Automated Genomic DNA Isolation from Oragene™ Saliva Collection Kits Using NiXTips®

HIGHLIGHTS: Fast, efficient, high throughput

NIX

INTRODUCTION

Saliva collection for genomic DNA extraction is a convenient and non-invasive procedure that is commonly used for genomic studies. However, saliva is a heterogeneous biofluid containing mucins, enzymes, microbes and inhibitory polysaccharides that presents inherent challenges for nucleic acid purification. Saliva collection tubes containing nucleic acid stabilization buffers have allowed for self-collection and storage at room temperature. For high throughput laboratories processing hundreds to thousands of saliva samples, an automated process for nucleic acid extraction using a liquid handler is required.

Commercial nucleic acid extraction protocols that employ protein precipitation as a cleanup prior to nucleic acid purification are not amenable to automation. Bind-wash-elute protocols utilizing magnetic beads have been developed but require additional hardware for processing. As an alternative, DPX Technologies has developed an automated DNA extraction protocol utilizing Oragene collection kits and patent-pending NiXTips on a Hamilton® liquid handler. Integration of all steps within the pipette tip reduces handling complexity, improves reproducibility, and ensures recovery of DNA with sufficient integrity for downstream molecular analysis. The demonstrated combination of yield, integrity, and functional performance establishes this method as a robust alternative to conventional workflows and a valuable tool for laboratories engaged in high-throughput genomic research.

MATERIALS AND METHODS

Human saliva specimens were collected using Oragene•Dx kits (DNA Genotek, *Cat #: OGD-600*). Following collection, tubes were homogenized by inversion to ensure uniform mixing of the saliva and stabilization buffer. Samples were incubated at 50°C for 2 hours to promote protein denaturation and nucleic acid release and then stored at ambient temperature as recommended in Oragene's protocol.

Genomic DNA was extracted from the saliva samples using DPX Technologies' NiXTips along with DPX's proprietary Lysis, Wash 1, Wash 2, and Elution Buffers. Extractions were performed with 1 mL NiXTips (*Cat #: NIX-HM1000-96*) on a Hamilton STARlet (**Figure 1**). Cell lysis and DNA binding were initiated by adding 125 µL of Lysis Buffer and 185 µL of isopropanol to 250 µL aliquots of sample. DNA binding was accomplished via ten aspirate and dispense cycles with the NiXTips. Bound DNA was purified by sequentially washing with Wash 1 Buffer once, Wash 2 Buffer twice, and then dried by air expulsion. DNA was subsequently eluted in 100 µL of Elution Buffer at 70°C.

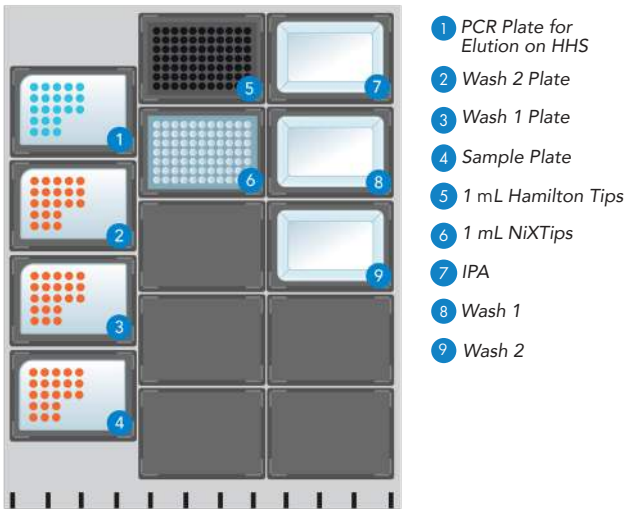

Figure 1. *Deck layout of the Hamilton STARlet method.*

RESULTS

Genomic DNA was extracted in duplicate from 6 volunteer samples using the NiXTips automated protocol. Spectrophotometric quantification using a Thermo Scientific NanoDrop demonstrated average DNA concentrations of 111 ng/µL, corresponding to total recoveries ranging from 3.7-35.6 µg per 250 µL of input sample. In addition, the average A260/280 ratio was 1.88 and the average A260/230 ratio was 2.09. These yields were consistent across biological replicates, reflecting protocol robustness (**Figure 2**).

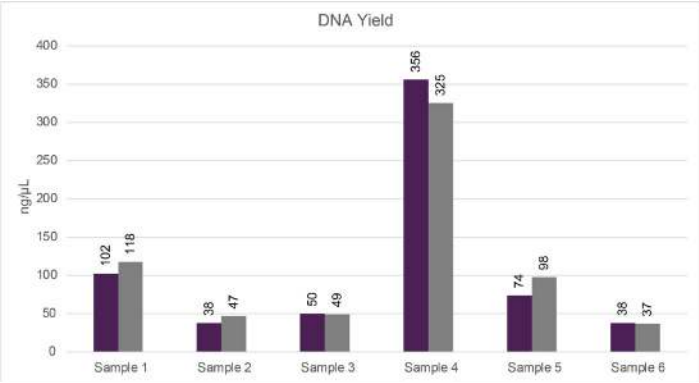

Figure 2. *DNA was extracted from six volunteers in duplicate using the NiXTips automated protocol and quantified with a NanoDrop spectrophotometer.*

Replicate samples (n=4) were extracted using NiXTips and a commercial magnetic bead protocol for comparison. Agarose gel electrophoresis confirmed the yield and integrity of the purified genomic DNA (**Figure 3**). The DNA extracts were run on an Agilent 5200 Fragment Analyzer which confirmed the high molecular weight of the purified DNA with an average size of greater than 48.7 kbp (**Figure 4**).

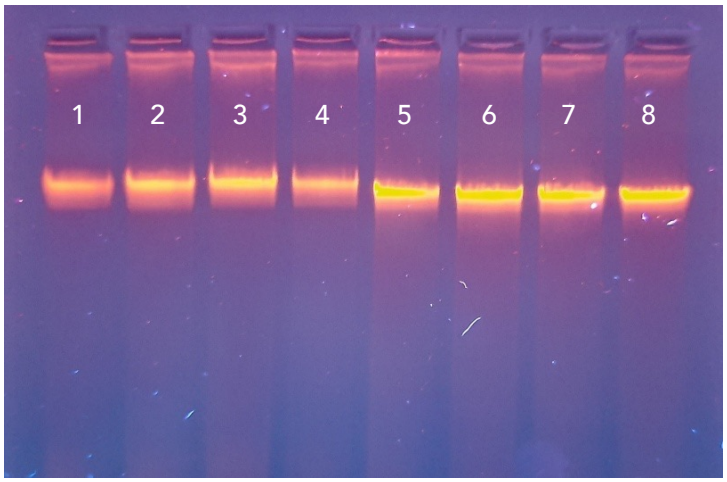

Figure 3. *Agarose gel (1%) of 4 replicates of genomic DNA purified by a commercial magnetic bead protocol (lanes 1-4) or the NiXTips automated protocol (lanes 5-8).*

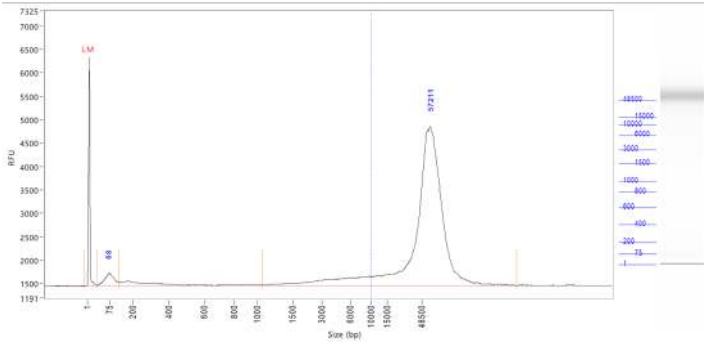

Figure 4. *A representative genomic DNA sample purified with the NiXTips automated protocol and run on an Agilent 5200 Fragment Analyzer. The average size of the DNA is greater than 48.7 kbp.*

Functional suitability was validated by PCR amplification of the ribonuclease P (RNase P) locus, a standard genomic DNA control. Amplification was uniformly successful, yielding Ct values between 24.5-25.5 (**Figure 5**), indicating efficient template availability and absence of significant inhibitory carryover. Together, spectrophotometric, electrophoretic, and functional data confirmed that DNA isolated from the NiX workflow was of high integrity and suitable for downstream molecular applications. The protocol is scalable up to 500 µL of sample.

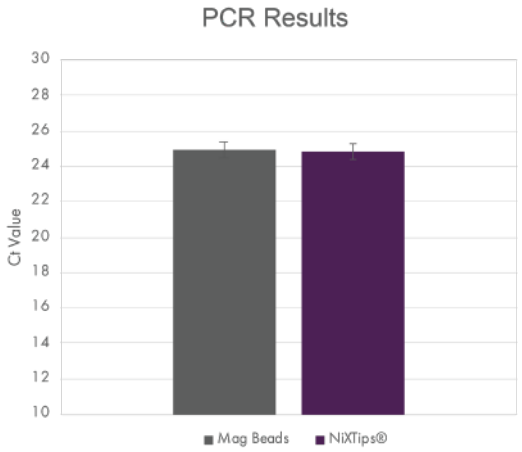

Figure 5. *Ct values from the 4 replicate samples extracted by a commercial magnetic bead protocol (gray) and the NiXTips automated protocol (purple). Error bars represent standard deviation.*

DISCUSSION

This study highlights the utility of DPX NiXTips for saliva-based genomic DNA extraction. The tip-based bind-wash-elute workflow consolidates processing steps within a single pipetting element, reducing opportunities for cross-contamination and DNA loss associated with transfer-dependent methods such as magnetic bead or silica column protocols. The NiX platform effectively mitigates these challenges, producing DNA of sufficient yield and purity for robust amplification.

Furthermore, the capacity to process 96 samples in under 36 minutes represents a substantial advance in throughput, aligning with the needs of large-scale population genomics, epidemiological surveillance, and biobank operations.

Available NiXTip Formats:

Automation	Volume	Amount
Hamilton	1 mL	96 tips/rack
Integra - Coming Soon!	1250 µL	96 tips/rack

For more information, visit www.dpxtechnologies.com or contact sales@dpxlabs.com.