



## Performance of CollectEject™ Oral Swab

### Introduction

Self-administered oral sample collections performed remotely can overcome challenges with onsite, invasive methods that are not convenient for the patient. Gentueri Inc. offers an inexpensive and user-friendly at-home sample collection kit to provide a solution to these challenges. The kit features the CollectEject Oral Swab designed to fit seamlessly into downstream processing while still providing ample DNA yield and excellent purity.

Suboptimal DNA extraction conditions could have negative effects on DNA yield and purity, leading to failures in downstream analytical methods such as q-PCR, Next Gen Sequencing, and microarrays.

This study aims to prove the Maxwell® RSC 48 Instrument used in conjunction with the Maxwell® RSC Buccal Swab DNA Kit is an effective method to extract a high yield of excellent-quality DNA with adequate molecular weight from buccal cells with the CollectEject Oral Swab.

### Materials

CollectEject Oral Swab	Elution Buffer
GenDry™ CE Pouch	Maxwell RSC Plungers
Promega Maxwell RSC 48 Instrument	Microcentrifuge
Promega Maxwell RSC Buccal Swab DNA Kit	Pipettors and pipette tips
Maxwell RSC Cartridges	Incubation Tubes (1.5ml)
Proteinase K Solution	Heating block (56°C)
Lysis Buffer	Parafilm® “M” Laboratory Film
Elution Tubes (0.5ml)	Nanodrop™ UV/Vis Spectrophotometer
Clearing Columns	(Thermo® Scientific)

### Methods

To determine average DNA yield, purity, and molecular weight from the CollectEject Swab, a DNA extraction protocol was conducted in-house, and the eluate was analyzed by the UW-Madison Next Generation Sequencing Core. DNA extractions utilized the Promega Maxwell RSC 48 Instrument and were performed as written in the *Promega Maxwell RSC Buccal Swab DNA Kit* Protocol.

All participants followed the Gentueri Standard Sample Collection protocol described below. As per standard collection protocol, participants waited 30 minutes after eating or drinking before collecting a sample.

The wrapper containing the CollectEject Oral Swab was opened until the indicated stop mark is reached. The CollectEject Oral Swab was removed from the wrapper carefully, ensuring to not press the green plunger or touch the swab head. The wrapper was not discarded. To begin the sample collection, the swab was placed under the tongue for 20 seconds. The swab head was then rubbed against the inside of both cheeks for 20 seconds each. The total swab collection duration was one minute. After collection was completed, the CollectEject swab was reinserted headfirst into the original wrapper, ensuring the swab head was secured in the non-peeled section of the package. The wrapper now containing the sample was inserted into the GenDry™ CE Pouch with the desiccant and resealed. The desiccant was not removed and the GenDry™ Pouches were completely sealed to allow for proper desiccation. The samples were



labeled, stored, and allowed a minimum of 24 hours to desiccate before the extraction was performed. Prior to the DNA extraction, the samples must be properly prepared to lyse the buccal cells according to the protocol described below.

To begin, 300 $\mu$ l of Lysis Buffer + 30 $\mu$ l of Proteinase K (PK) were mixed in a 1.5ml microtube for each sample processed for DNA extraction. One Clearing Column was placed into a 1.5 ml microtube for each sample. Each sample was carefully removed from the wrapper and one swab head was ejected into each Clearing Column. The 330 $\mu$ l solution of Lysis Buffer and Proteinase K was pipetted on to the swab head and the microtube was covered tightly with parafilm to prevent evaporation during incubation. All samples were incubated at 56°C for 20 minutes and then spun in the microcentrifuge at max speed (16,873 rcf) for two minutes. The microtubes were removed from the microcentrifuge and the Clearing Column and swab head were discarded.

To extract the DNA from each sample, the samples must be properly loaded into the Promega Maxwell RSC 48 Instrument.

One Maxwell RSC Cartridge was placed into the deck tray for each sample. The flow through from the microtube was pipetted into Well #1 and a Maxwell RSC Plunger was placed into Well #8 of each cartridge. 50 $\mu$ l of Elution Buffer was pipetted into a 0.5ml PCR tube and placed into the deck tray in the designated location. The Maxwell RSC 48 Instrument completed the DNA extraction and the 0.5ml PCR tubes containing the eluate were removed from the deck tray and stored at 4°C.

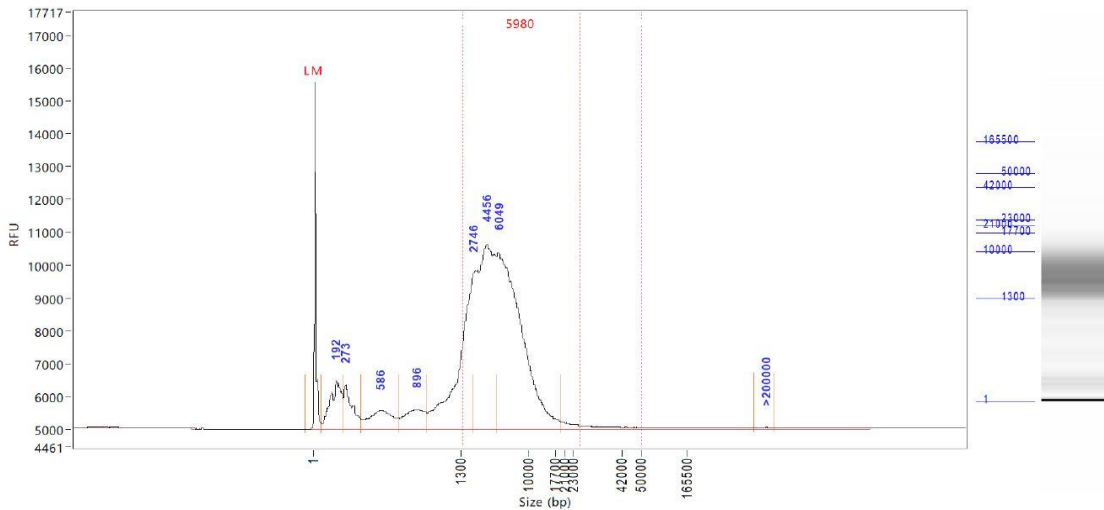
The extracted DNA was sent to the UW-Madison Next Gen Sequencing Core for analysis.

## Results

To assess the effectiveness of the Maxwell® DNA Extraction method on the CollectEject Oral Swab, the DNA extraction protocol and subsequent data analysis were conducted with the results described in Table 1. The electropherogram (Figure 1) shows significant peaks at 4.46 and 6.05 kb.

**Table 1. Average DNA Yield, Purity, and Size of Extracted DNA**

Average DNA Yield (ng/ $\mu$ l)	Average DNA Yield ( $\mu$ g)	Average Purity (A260/280)	Average Size (kb)
91.06 $\pm$ 26.06	4.55 $\pm$ 1.30	1.809 $\pm$ 0.044	4 - 6



**Figure 1. Electropherogram of DNA.** Representative graph generated by the FEMPTO Pulse.

## Discussion

Sample collection was simple, painless and can be performed at-home without the need for trained medical personnel. By following the sample collection protocol described above, ample biological material is obtained for downstream DNA analysis.

The results described above with the Gentueri CollectEject Oral Swabs prove adequate amounts of DNA, purity levels, and adequate molecular weight for downstream processes.

We observed variable DNA yields from buccal cells collected from multiples subjects, as expected from previous studies (unpublished data).

We recommend lab personnel follow sample collection protocols and the cell lysis and DNA extraction procedures described above to obtain consistent microgram amounts of DNA and high purity levels. This study utilized the Maxwell RSC 48 instrument and the Maxwell 48 Buccal Swab DNA Extraction kit, but similar DNA extraction kits should provide similar results.

## Conclusion

This work demonstrates a simple and effective buccal cell collection protocol and a cell lysis/DNA extraction procedure to provide approximately 5µg DNA, purity ratios of approximately 1.8 (A260/280), and an average size of 4-6 kb. We conclude that the CollectEject Oral Swab device is suitable for collection of oral/buccal samples providing sufficient DNA yields and good purity for downstream analyses such as Next Gen Sequencing, qPCR, and microarrays.

Additional studies are planned with other manufacturers' DNA extraction kits.

## Acknowledgements

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