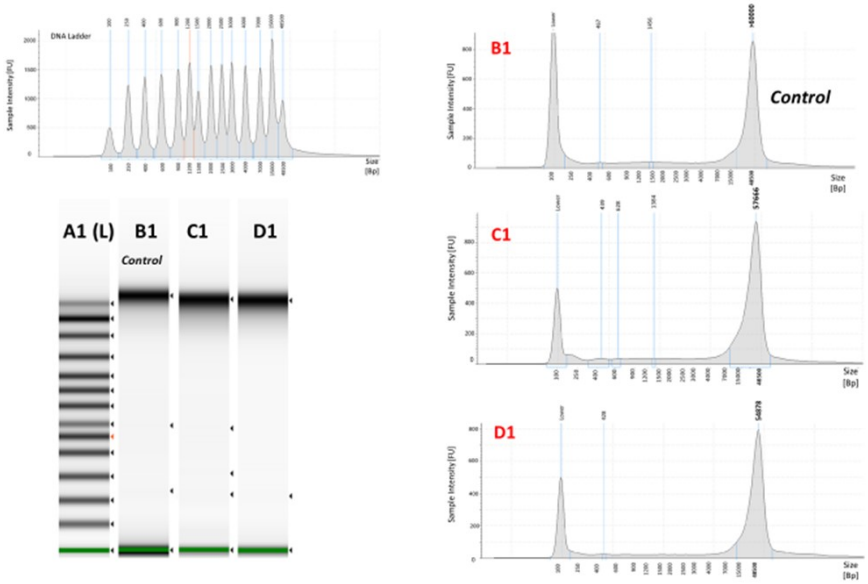

Figure 3. Tape Station analyses for gDNA extracted from ISWAB-DNA-1200 on the chemagic MSM I System as described in Figure 2.