JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, May 2010, Vol. 55, No. 3
doi: 10.1111/j.1556-4029.2010.01345.x
Available online at: interscience.wiley.com

TECHNICAL NOTE

CRIMINALISTICS

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Implementation and Validation of the Teleshake Unit for DNA IQ™ Robotic Extraction and Development of a Large Volume DNA IQ™ Method

ABSTRACT: Automated platforms used for forensic casework sample DNA extraction need to be versatile to accommodate a wide variety of sample types, thus protocols frequently need modification. In this study, DNA IQ™ methods previously developed for the Biomek® 2000 Automation Workstation were adapted for the Teleshake Unit using normal volumes and all deepwell extraction, and a large volume DNA IQ™ method developed. DNA purification without detectable contamination of adjacent reagent blanks is reported in the extraction of tissue samples containing several micrograms of DNA. Sensitivity and contamination studies demonstrated similar performance with the manual organic extraction method for bloodstain dilution samples. Mock casework samples demonstrated the effectiveness of the Teleshake and Teleshake large volume methods. Because of the performance and increased versatility of the DNA IQ™ extraction with these modifications, the Teleshake Unit has been implemented in both normal and large volume automated DNA extractions at the Virginia Department of Forensic Science.

KEYWORDS: forensic science, forensic DNA analysis, DNA purification, DNA IQ™, Teleshake, large volume, automation

The technology of automation as it applies to forensic science is continually changing (1–8). Software and hardware updates provide refinement of current methodologies to make the processes more efficient. Described here is the integration of an all deepwell DNA extraction method using the Biomek® 2000 Laboratory Automation Workstation (Biomek® 2000, Beckman-Coulter, Fullerton, CA) into a previously reported automated DNA extraction method. Moreover, an alternative semi-automated extraction procedure was developed, the large volume Teleshake method, using the deepwell hardware for analysis of low-level and problematic samples requiring a larger extraction volume (500 μL or larger).

One of the challenges associated with the implementation of automated DNA extraction methods into a forensic casework laboratory is the need to ensure its versatility to a wide variety of sample types. For example, casework samples may consist of tissue, which contains very high amounts of DNA or the opposite extreme, low-level samples such as skeletonized bone or a diffuse stain that requires the use of large extraction volumes. Previously, automated DNA extraction using the Biomek® 2000 did not have the flexibility to routinely accept these sample types without a following concentration step (9), because the extraction was completed in shallow-well 96-well plates. As such, overloading the system with samples containing microgram (μg) quantities of DNA was avoided for fear of contaminating the surrounding wells. For example, tissue samples were handled very cautiously, with only tiny amounts of sample utilized and caution employed when loading the robot plate. Even for routine casework sample extraction, blank columns of wells were used to separate each examiner's set of samples (10). Thus, a deepwell extraction method was warranted that could diminish the need for such extreme caution owing to hardware limitations. Moreover, the employment of an all deepwell extraction allows for the use of a vigorous shaking unit and reduces the need for pipettor mixing of the samples. This application of the deepwell extraction method became possible with the advent of a pronged heat transfer plate that sits atop the electronic heater (Promega Corporation, Madison, WI) which can effectively heat large sample volumes in a deepwell plate. Previous DNA extraction methods on the Biomek® 2000 in conjunction with the DNA IQ™ System (Promega) utilized a shallow-well Greiner plate (250 μL well volume) that sat atop a thermal exchange unit. The thermal exchange unit was connected by tubing to a waterbath, which was used to heat it.

A significant change to the previously reported automated DNA extraction method (1,2) allows for the extraction of samples requiring large volumes of incubation/extraction buffer such as diffuse stains or bone samples. Previously, samples up to 275 μL in the DNA IQ™ lysis buffer and only 100 μL in a proteinase K buffer could be accommodated with the standard deck setup. Following incubation of the extract with the DNA-binding paramagnetic resin and the placement of the sample plate onto a magnet, thus keeping the DNA bound to the resin fixed in the wells, the lysis buffer was

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Received 5 Nov. 2008; and in revised form 17 April 2009; accepted 19 April 2009.

removed to a waste plate and wash steps carried out. A constraint upon sample volume was a result of the need to limit the number of passes from the extraction plate to the waste plate that would be excessive if a large volume (greater than 500 μL) needed to be transferred (max. tip volume = 125 μL). Excessive liquid transfers not only increases the length of time needed for DNA extraction but also could potentially increase the opportunities for cross-contamination. The new method reported here accommodates larger sample volumes because the method aspirates the resin from the bottom of the extraction plate and, thus, transfers the resin to a new plate as opposed to re-suspending the resin-lysate-lysis buffer suspension and transferring the entire volume of liquid. This allows for a much more efficient use of processing time as well as permitting the use of larger volumes for the extraction—making the automated method more applicable to the wider variety of casework samples encountered in a forensic laboratory.

The innovation of transferring the DNA IQ™ resin by aspiration from the well bottoms was also applied to the standard volume extraction method. Previously, the standard extraction method accommodated sample (plus DNA IQ™ lysis buffer and resin) volumes up to 500 μL, but the new method increases this to 600 μL, although it could easily be increased beyond that volume. The large volume extraction method can accommodate the total volumes of *c.* 1.5 mL.

Materials and Methods

Hardware

A Biomek® 2000 Laboratory Automation Workstation was utilized for the robotic platform. Four new pieces of hardware were added to the robot deck: the V&P Scientific heating block (Promega, Madison, WI, P/N V6761), a deepwell heat transfer block (Promega, P/N V6741), a VARIOMAG® Teleshake shaker (Promega, P/N V6751), and the shaker integration plate (Promega, P/N V3691). Additionally, a Watlow electrical heater (St. Louis, MO) was utilized as a replacement to the waterbath used previously (1). The sample plate utilized is a Marsh 2.2-mL square well, conical bottom plate (Marsh BioProducts, Rochester, NY; P/N AB-0932). The Greiner U-bottom plate previously used (1), also referred to as the "working plate," was replaced with an ABgene 1.2-mL round-bottom deepwell plate (Surrey, U.K., P/N AB-0787).

Sample Preparation

Portions of human tissue samples (*c.* 75 mm^3) were utilized for the checkerboard contamination tests. Mock sexual assault samples were generated by preparing a semen dilution in sterile water for a final volume of 150 μL. Immediately after preparing the dilution, a vaginal swab was placed into the tube for *c.* 1 min to soak up the solution. The swab was then air-dried prior to use. Whole blood from a volunteer was mixed with sterile Type I water to create the desired dilutions for the sensitivity assays, followed by pipetting *c.* 1 mL of neat or diluted blood onto blood stain cards (Whatman®, Clifton, NJ) and the cards air-dried. The bloodstain dilution series was prepared with the following dilutions: 1:100, 1:200, 1:400, 1:800, 1:1000, 1:1200, 1:1400, 1:1800, 1:2000, 1:4000, 1:8000, and 1:10,000. To better simulate casework and to utilize the large volume extraction as intended for casework samples, sample sizes removed for DNA extraction were gradually larger as the blood stains became more dilute (1:100, *c.* 2 cm × 2.1 cm; 1:200, *c.* 1.8 cm × 2.13 cm; 1:400, *c.* 1.8 cm × 2.13 cm; 1:800, *c.* 1.8 cm × 2.17 cm; 1:1,000, *c.* 2 cm × 2.27 cm; 1:1,200, *c.* 2.1 cm × 2.27; 1:1,400, *c.* 2.2 cm^2; 1:1,800, *c.* 2.2 cm × 2.33 cm; 1:2,000, *c.* 2.3 cm × 2.23 cm; 1:4,000, *c.* 2.4 cm × 2.5 cm; 1:8,000, *c.* 2.5 cm × 2.6 cm; 1:10,000, *c.* 2.5 cm × 2.6 cm). Samples were incubated for 1–2 h at 56°C in 500 μL of a proteinase K containing buffer (1.2 mL TNE, 75 μL 20% sarkosyl, 225 μL Type I H_2O, 15 μL 20 mg/mL proteinase K).

Sensitivity Assays

The efficiency of the new robotic DNA IQ™ (Promega) method using the Teleshake system to bind and elute DNA was tested using standard 9947A DNA (Promega). A total of 50 μL of a 1 mg/mL solution of bovine serum albumin (BSA, Sigma–Aldrich, St. Louis, MO) plus 703 pg of 9947A DNA standard were pipetted into each well of the sample plate. BSA was added, rather than simply naked DNA, by recommendation of Promega Corp. because of an unexplained phenomenon negatively affecting the extraction with no proteins in the sample. The DNA samples were purified and eluted using a 24-sample method. The extracts produced were amplified using the maximum volume, 5 μL, without quantitating as the DNA concentrations were expected to be extremely small (≤ 20 pg/μL) and below the range of reliable quantitation using the method that was employed at that time by the Virginia Department of Forensic Science (VDFS) laboratory.

Contamination Tests

Checkerboard contamination tests were performed with tissue samples (four different donors using multiple samples from muscle, brain, heart, and liver—for a total of 56 tissue samples), 31 different mock sexual assault samples, buccal swabs from eight different donors, blood stains from eight different donors, and four different mock touch evidence samples. Sample wells were alternated with reagent blank wells; thus, for each sample listed, a corresponding blank well was included so that the total number of wells was double the number listed previously. Four separate runs were performed using the Teleshake upgrade, and all deepwell extraction and an additional two runs were performed to assess the large volume Teleshake methods. All blanks and most samples were taken through the entire short tandem repeat (STR) typing process to ensure that contamination was not observed in any of the samples. All samples were quantitated for DNA to ensure that DNA was successfully obtained from the sample wells containing biologic material. For the blanks, the maximum volume of eluate was added to the amplification reaction (5 μL), consisting of 40% of the total amplification volume, and 2 μL of the PCR product added to the gel loading cocktail (33% of the total volume).

An additional contamination test was performed using the normal Teleshake extraction protocol. The samples tested consisted of six mock sexual assault samples, four cigarette butts, four blood stains, and four buccal samples. Samples were loaded in a diagonal pattern across the plate, and blank wells adjacent to the sample wells were assessed for contamination (23 blanks total). All samples and blank wells were taken through the entire STR typing process as described earlier.

Bone Sample Preparation

Bone samples were prepared by cleaning the outside of the bone with a proteinase K solution (1.2 mL TNE, 75 μL 20% sarkosyl, 225 μL Type I H_2O, 15 μL 20 mg/mL proteinase K). The proteinase K solution was applied to the bone surface using a Kimwipe. The bone was placed in a plastic bag and incubated at 56°C for

30 min. After incubation, the surface of the bone was cleaned with 95% ethanol. *c.* 0.05–0.1 g of bone material was collected by an electric drill (11).

For the large volume DNA IQ™ extraction, bone digest buffer (500 μL) (50 mM Tris, 100 mM NaCl, 250 mM EDTA, 2% sarkosyl) was added to the bone powder and the sample incubated at 56°C overnight. The large volume extraction method was completed on the sample, as described later. For the organic extraction, 500 μL of phenol/chloroform/isoamyl alcohol was added to the bone sample after pelleting and the process performed as described (12).

Normal Volume DNA Extraction

The normal or standard volume DNA extraction using the Biomek® 2000 robotic platform is essentially as described (1); however, modifications were made to the process when the system was adapted to the Teleshake shaker, electronic heater, and all deepwell extraction process. Figure 1 describes the sample processing steps for the normal and large volume DNA IQ™ methods in detail. During the development of the large volume DNA IQ™ DNA extraction process using the Biomek® 2000 platform, a modification of the process was developed to efficiently transfer the DNA IQ™ resin to the "working plate" which sits atop the magnet. This same modification was incorporated into the normal volume DNA extraction method. This involved changing the approach to transferring the resin to the working plate. After mixing the sample lysate, by both shaking and pipette mixing, the resin is allowed to settle naturally by gravity (5 min incubation) only to be aspirated from the bottom of the conical well and transferred to the working plate in two consecutive passes. After those first two passes of resin, two additional passes of resin transfer were completed with the transient use of the magnet to concentrate and pull the dispersed resin particles to the bottom of the well. The use of two passes to transfer the resin was not required by the tip volume (tip maximum = 125 μL), but by optimal recovery of resin (data not shown). For these final two resin transfer passes, the sample plate was removed from the magnet at robotic platform position B5 and moved to robotic platform position A5 (Fig. 2) for aspiration of the resin from the well bottoms and subsequent transfer to the working plate atop the magnet. This modification allowed for the accommodation of increased volumes by the normal method for samples incubated in DNA IQ™ lysis buffer or proteinase K buffer without requiring increased passes from the sample plate to the working plate. Both the normal and large volume extraction methods utilized 40 μL of elution buffer, of which *c.* 35 μL was typically recovered.

Large Volume Sample Preparation for DNA Extraction

The primary differences between the standard volume robotic DNA IQ™ sample extraction and the large volume sample extraction are the volumes of sample accommodated and the DNA IQ™ resin is added to the large volume extractions manually off-robot for optimal mixing. A scaled-up version of the proteinase K containing buffer (*c.* 500 μL) or the entire nonsperm fraction in a proteinase K containing buffer described elsewhere (13) was used in conjunction with the DNA IQ™ system for the large volume digest. For each sample, the buffer components were as follows: 187.5 μL of TNE, 187.5 μL of Sterile Type I water, 62.5 μL of 20% sarkosyl (Sigma–Aldrich), 50 μL of 0.39M DTT (Sigma–Aldrich), and 25 μL of 20 mg/mL proteinase K (Sigma–Aldrich), for a total volume of 512.5 μL or the entire nonsperm cell digest extract or 500 μL was used.

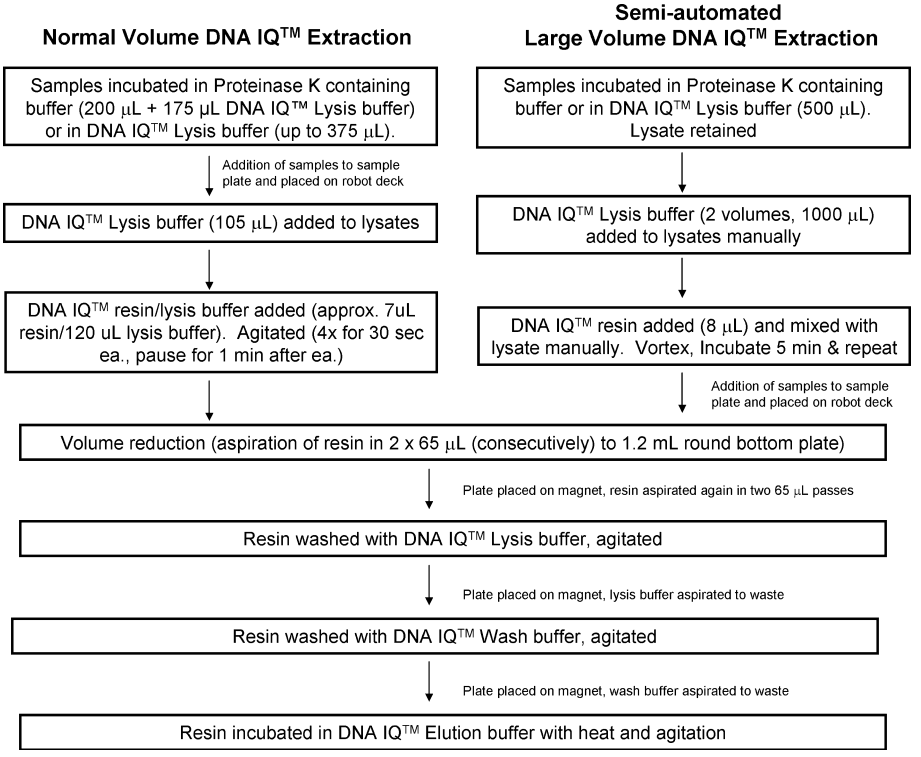

FIG. 1—*Flow chart depicting the steps involved in the semi-automated extraction of DNA using the normal and large volume DNA IQ™ System. The two methods converge at the volume reduction step, where the resin is aspirated to a new 96-well plate (the working plate).*

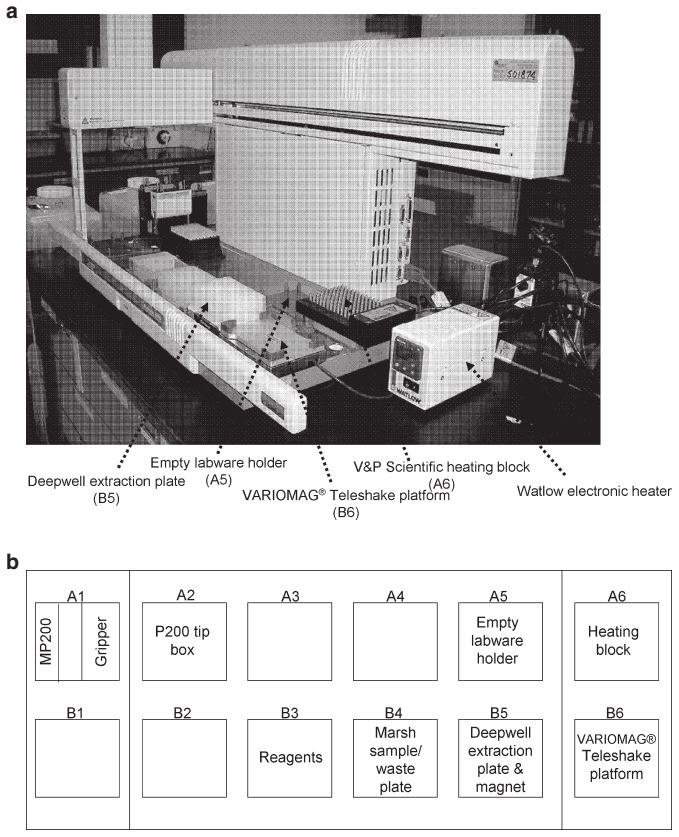

FIG. 2—*Setup of the Biomek® Laboratory Automation Workstation for the DNA IQ™ Teleshake extraction. a) Photograph of the deck adapted for an all deepwell extraction. The Watlow electronic heater is evident to the right of the robotic platform. At the far right of the robotic platform, the V&P Scientific heating block with deepwell heat transfer block (rear) and VARIOMAG® Teleshake platform (front) are evident. At deck position A5, an empty gray labware holder is positioned to the left of the pronged deepwell heat transfer block (rear, far right). The availability of an open position for placing the sample plate is necessary for the transfer of resin from the bottom of the wells. b) Diagram of the deck.*

Samples were incubated for 1–2 h at 56°C and the cuttings spun in the recessed lids or spin baskets to collect all of the liquid. Each lysate contained in a 1.5-mL tube was then transferred to a 2-mL tube and 1.0 mL of DNA IQ™ lysis buffer with DTT (75 μM final concentration) added, followed by 8 μL of DNA IQ™ resin. Each sample was vortexed at high speed for 30 sec, incubated for 5 min, followed by a second vortex for 30 sec. For the semi-automated large volume extractions, the lysate-resin suspensions were loaded manually into a deepwell sample plate and the purification continued on the robot using the large volume extraction method, as delineated in further detail in Fig. 1. For manual large volume extractions, the lysate-resin volume was reduced by transferring the suspension to a labeled 1.5-mL tube on the DNA IQ™ magnetic stand. Once the resin was pulled to the side of the tube, the lysate was aspirated and disposed of. This was repeated until all of the resin contained in the suspension was loaded into the 1.5-mL tube. The DNA IQ™ manual extraction process was continued according to the manufacturer's recommendations, beginning with the addition of 100 μL of lysis buffer to each tube for a lysis buffer wash step (14). Both the manual and semi-automated large volume extraction methods utilized 40 μL of elution buffer, of which *c.* 35 μL was typically recovered.

DNA Quantitation

DNA samples were quantitated using the AluQuant® Human DNA Quantitation System (Promega) as described in the VDFS procedures manual (15). The ThermoLab System Luminoskan (Luminoskan Ascent, Thermo Labsystems, Vantaa, Finland) luminometer in conjunction with the Ascent software program were used for luciferase injection and data capture. The quantitation data were generated using the AluQuant® Calculator version 3.0 (Promega).

PCR Amplification

STR amplifications were performed using the PowerPlex® 16 BIO amplification kit (Promega). Amplification reactions were prepared using half-volume PCRs (12.5 μL) and a maximum of either 0.75 ng total DNA or, if less concentrated than 0.75 ng/5 μL of DNA, then the maximum volume of 5 μL was utilized as described (9). The amplification conditions were adjusted to accommodate the half reactions as follows: 95°C hold for 11 min, 96°C hold for 1 min, 10 cycles of: 94°C, 30 sec; 60°C, 30 sec; and 70°C, 45 sec, 21 cycles of: 90°C, 30 sec; 60°C, 30 sec; and 70°C, 45 sec, a 60°C hold for 30 min, and an indefinite 4°C hold.

Separation and Detection of STR Fragments

STR amplification products were separated on a 6% PAGE PLUS polyacrylamide gel (Amresco, Solon, OH). The postamplification reagents were provided in the PowerPlex® 16 BIO kit. The manufacturer's directions were followed for preparation of the samples. The polyacrylamide gels were prerun for *c.* 25 min at 60 W. After addition of the loading cocktails to the wells, electrophoresis was continued for *c.* 2 h at 60 W. Detection of the PCR products was performed using the Hitachi FMBIO® III+ Fluorescent Imaging System and the FMBIO® Analysis software program (Tokyo, Japan). Color separation and typing analysis was completed using the ImageAnalysis program version 3.0.0.79 (MiraiBio, Inc., San Francisco, CA), as described in the Promega PowerPlex® 16 BIO Technical Manual (16).

Statistical Analyses

Student's *t*-tests were utilized to determine the statistical significance ($\alpha = 0.05$) of the data.

Results and Discussion

The Teleshake upgrade to the Biomek® 2000/DNA IQ™ System incorporated the VARIOMAG® Teleshake shaker, the V&P Scientific Heating Block, the Deep Well Heat Transfer Block, and the conversion to an all deepwell extraction. Figure 2 displays the deck of the Biomek® 2000 robot with the current modifications. The VARIOMAG® Teleshake shaker and V&P Scientific Heating Block (heat transfer plate) are positioned on the robot deck at B6 and A6, respectively. The heat transfer plate provides efficient heat transfer to larger volumes in the deepwell plate attributed to the metal prongs.

Normal Volume Teleshake

Initial testing involved the redesign of the automated DNA IQ™ methods to the new hardware and plasticware. An initial method

designed to accommodate the electronic heater, Teleshake shaker, and all deepwell extraction was provided by Promega Corporation. Once the method was installed and calibrated, initial testing was carried out to assess the performance (data not shown). The rpms for the Teleshake shaker were reduced for the step where the sample lysate was mixed with the resin and DNA IQ™ lysis buffer (the step where DNA binds to the resin) from 1050 to 800 to reduce the risk for aerosol formation, as this step contains the most unbound DNA of any step in the DNA purification process. Once the optimal parameters were set, sensitivity and contamination tests were performed to show the functionality of the method and to compare to our previous robotic DNA IQ™ extraction method.

Sensitivity and Efficiency of DNA Purification—To determine whether the Teleshake method was binding and eluting DNA uniformly on the plate, a 24-sample method was run containing a 1 mg/mL solution of BSA and 9947A DNA (703 pg per well) in the first three columns of a 96-well sample plate. The DNA was eluted in a total volume of 40 µL. The eluted DNA was amplified using PowerPlex® 16 BIO, and the extent of the profiles recovered assessed. The maximum volume, 5 µL, was placed into the half-volume PCR. Thus, if 100% efficiency were achieved, each well would yield *c.* 20 pg/µL and 100 pg would be placed into each amplification reaction. All 24 sample wells produced nearly identical results on the STR typing gel—strong partial or full profiles (data not shown). An average of 22 (± 2) out of a maximum of 25 alleles was detected per sample. This was expected if the DNA binding and elution of the Teleshake procedure was efficient and is comparable to the typing success observed using the unmodified DNA IQ™ method previously employed. While it is unlikely that 100% efficiency was achieved, the efficiency was extremely high (unpublished data). The small degree of variability in the profiles observed across the 24 low level samples indicates that the efficiency of binding and eluting DNA was sensitive, consistent from well to well, and sufficient for our purposes. It is interesting to note that when this same experiment was repeated using 9947A in DNA IQ™ lysis buffer, with no added BSA, several wells failed to yield any DNA profile when assessed by PowerPlex® 16 BIO typing (data not shown). This is an unexplained phenomenon, but previously observed by others (Bob McLaren, Promega Corp., personal communication).

Contamination Tests—A main goal in the incorporation of the Teleshake modification into the forensic DNA laboratory was the modification to an all deepwell extraction. The previous method utilized a polystyrene *U*-bottom plate (wells *c.* 10.3 mm depth and a 250-µL well volume) for DNA purification. The new method, however, utilizes a 1.2-mL polypropylene deepwell plate (wells *c.* 39.5 mm depth) which decreases the likelihood for contamination on the robot deck as a result of the formation of aerosols from concentrated samples during the shaking steps. With the previous extraction protocol (1), examiners were urged to severely limit samples likely containing large quantities of DNA, such as tissue samples. In addition, these samples were sequestered away from other evidentiary samples to diminish any possibility of cross-contamination. Thus, a primary goal was to demonstrate the use of the all deepwell extraction with tissue samples without the evidence of contamination. Using an initial mixing step (of cell lysate, DNA IQ™ lysis buffer, and resin) shaker speed of 800 rpm, no contamination was observed via STR typing in any of the blanks of the

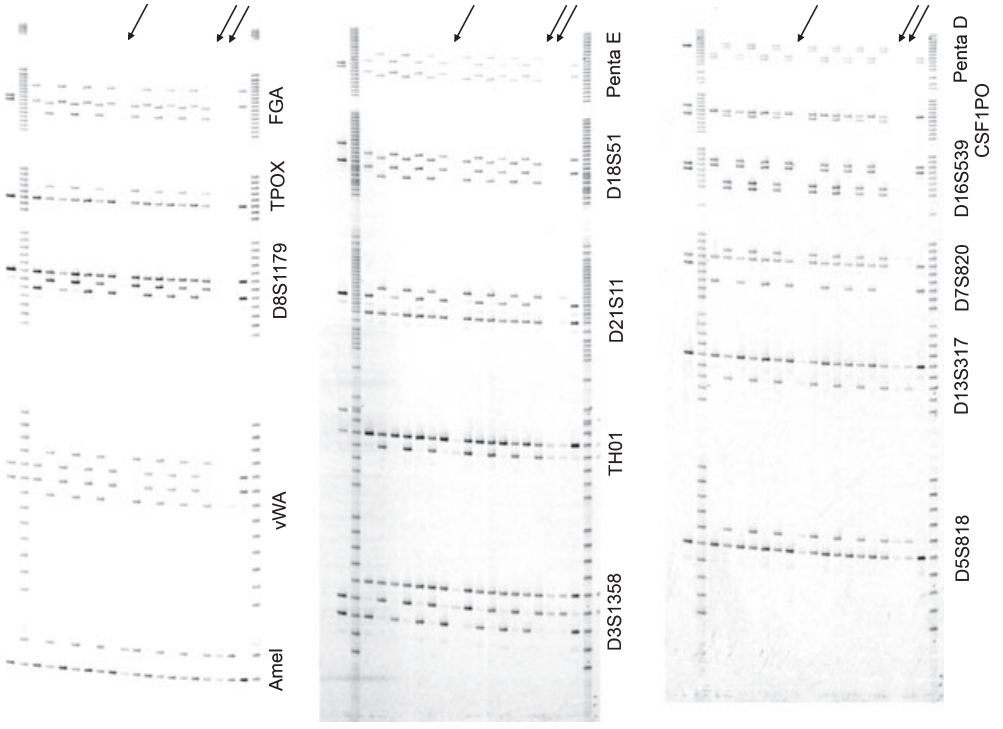

FIG. 3—*PowerPlex® 16 BIO typing gel images of a tissue checkerboard test to show validity of deepwell extraction for reducing contamination susceptibility with samples containing large amounts of DNA. Two tissue donors were utilized for this study, as is evident from the short tandem repeat (STR) typing results. The left panels contain the channel 1 scan (Rhodamine RedTM –X), the middle panel contains the channel 3 scan (Fluorescein), and the right panel contains the channel 4 scan (JOE). Channel 2 containing the ILS 600 is not shown. Arrows point to a single sample extracted in three different wells of the 96-well plate. Gross evaluation of the tissue sample, quantitation results, and STR profiling all suggest degradation of the tissue sample and is not meant to be a reflection of the extraction method.*

two 56-sample checkerboard tests. Results from the tissue samples of the checkerboard test are shown in Fig. 3 (data not shown for blanks).

As the DNA IQ™ resin has a limited capacity to bind DNA, it was presumed that the majority of the DNA from the very concentrated tissue samples was disposed of into the waste plate. To accurately assess the quantity of DNA accommodated by the new DNA IQ™ extraction process during the initial lysate and resin mix steps using the Teleshake shaker, the lysate waste was retrieved from the waste plate from a 24-sample tissue checkerboard test. Sixteen of the lysates were manually extracted using phenol/chloroform followed by Microcon® clean-up and a 1:10 dilution made for AluQuant® quantitation. DNA concentrations for the lysates containing unbound DNA ranged from 3.0 ng/μL to 110 ng/μL (40 μL elution volume). Thus, the total amount of DNA in the wells tested ranged from greater than 120 ng to greater than 4.35 μg, as these DNA estimates do not include the DNA captured by the DNA IQ™ resin during the Teleshake DNA IQ™ extraction process (data not shown). Thus, the new Teleshake deepwell extraction method is capable of handling greater than 4 μg of DNA in a sample well without contamination of an adjacent well during the robotic process. While this does not simulate our anticipated routine casework experience, it tests the range of the system and demonstrates that the new system is more forgiving when dealing with DNA-rich samples than the previous shallower-well extraction plate (unpublished data).

An undesirable consequence of the switch to the deepwell plate appears to be a greater incidence of PCR inhibition. Using the old hardware and shallow polystyrene plate, PCR inhibition was extremely rare. Using the deepwell polypropylene plate, it has been observed both with low concentration mock and actual casework samples (data not shown). An estimate of the inhibition rate is difficult to ascertain, as inhibition is only typically observed in samples that are not diluted prior to amplification setup. However, in a 40-sample run where all DNA extracts were amplified using the maximum volume of 5 μL (40% of the amplification reaction volume), inhibition has been observed in a maximum of five samples. In casework runs, this would be an overestimate of the inhibition rate, as many samples are diluted prior to amplification setup. Incomplete drying during the alcohol wash step is one possible explanation; however, the drying time was extended from 5 min to 10 min and an additional wash aspiration step was added, but the sporadic inhibition of low DNA yield samples was not eliminated. Another possible explanation might be that the DNA IQ™ lysis buffer may not be completely and uniformly removed during the subsequent wash steps and then is co-eluted with the purified DNA. Leeching of an inhibitory substance from the deepwell working plate into the eluent has been ruled out by performing solely the elution step minus any sample, and assaying for inhibition by adding 250 pg of 9947A and 5 μL of eluent to each amplification reaction, and typing the samples for PowerPlex® 16 BIO. No inhibition was observed (data not shown). Further investigation into the cause of this inhibition is currently underway.

Large Volume DNA IQ™ Purification

To accommodate diffuse stains over a large area or other samples requiring a larger volume, such as the entire nonsperm fraction from a sexual assault sample, a large volume DNA IQ™ method was developed, both manually and robotically. The large volume DNA extractions were designed to accommodate samples requiring 500 μL or greater of a proteinase K containing digest buffer.

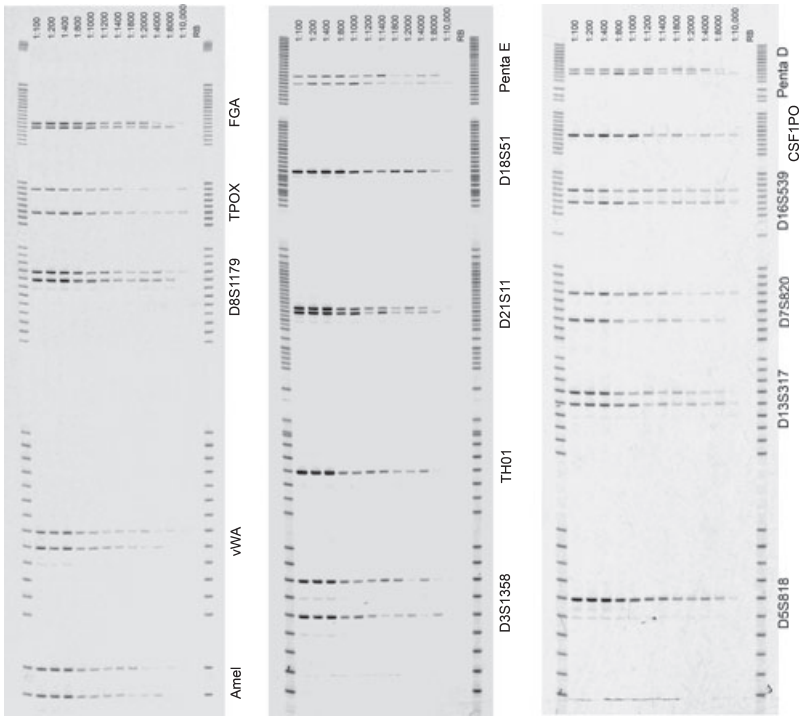

FIG. 4—*PowerPlex® 16 BIO typing gel image of a series of blood dilutions on filter paper extracted manually with the large volume DNA IQ™ DNA purification method. The left panels contain the channel 1 scan (Rhodamine RedTM –X), the middle panel contains the channel 3 scan (Fluorescein), and the right panel contains the channel 4 scan (JOE). Channel 2 containing the ILS 600 is not shown. Loci names are indicated to the right of the images. The dilutions 1:100–1:10,000 blood (v/v in H_2O) are labeled along the top of the gel. RB = Reagent Blank.*

Manual Large Volume Extraction—Optimization of the large volume method was completed manually prior to introduction to the robotic platform. A series of bloodstain dilutions, as described in the Materials and Methods section, was utilized to demonstrate the sensitivity of the method for DNA extraction. The data, shown in Fig. 4, demonstrate that the method can successfully accommodate large, diffuse stains and purify the DNA efficiently. A full Powerplex 16® BIO profile was obtained for dilutions from 1:100 to 1:2000, with the exception of drop out of one allele in the 1:1800 sample. Strong partial profiles were obtained for the remaining samples: 1:4000, 1:8000, and 1:10,000. This sensitivity is nearly identical to that obtained in an organic extraction of the same samples completed in accordance to the VDFS protocol (data not shown).

Semi-automated Large Volume Teleshake—The semi-automated large volume DNA IQ™ method is similar to the normal volume method with a couple of adaptations. To accommodate a larger volume of a proteinase K or other non-DNA IQ™ lysis buffer (up to 500 μL), two volumes of DNA IQ™ lysis buffer are added manually to the sample to bring the total concentration of guanidinium isothiocyanate to an optimal concentration for DNA binding to the DNA IQ™ resin, as outlined in Fig. 1. The normal volume of DNA IQ™ resin (8 μL) is added, and the samples vigorously vortexed, and incubated 5 min, and then repeated prior to manually loading the samples into the deepwell plate for robotic DNA purification.

Automated volume reduction is accomplished by gravitational sedimentation of the resin to the bottom of the wells of the polypropylene plate and transfer of the resin to a new polypropylene plate (the working plate) in two consecutive 65-μL steps. This ensures that *c.* 90–95% of the resin is transferred in a minimal volume (130 μL). The final two transfers of resin use the Magnabot transiently to bring the remaining dispersed resin to the bottom of the conical wells prior to transfer to the "working plate." Subsequent wash and elution steps are performed as in the normal volume method.

The aspiration of resin from the well bottoms decreases sample processing times and simplifies the method substantially. For example, a 40 sample large volume robotic method takes slightly less time than a normal volume 40 sample robotic method as the resin is mixed with the sample lysate prior to loading onto the robot (*c.* 1 h). If re-suspension of the resin-lysate mixture was required for the volume reduction step for the 40 sample large volume method, an additional hour (approximately) would be added to the process (unpublished data). The amount of time would increase proportionately as more samples are processed; thus, the time savings are greater for methods purifying a larger number of samples.

Prior to adapting the large volume DNA IQ™ method to the new Teleshake hardware and all deepwell extraction, extensive testing of the semi-automated large volume DNA IQ™ method was performed using the old hardware and Greiner plate (as the working plate). A total of 48 sensitivity (bloodstain dilutions), 76 mock sexual assault (forearm, abdomen, breast, rectal, lip, thigh, neck, and calf swabs—all mixed with different semen dilutions), and 16 mock casework samples (soft drink can swabs, cigarette butts, lip, and forearm swabs) were tested, and all samples, associated reagent blanks, and blank wells for assessing contamination, were taken through the entire STR typing process (data not shown, total number of samples typed = 176). The majority of the samples were created whereby consumption of the entire sample was required for optimal results (e.g., a breast swab). Direct comparisons with organic extraction of an identically sized portion removed from the same sample or a duplicate of the sample were performed for 48 of the mock sexual assault samples with similar PowerPlex® 16 BIO typing results obtained (data not shown).

Sensitivity—The sensitivity of the semi-automated large volume method was compared to that of the manual large volume method. Similar results were obtained on the robotic platform (data not shown) with only slight variations (e.g., one series showed slightly less sensitivity and a second series showed slightly more). Because different blood donors were used to create these sensitivity series, these minor variations are not unexpected.

Contamination Studies—Contamination studies were performed using two different 40-sample checkerboard extractions with known samples such as buccal swabs, bloodstains, and mock sexual assault samples, for a total of 31 samples, to simulate casework. No evidence of contamination was observed in any of the blanks or reagent blanks (data not shown).

Mock Casework—The large volume DNA IQ™ extraction method was developed for the occasional forensic samples that require larger than ordinary volumes of a lysis buffer for DNA extraction. Most commonly, this involves low-level samples that are dispersed over a large area, bone and teeth samples, nonsperm fractions from sexual assault cases where the entire 500 μL is needed to obtain a profile such as rectal swabs and, potentially, samples containing contaminants. Frequently, this involves a proteinase K digestion in a large volume (up to 500 μL). The utility of the large volume DNA IQ™ purification was demonstrated by the extraction of DNA from fully skeletonized remains (four different remains). The DNA IQ™ large volume extraction was directly compared to a manual organic extraction from the same bone samples. Similar results were obtained using both methods for all four bone samples tested (Table 1 and data not shown). Thus, the large volume DNA IQ™ Teleshake method developed allows for the robotic processing of sometimes problematic casework samples that would have previously required manual extraction.

Minimizing the DNA Yield Reducing Effects of Commonly Encountered Contaminants—An earlier study performed at VDFS demonstrated that some commonly encountered contaminants, such as contraceptive gel, could reduce the DNA yield for samples

TABLE 1—*PCR amplification results using the PowerPlex® 16 BIO System for a bone sample.*

Locus	Semi-Automated Large Volume DNA IQ™	Manual Organic
FGA	+	±
TPOX	+	±
D8S1179	+	+
vWA	±	+
Amel	+	+
Penta E	±	±
D18S51	±	±
D21S11	+	+
TH01	+	+
D3S1358	+	+
Penta D	+	+
CSF1PO	+	+
D16S539	+	+
D7S820	+	+
D13S317	+	+
DS5818	+	+

Key: +, complete DNA type; ±, allelic dropout.

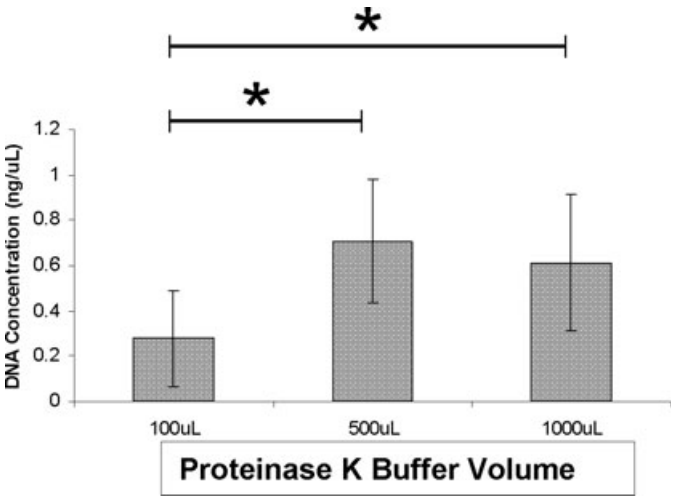

FIG. 5—*The effect of dilution volume on the DNA yield. Samples were digested in the same volume of proteinase K buffer (100 μL) followed by addition of varying volumes of proteinase K buffer prior to DNA IQTM extraction. Shown is the average and standard deviation of three different blood samples, each tested in triplicate for each volume. Statistical significance at* $p < 0.005$*, as determined by the Student's* t*-test, is indicated by the *.* $n = 9$.

extracted using the DNA IQ™ System (unpublished data). During the study, it was observed that when bloodstain samples contaminated with various compounds and the uncontaminated control bloodstains were subjected to DNA purification using the large volume method, DNA yields increased over those produced using the standard DNA IQ™ extraction procedure. An experiment was designed to determine whether the yield-enhancing effects of the large volume method for the unadulterated bloodstains was because of the dilution of a competing factor (for binding to the DNA IQ™ resin) or whether the proteinase K digest was simply more effective at a larger volume.

Bloodstain cutouts (3 mm^2) were prepared in triplicate from three different bloodstain donors ($n = 9$). All samples were incubated in a 100-μL volume proteinase K buffer at 56°C for 1 h. After the 56°C incubation, the samples were placed into a rack at room temperature. One set of samples was undiluted, the volume of the second was increased to 500 μL with proteinase K buffer, and the volume of the third set was increased to 1000 μL with proteinase K buffer. All samples were then carried through the large volume DNA IQ™ extraction method manually beginning with the addition of DNA IQ™ lysis buffer (two volumes). The volumes of added DNA IQ™ lysis buffer were increased proportionately; the volume of DNA IQ lysis buffer added to the undiluted samples was 200 μL, to the 500 μL dilution was 1000 μL, and to the 1000 μL dilution was 2000 μL. All dilution sets received the same volume of DNA IQ™ resin, 8 μL. The measured DNA yield was the highest for the 500-μL dilution sample, and it dropped slightly for the 1000 μL dilution (Fig. 5). The data indicate that a significant increase in DNA recovery can be obtained from the use of the large volume method when extracting DNA from whole blood.

The volume effect observed is believed to be a result of the dilution of competing factors for binding sites on the DNA IQ™ resin. The DNA IQ™ resin has a limited binding capacity (14) and, although the DNA IQ™ extraction process is optimized specifically for DNA binding, proteins also bind efficiently to the silica-coated resin (17). Proteinase K is a nonspecific serine protease that produces peptides as well as hydrolyzed amino acids (18). Thus, it is conceivable that proteinase K digestion of the bloodstain sample may increase the DNA yields over incubation solely in the DNA IQ™ buffer by cleaving the hemoglobin and diluting out the concentrated peptides, resulting in a higher DNA-binding efficiency. If it were simply that the proteinase K digest was more effective at a larger volume, then all three dilutions tested would have approximately the same DNA yields as the proteinase K digestion was carried out in the same volume for all dilutions, 100 μL. Indeed, concentrated bloodstains, particularly flakes of blood, can be challenging using the DNA IQ™ System, and examples of such samples producing no detectable DNA yield have been encountered with both casework and mock casework testing (unpublished data).

Conclusions

Both the standard and large volume Teleshake methods have been successfully demonstrated to perform DNA extractions with high efficiency, high sensitivity, and reduced susceptibility for contamination because of the all deepwell extraction, more efficient shaker, and electronic heater. We did observe an increased incidence of PCR inhibition which is related in some manner to the employment of the deepwell "working plate." The transfer of the DNA IQ™ resin, as a volume reduction step, to the "working plate" is a useful means to move the resin and attached DNA into a new plate for the DNA purification and away from the leftover waste lysate material. The transfer of the resin by aspiration from the bottoms of the sample wells allows for an accommodation of larger volumes of sample liquid in the sample plate, as it removes the need for the resuspension step for the resin-lysate mixture for transfer and, thus, reduces the number of passes required for the large volume method to transfer all of the resin to the "working plate." The aspiration of resin from the well bottoms decreases sample processing times and simplifies the method substantially. As a result, this processing flow was also incorporated into the standard Teleshake DNA IQ™ extraction process. Finally, the large volume method may be useful for obtaining higher DNA yields from neat bloodstains. This is likely because of the diluting out of competing factors for binding to the DNA IQ™ resin, such as hemoglobin peptides.

Acknowledgments

The authors thank Allan Tereba and Bob McLaren, both from Promega Corporation, for many helpful suggestions and experimental ideas.

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