Fully automated DNA extraction solution from Omega Bio-tek using saliva stabilized in Biomatrica's Salivagard® HT DNA collection tubes

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Introduction

Non-invasive sampling, along with ease and logistics of sample collection, make saliva an appealing biospecimen for molecular analyses, supporting applications such as diagnostics, pharmacogenomics, and biomarker discovery. Biomatrica's Salivagard® HT DNA tubes are specifically designed for safe, intuitive and user-friendly saliva collection and stabilization. From a performance perspective, they not only take a molecular level snapshot at the point-of-collection by preserving DNA integrity, but they also support long-term ambient storage and non-biohazardous shipping conditions. The tubes are LIMS-compatible and include a pierceable cap for seamless integration with automated platforms, eliminating time-consuming decapping and/or sample transfer steps. For high-quality, high throughput DNA extraction from saliva, Omega Bio-tek offers a magnetic bead-based kit, the Mag-Bind® Blood & Tissue DNA HDQ 96 Kit (M6399), automatable on Hamilton Microlab® STAR™. The Omega/Hamilton automated protocol is capable of extracting DNA from 96 samples in under 90 minutes. Outlined below is a complete workflow solution from sample collection with Salivagard® HT DNA tubes to sample transfer and DNA purification utilizing Omega Bio-tek's bead chemistry, with all the liquid handling steps fully automated on Hamilton Microlab® STAR™.

Materials and Methods

Saliva was collected from eight independent donors into Salivagard® HT DNA tubes following the manufacturer's instructions. Upon collection, the samples were stored at room temperature for a day before DNA was extracted using Omega Bio-tek's Mag-Bind® Blood & Tissue DNA HDQ 96 Kit (M6399). The automated protocol commenced on the Hamilton STAR™ with 250 µL of the saliva sample from Salivagard® HT DNA tubes being transferred into a 96-well deep-well plate. 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. Purified DNA was eluted in 100 µL and quantified using Promega's QuantiFluor® dsDNA system. Size and integrity of the isolated genomic DNA was analyzed on Agilent's TapeStation® 2200 with a genomic DNA tape. The suitability of the extracted DNA for downstream applications was examined by performing real-time PCR using human-specific primers on 10-fold and 100-fold dilutions of the purified DNA. Agilent's Brilliant III 2X SYBR® mix was used as the master mix following a standard amplification protocol on the ABI 7900.

Results and Discussion

The purified DNA yield from the Salivagard® HT DNA samples are shown in Figure 1. The differences in the DNA yield can be attributed to inter-donor variability. The average A260/A280 ratio was ~1.7±0.1 indicating good quality of extracted DNA. TapeStation® analysis was performed on the purified DNA to determine the size of the genomic DNA that was extracted as well as its integrity. TapeStation® 2200 analysis software automatically calculated each sample's DNA Integrity Number (DIN). It typically ranges from 0 to 10 (10 being highest integrity) and is determined on the basis of genomic DNA sample fragmentation. Figure 2 shows the TapeStation® analysis performed on the extracted DNA from the saliva samples stored in Salivagard® HT DNA tubes. The purified DNA across the 8 samples migrated as a well-defined band without fragmentation and was above the largest ladder peak (48,500 bp), with TapeStation® software analysis determining it to be over 60 kb. The DIN values ranged between 7.8-9.3, suggesting high-quality, intact DNA with minimal degradation.

The quality of the DNA obtained from each extraction was determined based on the Ct values generated from a real-time PCR reaction. Figure 3 shows the average Ct values obtained on 10-fold and 100-fold dilutions of the purified DNA using human-specific primers. The Ct values across all the dilutions indicate positive amplification and correspond well with the yields shown in Figure 1. Typically, Ct values of samples whose concentration differs by a factor of 10 are 3.3 cycles apart. The average ΔCt value between the 100-fold and 10-fold was ~3.5 indicating no inhibition.

Conclusions

Our results demonstrate that DNA obtained from saliva samples collected and stored in Biomatrica's Salivagard® HT DNA tubes is of high-quality and integrity. Omega Bio-tek has developed a rapid, reliable, fully automated solution by translating their chemistry onto the Hamilton's Microlab® STAR™ platform that can extract 96 samples in less than 90 minutes. Biomatrica's Salivagard® HT DNA tubes together with the Omega/Hamilton protocol provide a complete workflow solution from sample collection and storage through processing and purification that is user-friendly, simple and fast.

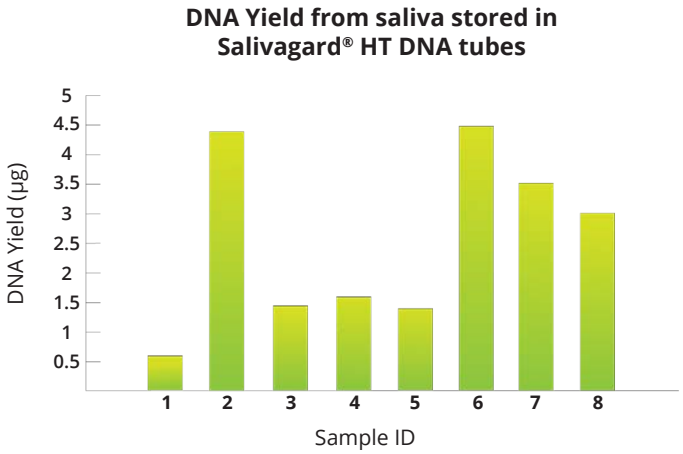

Figure 1. DNA was extracted from 250 µL of saliva stabilized in Salivagard® HT DNA tubes using Omega Bio-tek's Mag-Bind® Blood & Tissue DNA HDQ 96 Kit (M6399). The eluted DNA was quantified using Promega's QuantiFlour® dsDNA system.

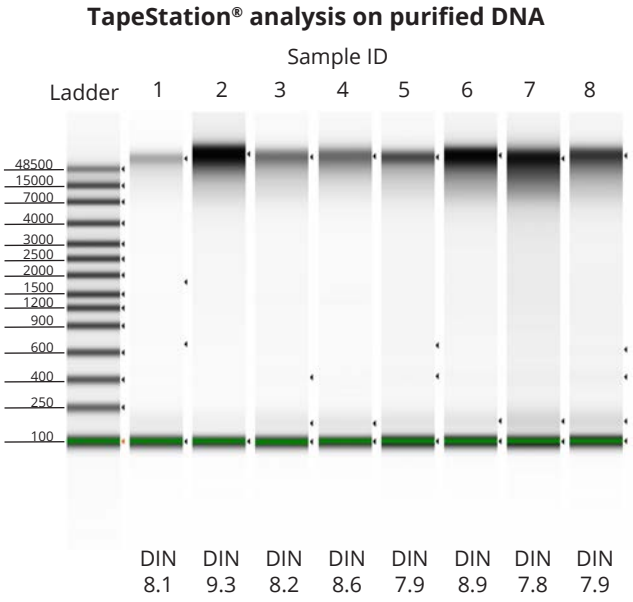

Figure 2. TapeStation® analysis performed on the extracted DNA from 250 µL of saliva stabilized in Salivagard® HT DNA tubes.

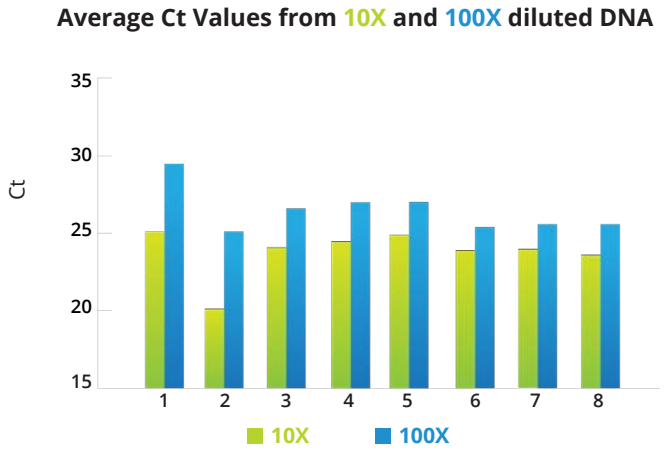

Figure 3. Average Ct values obtained amplifying the purified DNA from stabilized saliva using Omega Bio-tek's kit.

Product Information

Company	Product No.	Description
Biomatrica	21008-048	Salivagard® HT DNA Tubes
Omega Bio-tek	M6399-00	Mag-Bind® Blood & Tissue DNA HDQ 96 Kit (1 x 96 Preps)
	M6399-01	Mag-Bind® Blood & Tissue DNA HDQ 96 Kit (4 x 96 Preps)

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