

High Throughput Workflows for DNA Extractions from Oral Samples

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Introduction

With advances in genomics research and clinical diagnostics, there is a need for rapid, high throughput and cost-effective technologies for purifying high quality DNA from cheek samples. DNA Genotek's ORAcollect (Figure 1) offers a painless, non-invasive method for collection of oral cavity DNA samples. DNA from these samples has been extracted using Omega Bio-tek's Mag-BIND® Blood & Tissue DNA HDQ 96 Kit (M6399). Here, we demonstrate a streamlined workflow for processing up to 96 ORAcollect oral samples in a single run on 2 open-ended automation platforms, Hamilton Microlab® STAR™ and Qiagen BioSprint® 96.



Figure 1. ORAcollect (OC-100) Collection Kit for human oral samples.

Materials & Methods

Cheek swab samples were collected from 8 independent donors into ORAcollect tubes. These tubes come pre-filled with a proprietary bacteriostatic reagent which not only inhibits the growth of bacterial, but also lyses and preserves the sample DNA. The DNA isolation from these samples was then carried out using Mag-BIND® Blood & Tissue DNA HDQ 96 Kit from Omega Bio-tek, skipping the lysis step. The Mag-BIND® HDQ beads provide quick magnetic response and allow for automation. The workflow for DNA extraction using Hamilton and Qiagen platforms is outlined in Figure 2.

The workflow with Qiagen involved a few manual steps (~2) whereas with Hamilton, it was totally hands off. Both the workflows were comparable in terms of overall protocol time (~55-65 minutes). Also, the ORAcollect tubes contain barcodes that can be decoded by most commercially available scanners and can be integrated into the Hamilton workflow, ensuring full sample traceability throughout the process.

DNA yield extracted on Hamilton and Qiagen was quantified using Promega's QuantiFluor® dsDNA system. To test for the integrity of the purified DNA and its direct suitability for downstream applications, real-time PCR was performed on triplicates on undiluted, 10-fold and 100-fold diluted DNA

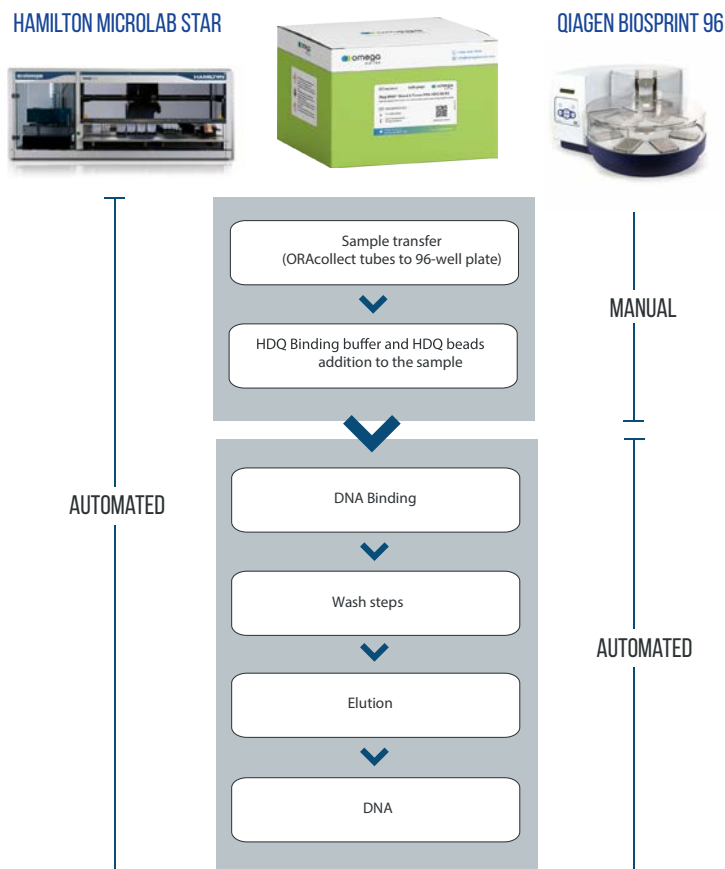


Figure 2. Schematic of the DNA extraction workflow.

extracts. Agilent's Brilliant III 2X SYBR® mix and universal human primers were used following a standard amplification protocol on the ABI 7900.

Results

DNA yield from ORAcollect samples, when extracted on Hamilton Microlab® STAR™ and Qiagen BioSprint® 96 are shown in Figure 3.

C_t values of the undiluted samples extracted on either of the automation platforms are as listed in Table 1 and they indicate positive amplification. There was no detectable fluorescence in the no template control wells. Typically, C_t of the samples whose concentration differs by a factor of 10 are ~3.3 cycles apart. The average ΔC_t between the 10-fold and undiluted samples was lower than 3.3 for both Hamilton and Qiagen, indicative of presence of inhibitors. As expected, the average ΔC_t values at higher dilutions (100-fold and 10-fold) were 3.21 and 3.31 for the same, indicating good PCR efficiency.

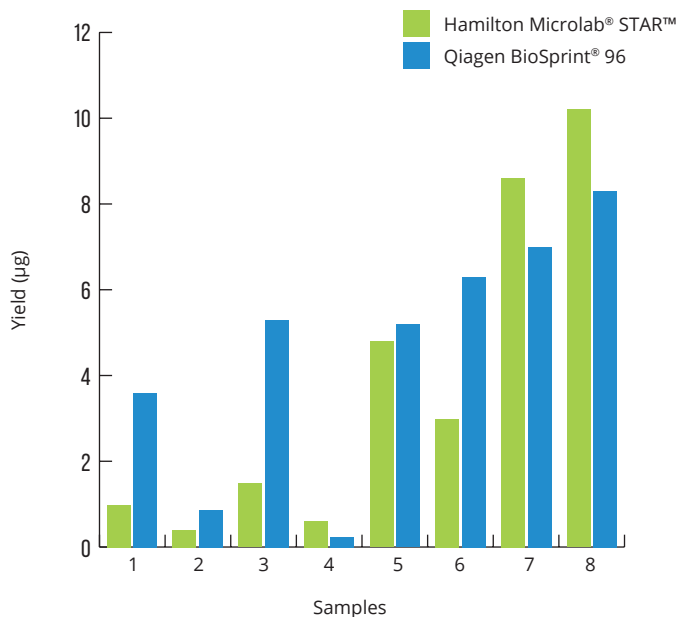


Figure 3. DNA yield.

Table 1. C_i values of undiluted DNA extracts run in triplicate.

Sample	Hamilton Microlab STAR	Qiagen BioSprint 96
1	21.85 ± 0.13	21.25 ± 0.09
2	23.60 ± 0.11	22.84 ± 0.08
3	22.58 ± 0.09	20.57 ± 0.36
4	23.62 ± 0.14	24.30 ± 0.12
5	22.16 ± 0.15	20.53 ± 0.08
6	21.42 ± 0.19	20.37 ± 0.23
7	21.40 ± 0.51	21.05 ± 0.56
8	20.40 ± 0.18	20.08 ± 0.03

Conclusions

A rapid, reliable and high throughput solution for extracting DNA from ORAcollect samples, in conjunction with Omega Bio-tek's extraction chemistry, was ported onto 2 open-ended automation platforms, Hamilton's Microlab® STAR™ and Qiagen's BioSprint® 96. The DNA obtained was of sufficiently high quality and suitable for a variety of downstream applications.

Product Information

Description	Product No.	Preps
Mag-BIND® Blood & Tissue DNA HDQ 96 Kit	M6399-00 M6399-01	1 x 96 4 x 96
DNA Genotek's ORAcollect	OC-100	100



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