New DNA Extraction Methods for Casework Evidence

Martha F. Burger, Todd W. Bille, and James W. Schumm
The Bode Technology Group, Springfield, VA 22150 USA

ABSTRACT

To ease the national backlog of crime scene evidence requiring DNA analysis, we have adopted methods that support high throughput processing of casework samples. In recent years, we have developed a new device for simplified reference sample collection, new protocols for a rapid pre-screen for Y chromosome DNA, and a microplate-compatible human-specific quantification method (Fox et al., 2003). Here we describe development of two new technologies to improve critical DNA extraction processes in casework analysis.

First, we have developed a companion reagent and method for the buccal cell collection device described two years ago at this meeting. In one 30-minute incubation step, treatment of a filter paper punch from the device with this new reagent releases enough DNA to perform dozens of amplification reactions. The ability to quantify the eluted DNA prior to amplification supports DNA concentration adjustments that produce a high frequency of reliable profiles. This overcomes a deficiency of other methods that lack the quantification step, often generating profiles that are too strong or too weak to interpret. This difference is especially advantageous when working with saliva extracts.

Second, we describe a novel differential extraction method that utilizes principles of preferential lysis (Gill et al. 1985), physical separation (Yoshida et al., 1995), and employs a filtered microplate to separate epithelial and sperm DNA fractions. This method takes advantage of size and shape differences between epithelial and sperm cells. The epithelial cells are preferentially lysed and the released DNA flows through a 96-well filter during centrifugation. The filter has a pore size that allows for the physical, not chemical or ionic, separation of the epithelial DNA from the larger intact sperm cells. Following cleansing washes, the remaining sperm cells are lysed and the DNA released into a separate microplate. Clean separation and effective recovery of both epithelial and sperm fractions in the 96-well format make the method suitable for automated or manual handling of many samples.

Incorporation of these methods into the forensic laboratory maintains high quality while allowing more samples to be analyzed by the same personnel in the same work space without major capital investment in robotic equipment. Thus, the costs of eliminating the casework backlog are lowered.

INTRODUCTION AND BACKGROUND

It has recently been reported that as many as 542,000 crime scene samples are awaiting analysis in the United States (Smith Alling Lane, 2004). Of these, 169,000 samples are from rape victims (Smith Alling Lane, 2004.). In this article, we describe two important tools to support elimination of this backlog.

The first is a combination of a device and reagent to enable rapid collection and DNA extraction of reference samples to be obtained from crime victims, convicted offenders, arrestees, and suspects. The Bode Buccal DNA Collector was developed two years ago (Fox et al., 2002) and is a rapid and reliable method that requires minimal training for use (Figure 1; Schumm et al., 2004). Furthermore, it has now been shown that DNA samples stored in simple paper envelopes at room temperature allow generation of full profiles years later (Burger et al, submitted).

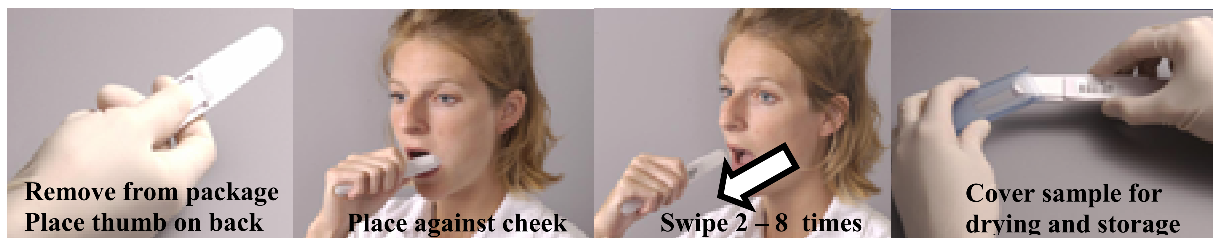

Figure 1. Summarized instructions for use of the Bode Buccal DNA Collector.

Here we describe the development of a new companion product that supports one-step elution of DNA from Collector samples into Bode*Elute*™ DNA Elution Buffer, in a format ready for amplification. Ninety-six samples can be extracted in 35 minutes with only 5 minutes of analyst attention required. DNA elution into liquid provides a means to quantify the retrieved DNA, an important aspect of work with the highly variable yields of buccal samples.

Historically, cotton swabs have been used for numerous sample collections. Bode*Elute* Buffer has also been shown to release DNA effectively and efficiently from this substrate, providing a convenient method for cotton swab DNA extraction.

The second tool described in this work is still under development. This is a 96-well compatible format for performing differential extraction of epithelial and sperm fractions of rape victim samples. It relies in large part upon the traditional differential lysis processes of Gill et al. (1985) and centrifugation of Yoshida et al. (1995). However, the identification of a filtered microtiter plate that supports retention of the sperm cells following the differential lysis of epithelial cells and subsequent centrifugation step provides a novel approach to separation.

REFERENCE SAMPLE COLLECTION AND PREPARATION

Anyone who works with buccal cell collection learns quickly that the number of cells collected from one individual to another varies widely. We recently collected samples from 40 individuals who exhibited almost a 10-fold variation in DNA yields (Figure 2A). Furthermore, two samples taken consecutively from different cheeks from the same individual displayed up to a 5-fold difference (Figure 2B).

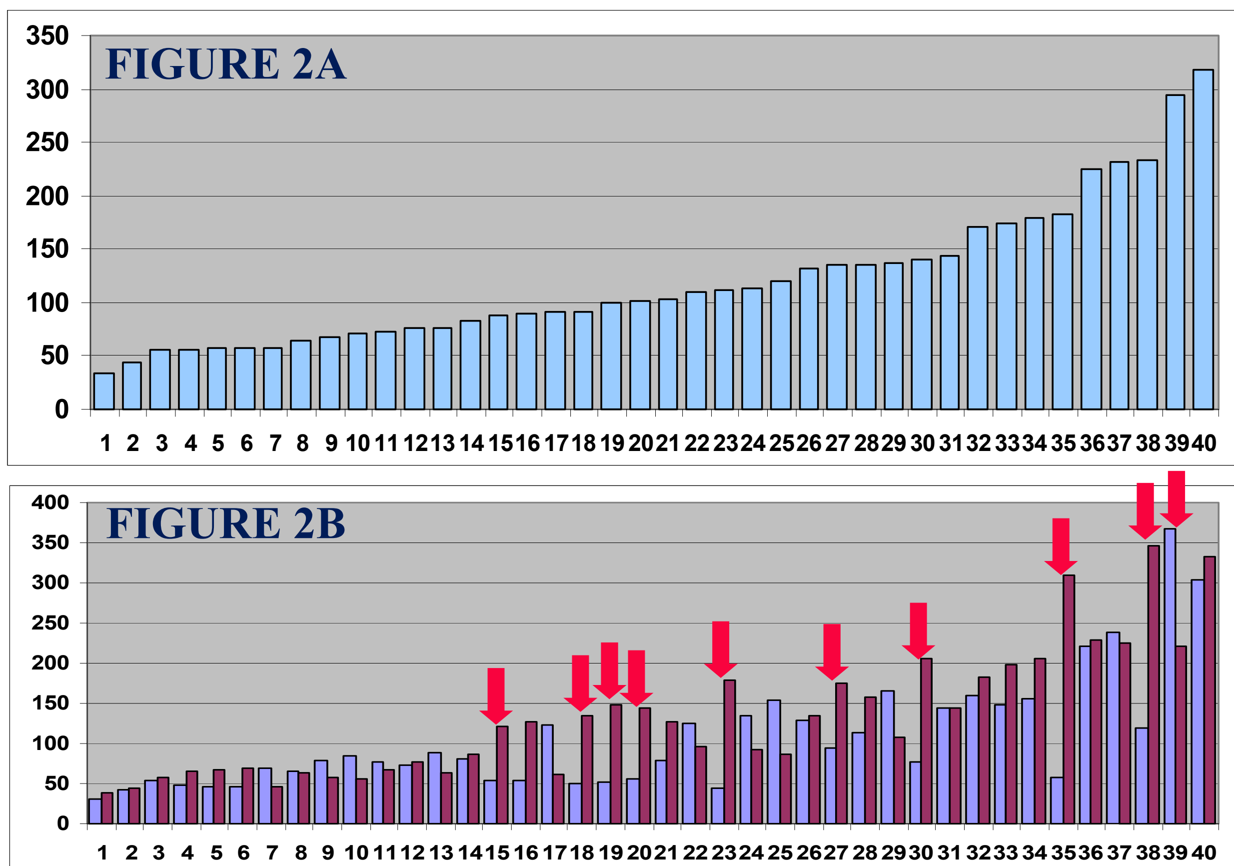

Figure 2. DNA samples were collected from 40 different individuals (numbered 1 to 40). The yield for each individual, in total nanograms DNA from a ¼" punch, is measured on the Y-axis. Figure 2A displays the average of two collections taken from opposing cheeks of each individual. Figure 2B displays each collection from each cheek separately. Arrows indicate significant differences in the yield observed from the two cheeks of one individual.

With some collection methods, yield variation is compounded by the fact that the sample is first collected onto a swab and then transferred to a flat format that is more convenient for sample handling and long term storage. This approach not only leads to even more variability in the sample collection process, but also cuts the amount of sample obtained due to loss of material in the transfer process itself.

One way to manage all this variability in sample collection efficiency is to elute the sample into solution and perform subsequent DNA quantification. The use of Bode***Elute*** DNA Elution Buffer (The Bode Technology Group, Springfield, VA) makes this a rapid and simple process. First, the analyst prepares a one-quarter inch punch from each collected sample and places it into a microtiter plate well. This step can be performed either manually or with an automated punch instrument that tracks the bar code identification on the Collector handle and creates a printout of the location of each sample within the plate.

Then 100 µl Bode***Elute*** Buffer is added to each well. The plate is covered with a semi-permeable membrane (provided in the kit) to limit sample evaporation, and the sample is heated to 55ºC on a heat block for 30 minutes. The eluted DNA is now present in the Bode***Elute*** Buffer (generally about 50 µl at this point) and available for either quantification or multiplex STR amplification. Yields from this procedure range from 20 ng to 532 ng with a median yield of 96 ng, that is, plenty of material to perform as many amplification reactions as necessary (Table 1).

190 Samples	**Minimum**	**Maximum**	**Average**	**Median**
Concentration	**0.5 ng/ml**	**9.5 ng/ml**	**2.2 ± 1.6 ng/ml**	**1.8 ng/ml**
Total DNA	**20.0 ng**	**532.0 ng**	**120.3 ± 86.6 ng**	**95.7 ng**

Table 1. Characteristic DNA concentrations and yields determined from 190 samples following Bode***Elute*** processing of a ¼" punch from the Bode Buccal DNA Collector.

There are several advantages to this approach versus other popular methods. First, only about 5 minutes of analyst time is required to extract a plate of 96 samples. By comparison, FTA® Purification Reagent extraction (Brentford, West London, United Kingdom) to prepare DNA on sample punches can take 45 minutes of analyst time. At the same time the Bode***Elute*** procedure requires preparation of only one sample to set up for two (or more) multiplex amplifications, while the FTA procedure requires a separate extraction for each multiplex. Furthermore, if a first sample amplification is too strong or too weak, the Bode***Elute*** extract can be adjusted accordingly while the on-punch method requires a new extraction.

Most important of all, the elution of the sample DNA into liquid during the Bode***Elute*** extraction provides material for quantification. This, in turn, allows introduction of precise and desired amounts of DNA in downstream amplification reactions leading to a higher percentage of successful amplifications and substantially limiting the need for sample re-runs. Table 2 indicates the high success rates with each of the popular commercial multiplex systems.

Multiplex Kit	**Complete Profiles**	**Complete Profiles**
	First Attempt	**Repeated**
PowerPlex 16	97%	100%
Profiler Plus	97%	100%
COfiler	92%	100%
Identifiler	91%	97%

Table 2. Multiplex amplification success rates for full profiling with commonly employed STR multiplex kits, in first attempts and repeated attempts from a single extraction per sample.

One caution to keep in mind when using this procedure is that the Bode***Elute*** Buffer itself contains amplification inhibitors (Burger et al., submitted). Therefore, we recommend that the addition of eluted reagent not exceed 10% of

the volume of any amplification reaction. This only rarely limits options to proceed because DNA yields are typically 1 to 3 ng/µl with the Bode*Elute* Buffer.

Excellent DNA profiles are commonly generated using the Bode*Elute* Buffer for extraction. Figure 3 displays examples of profiles generated from material eluted from either Bode Buccal DNA Collectors or from cotton swabs. In either case, complete profiles are easy to obtain.

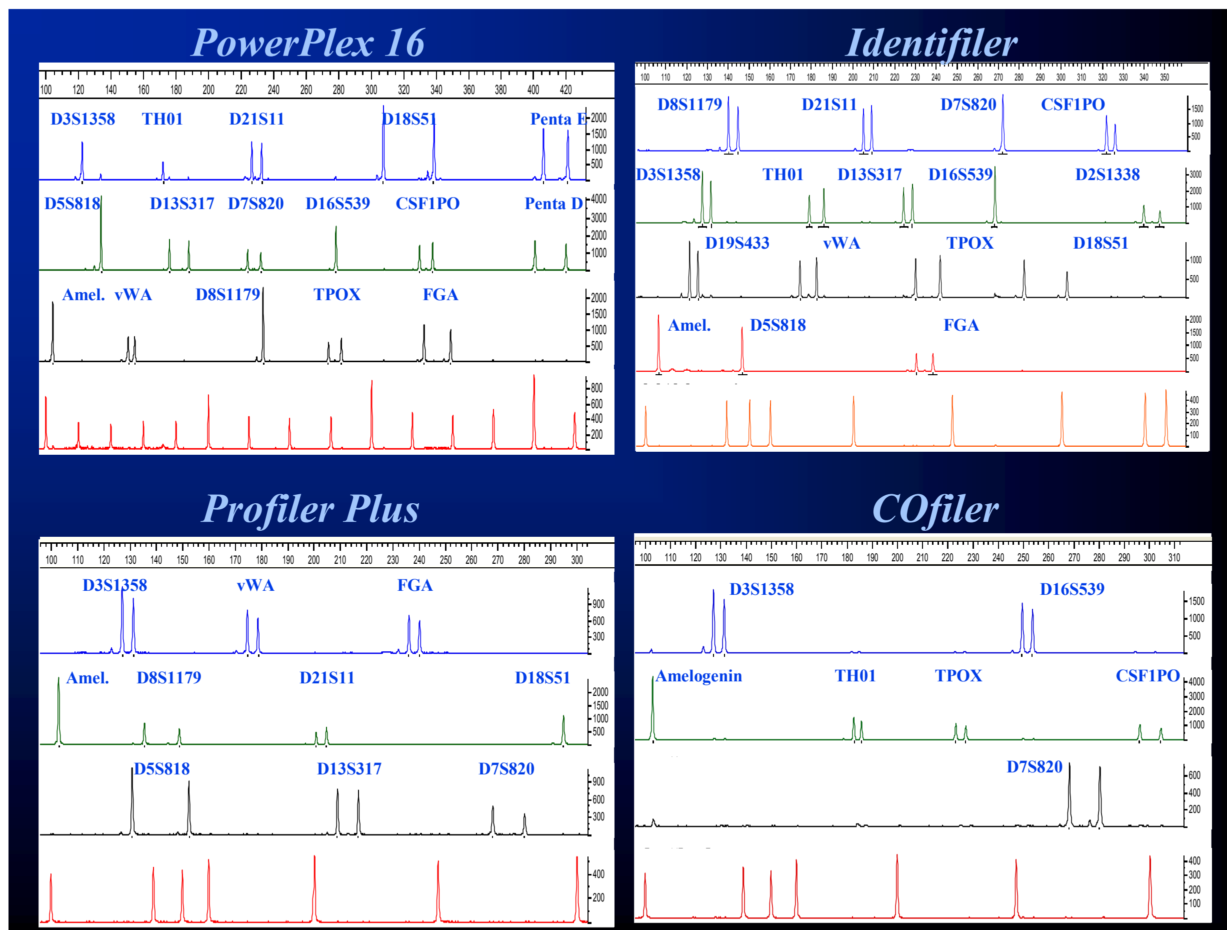

Figure 3. Profiling results observed following Bode***Elute*** DNA Elution Buffer extraction. The PowerPlex 16® (Promega, Madison, WI) and Identifiler™ (Applied Biosystems, Foster City, CA) amplification results were obtained following elution from Bode Buccal DNA Collector samples. The Profiler Plus® and COfiler® (Applied Biosystems) results were obtained following elution from cotton swabs (using 150 µl instead of 100 µl buffer to account for the increased absorbance of this sample type).

The availability of the Bode Buccal DNA Collector has already improved simplicity and effectiveness of taking and processing DNA samples. This is especially true with regard to remote site collection such as arrestee sample collection or in some convicted offender site collections. The introduction of a reagent designed to simplify handling and preparation of Bode Buccal DNA Collectors and cotton swabs for multiplex amplification will greatly assist the processing of these samples.

96-WELL DIFFERENTIAL EXTRACTION METHOD

Commonly used methods for differential extraction of rape sample materials employ the method of Gill et al. (1985) that includes a method preferentially lysing epithelial cells to separate them from more resistant sperm cells. However, cross-contamination between the two fractions occurs with this method. In 1995, Yoshida et al. employed centrifugation after the epithelial cell lysis step and prior to sperm cell lysis to improve separation.

Here we describe an alternative method more closely related to that recently described by Garvin (2003). We take advantage of size and shape differences between epithelial and sperm cells. The epithelial cells are preferentially lysed in a 96-well filter microplate with pores large enough to let DNA pass, but small enough to retain sperm cells. DNA from the lysed epithelial cells flows through the filters during a centrifugation step while sperm cells are retained. Following filter washes, a harsher lysis solution (containing DTT) releases DNA from the sperm. Another centrifugation causes the sperm DNA to flow through into a separate collection plate. Following separation, DNA from each fraction is purified using standard extraction protocols with the DNA IQ™ System (Promega Corporation, Madison, WI).

Using mock rape kit samples, we analyzed the extraction of oral swabs from female contributors carrying varying known concentrations of male sperm samples. Figure 4 displays the Profiler Plus profile for the respective sperm and epithelial fractions from a representative set of oral swabs containing 20 µl of 1:4, 1:16, and 1:64 dilutions of sperm sample. Excellent separation of the two fractions is observed. Results are comparable to separations performed with the more traditional "epithelial lysis-centrifugation in tube-wash-sperm lysis-extraction of both fractions" using the Gill et al. and Yoshida et al. approaches described above (data not shown).

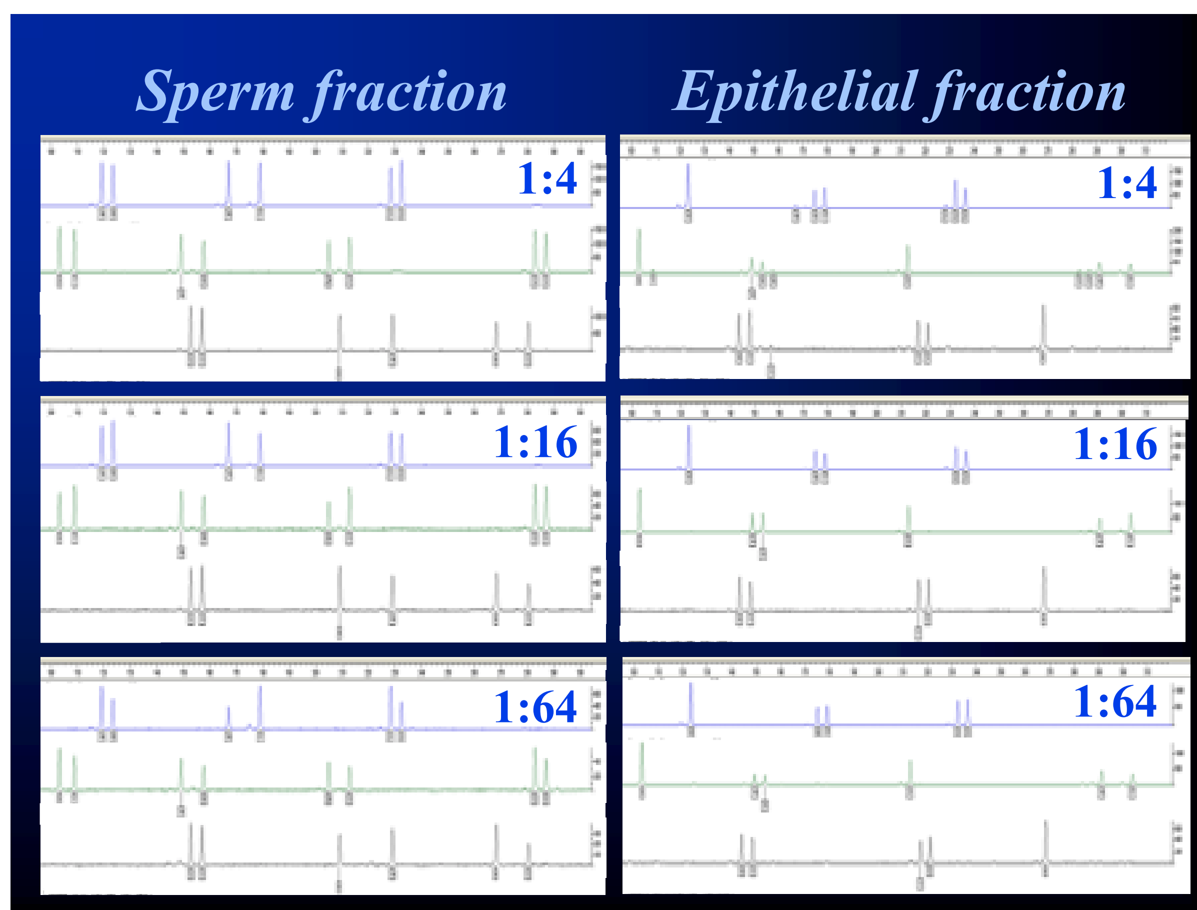

Figure 4. Oral swabs containing 20 µl of 1:4, 1:16, and 1:64 dilution of a sperm sample preparation were extracted as described in the text. Following quantification and adjustment of the samples to 1 ng template, samples were amplified with Profiler Plus reagents. A representative set of profiles is shown here. An additional swab containing no semen was tested and displayed no cross-contaminating female DNA in the male fraction (data not shown).

The advantages of this approach are substantial. The method significantly reduces sample manipulation, there is no tube labeling required, it may offer greater sensitivity, and there is substantially less hands-on time for the analyst.

ACKNOWLEDGMENTS

The authors wish to thank Eun Young Song and Robyn Wingrove for their constructive assistance in the development of the methods described here and Mike Cariola and Courtney McCoppin for methods evaluation once developed.

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