International Congress Series 1261 (2004) 550–552

www.ics-elsevier.com

Collecting and processing buccal cell samples

James W. Schumm*, Eun Young Song,
Martha Burger, Jangbir Sangha

The Bode Technology Group, Research and Development, 7364 Steel Mill Road, Springfield, VA 22150, USA

Abstract. Recently, we developed a new device and method of buccal sample collection. This device supports efficient sample collection, accessioning, and preparation for DNA amplification. The collection process is very simple and an individual can be trained in its effective use in just a few moments. New extraction methods were developed for use with the collector to provide rapid preparation of samples for amplification.

Keywords: Buccal; Sample; Forensic; Product; Short tandem repeat; Validation

1. Introduction

There is a vital need for a reliable, noninvasive, and efficient method of DNA sample collection that feeds directly into automated downstream processes. Blood collection and deposition onto membranes currently provides an reliable automation-compatible collection method, but this approach is invasive and expensive, requiring trained personnel. Buccal cell collection with cotton or Dacron swabs retains sufficient sample while providing a less expensive and less invasive alternative. However, current buccal cell collection methods either require a secondary transfer of collected cells to a flat membrane with concordant risk of material loss or are not readily adapted either to automated tracking or automated extraction. We describe a new device to overcome these shortcomings employing direct collection of buccal samples for automation-compatible analysis and long-term storage.

2. Materials and methods

The Buccal DNA Collector, Bode**X**tract DNA Extraction Reagent, and Bode**E**lute DNA Extraction Reagent were obtained from The Bode Technology Group (TBTG) (Springfield, VA) and used as recommended. Amplification was performed using Profiler Plus, COfiler (Applied Biosystems, Foster City, CA), or PowerPlex 16 System

* Corresponding author. Tel.: +1-703-644-1200x303; fax: +1-703-644-2319.
E-mail address: james.schumm@bodetech.com (J.W. Schumm).

0531-5131/
doi:10.1016/S0531-5131(03)01485-7

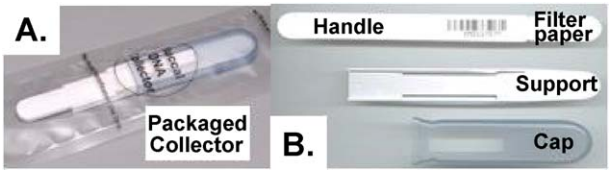

Fig. 1. Assembled and packaged Bode DNA Buccal Collector (A) and separated components (B).

(Promega) as described. Amplification products were separated and detected using the 3100 Genetic Analyzer and analyzed with GeneScan and Genotyper (Applied Biosystems).

3. Results and discussion

We designed, developed, manufactured, characterized, and validated a new device and accompanying protocols for rapid and simple buccal cell collection (Fig. 1). The collector is assembled, packaged, and sterilized for transport prior to use (Fig. 1A). It consists of three component parts: (1) a plastic handle holding a piece of filter paper, (2) a plastic support piece, and (3) a cap (Fig. 1B). The support snaps onto the handle to provide a firm support behind the filter paper during sample collection. It can be readily removed for sample analysis and storage.

To use the collector, one places a thumb on the back of it, puts the filter paper firmly against the inside of the cheek, and swipes with pressure against the cheek while pulling the device out of the mouth. This is repeated several times. Then the cap is placed over the filter paper to keep it clean during drying or transport (Fig. 2).

We have learned the following useful information after performing experiments to characterize and validate the collection process: (a) All positions on the collector collect enough sample material to provide reliable profiles. However, DNA yields are approximately twice as much near the tip of the paper versus near the handle. (b) Samples collected and stored in paper envelopes at room temperature for 12 months provided full profiles in 100% of cases (52 of 52) with allele peak heights similar to those in the original test profiles. (c) The number of cells collected is several-fold fewer than with blood collection. (d) Different individuals vary significantly in cell deposition generally yielding between 15- and 300-ng DNA from a 6-mm punch extracted with BodeElute. (e) Two- to threefold variation in DNA yield was observed from the same person collected on 4 consecutive days. (f) Using either two or eight swipes of the cheek

Fig. 2. Summarized instructions for use of Bode DNA Buccal Collector.

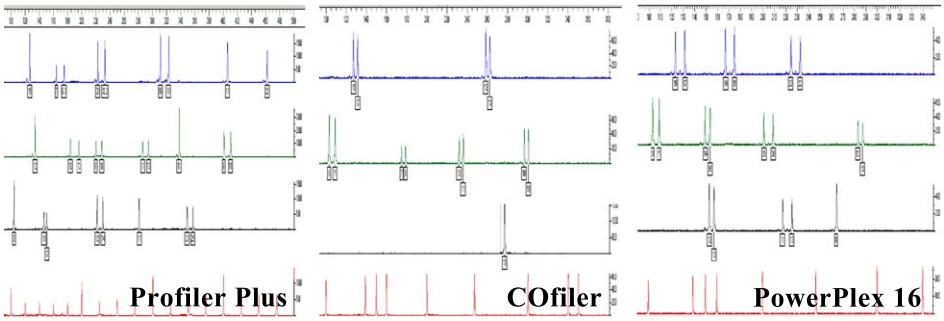

Fig. 3. Commercial kits were used to amplify punches from the Bode Buccal DNA Collector prepared using Bode**X**tract DNA Extraction Reagent.

generated full profiles. However, the DNA yield from two swipes was, on average, one-half to two-thirds of the yield of eight swipes; (g) Five consecutive collections of eight swipes each from the same cheek all yielded full profiles. However, the second and subsequent collections averaged yield of about one-third to two-thirds of the initial collection.

To simplify the extraction of DNA from the Buccal DNA Collector, two extraction reagents have been prepared. The Bode**X**tract DNA Extraction Reagent is used in preparation of small punches from the Buccal DNA Collector that are to be used directly in an amplification reaction. Protocols for preparation of one 3-mm punch or alternatively two 1.2-mm punches have been developed. The Bode**E**lute DNA Extraction Reagent is a one-step process that removes DNA from a 6-mm punch from the Collector. The DNA contained within the approximately 40-μl elution volume is immediately available for amplification without additional processing. Both extraction methods require minimal hands-on processing time, are compatible with tube or microplate format, and neither requires instrumentation beyond a plate heater.

Following collection and DNA extraction, samples prepared with Bode**X**tract were amplified with Profiler Plus and COfiler (Applied Biosystems) and with PowerPlex 16, PowerPlex 1.1, and PowerPlex 2.1 (Promega). All systems provided complete profiles. Examples of three of these multiplex systems amplified from punches prepared with Bode**X**tract are displayed in Fig. 3.

4. Conclusions

We have designed, developed, manufactured, and characterized a Buccal DNA Collection device for collection, processing, and storage of buccal cell samples. Training in collection and use of the collection device is extremely simple and reliable. Two DNA extraction reagents have been formulated for use with the Collector to allow amplification of processed punches either directly or following elution into an amplification-compatible reagent solution. These materials and methods provide convenient approaches to both large and small reference sample and databanking analysis requirements.