Technical Note

Optimizing DNA Yield from Buccal Samples with CollectEject™ Oral Swabs Using Currently Available Extraction Technologies

The purpose of this document is to describe the benefits of using Gentueri's CollectEject™ Oral and Large Oral Swabs for the non-invasive collection of buccal cell samples intended for genetic or forensic testing. Data generated by an independent testing laboratory will demonstrate that large DNA yields can be achieved when CollectEject Oral Swabs are integrated into both common manual and automated nucleic acid extraction procedures.

Gentueri's CollectEject Oral sample collection product comes in two sizes: the CollectEject Oral Swab and the CollectEject Large Oral Swab. Both devices feature an ejectable cellulose swab head, allowing for easy sample handling, eliminating the need to break or cut a swab handle prior to sample processing. These size options allow the user to select the swab head most appropriate for preferred sample extraction procedures. The individually wrapped swabs are treated with ethylene oxide to ensure sterility. Both products are packaged in boxes of 100 or cases of 1,000.

Dimensions

Swab Type	Swab Head Length	Swab Head Width	Swab Head Height
CollectEject Oral	0.63 in or 16 mm	0.07 in or 1.82 mm	0.25 in or 6.25 mm
 *Assembled CollectEject Oral Swab*			
CollectEject Large Oral Swab	0.87 in or 22 mm	0.07 in or 1.82 mm	0.25 in or 6.25 mm
 *Assembled CollectEject Large Oral Swab*			

Test Details/Goals

Testing was performed by Megan Foley of The Center for Forensic Science Research and Education in Willow Grove, PA. Test procedures were not designed to allow comparison of the two extraction methods, but rather to demonstrate that CollectEject Oral Swabs could be successfully used with multiple extraction technologies.

Sample Collections

Buccal cell samples were collected using Gentueri's CollectEject Oral Swabs by rubbing the swab on the inside of an individual's cheek for fifteen seconds. The collected swabs were then stored using a SwabSaver® tube. The desiccant in the SwabSaver promotes rapid drying of the buccal cell samples within a controlled environment. This highlights its ability to: (a) minimize degradation of the nucleic acids and (b) enable greater extraction yields, thereby providing more options for genetic analysis. Desiccating samples were stored at room temperature until extraction.

Extraction Methods

Buccal cell samples collected with Gentueri's CollectEject Oral Swabs were extracted using one of two extraction systems.

1. Applied Biosystems® PrepFiler *Express*™ Forensic DNA Extraction Kit releases and lyses the buccal cells on the CollectEject swab head. Duration and speed of agitation during the lysis step were varied during the testing. The resulting lysates were extracted with the Applied Biosystems Automate Express Forensic DNA Extraction System.
2. The Maxwell® RSC Buccal Swab DNA Kit releases and lyses the buccal cells on the CollectEject swab head. The temperature and duration of the lysis step were varied during the testing. Extractions with the Maxwell kit were completed per the kit protocol without the use of an automated liquid handling system.

For the first method, buccal cell release and lysis were performed using the PrepFiler *Express* Forensic DNA Extraction Kit (Applied Biosystems, Waltham, MA). Each CollectEject swab head was ejected into 500µL of the manufacturer-recommended extraction mastermix in the spin basket of a SwabSaver. The lysis incubation was performed on an Eppendorf Thermomixer® F2.0 (Eppendorf, Hamburg, Germany) with agitation at various speeds and durations (constant temperature of 70C). Sample tubes were centrifuged for 2 minutes at room temperature and 13,000 RPM to gather the condensate and separate out the swab head.

Extraction Methods, Continued

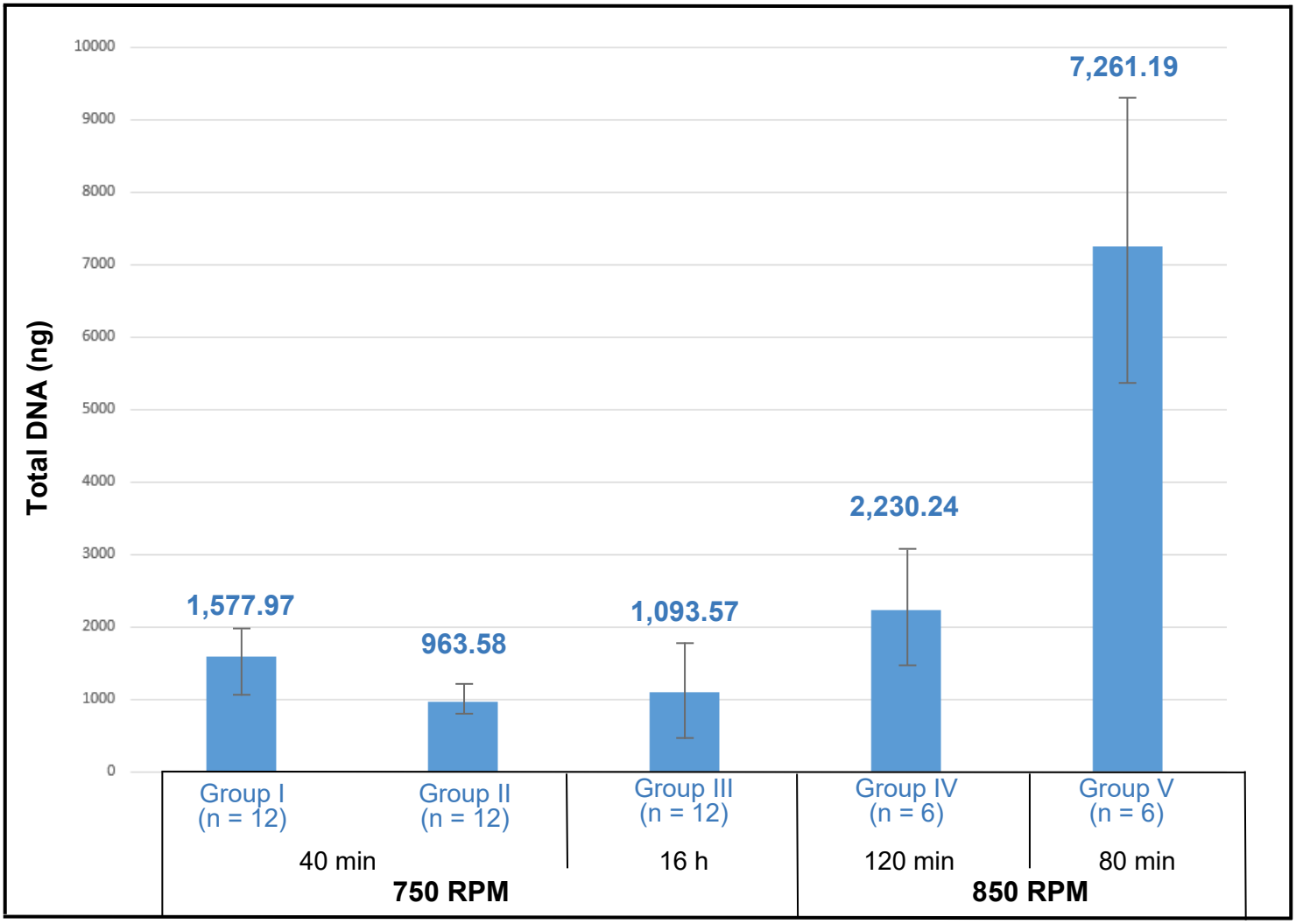
Following the centrifugation step, the spin basket holding the swab head was removed and the flow-through was transferred into a hinge-less PrepFiler™ sample tube. The lysates were then extracted on the Automate Express™ Forensic DNA Extraction System. Manufacturers' guidelines were followed for instrument set-up. An elution volume of 250 µL was selected. Extracted samples were stored at -15°C. Quantitation was performed using real-time PCR utilizing the Quantifiler™ Trio DNA Quantification kit (Applied Biosystems) on the Applied Biosystems™ 7500 Real-Time PCR System.

For the second method, buccal cell samples were extracted using the Maxwell® RSC Buccal Swab DNA Kit (Promega, Madison, WI). Each CollectEject swab head was ejected into 330 µL of the manufacturer-recommended master mix in the spin basket of a SwabSaver® and subsequently lysed at one of sixteen different duration and temperature combinations. The lysis step was performed using the Fisherbrand™ Isotemp™ Heating block (Thermo Fisher Scientific, Waltham, MA). Extraction of the lysed samples was completed per Maxwell kit protocol without the use of an automated liquid handler. Final elutions were performed in 50 µL. Extracted samples were stored at -20°C. DNA concentration (ng/µL) and DNA purity (A260/A230, A260/A280) were determined by sampling each Maxwell extraction with the DenoVix® DS-IIFX Nanodrop system (Wilmington, DE).

Data

Yield data for the samples extracted using the PrepFiler *Express*™ Extraction system are shown in Table 1 on page 4. Yield ranges, yield averages, and the number of samples/group are displayed for each of the five combinations of lysis time and agitation speed.

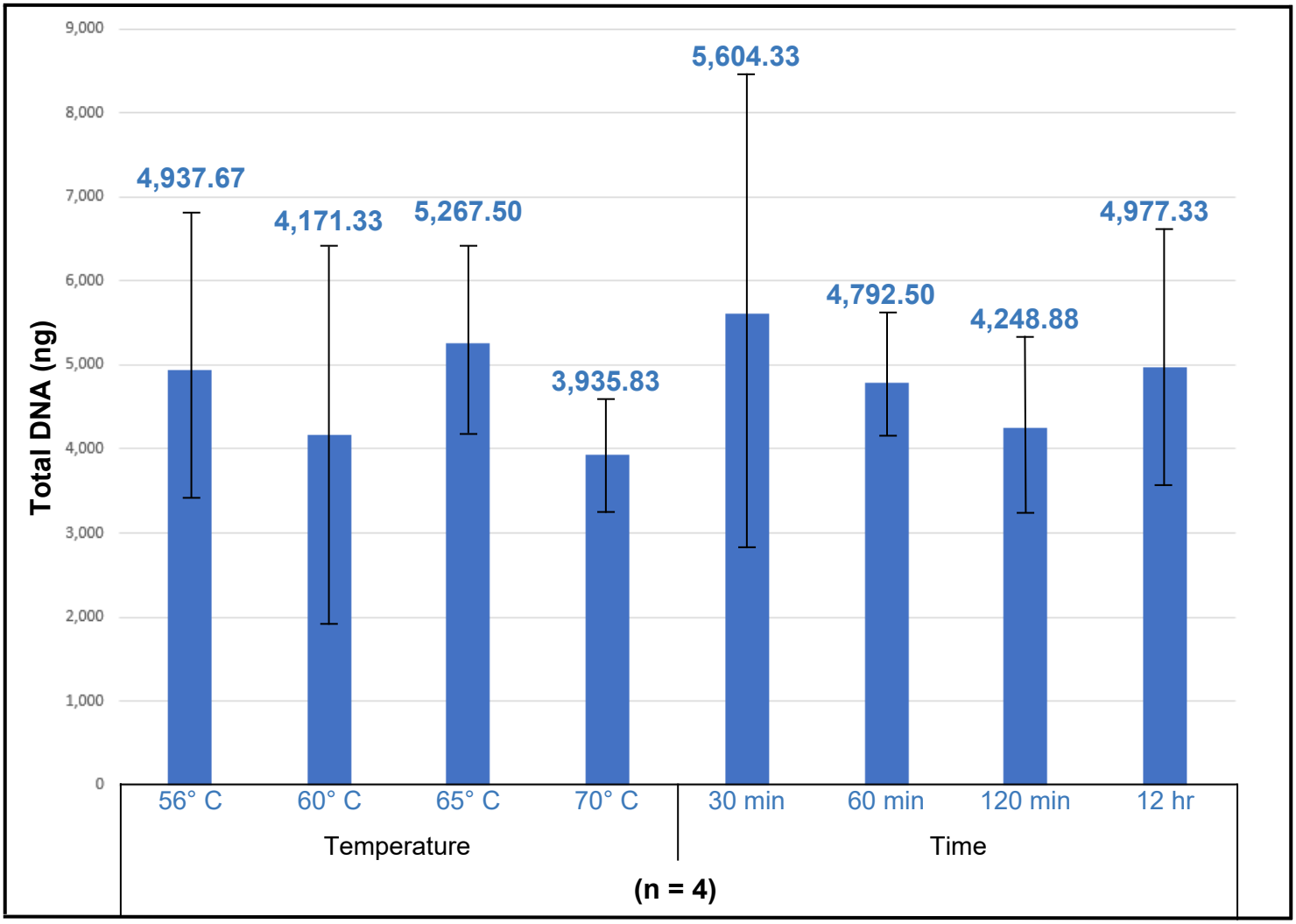
Table 1: PrepFiler *Express*™ Extraction



Data

Yield data for the samples extracted using the Maxwell® RSC Buccal Swab DNA Kit are shown in Table 2 on page 5. Yield ranges and averages are displayed for each of the eight combinations of lysis time and lysis temperature.

Table 2: Maxwell® RSC buccal Swab DNA Kit



Conclusion

Data generated with PrepFiler *Express*™ DNA Extraction system shows how optimization of a single extraction step (in this case, the duration of the lysis step) can allow the CollectEject™ Oral Swab to be successfully integrated into an otherwise low yielding automated extraction method. Lysis incubation parameters of 70°C at 850 RPM for 80 minutes lead to yields that would be suitable for most downstream applications. The successful amplification-based quantitation of the PrepFiler extracts suggests the possibilities of successful downstream genetic testing.

The Maxwell® RSC Buccal Swab DNA kit extract yields are much more uniform across all lysis parameters tested. A lysis step of 65°C for 30 minutes leads to the highest yield average, but all parameters tested with the CollectEject Oral Swab yield nucleic acid amounts suitable for most downstream testing.

Future iterations of the CollectEject Oral Swab will include packaging that allows desiccation and room temperature storage of buccal cell samples without the need for additional equipment.

The Gentueri CollectEject Oral Swab was designed to capture the advantage of non-invasive buccal cell collection for genetic analysis. The data shown here demonstrate the end user's option to combine that advantage with the time and cost savings associated with current manual and automated nucleic acid extraction systems, allowing for increased testing opportunities and throughput rates.