Published in final edited form as:

JALA Charlottesville. 2008 February ; 13(1): 40–48.

An instrument for automated purification of nucleic acids from contaminated forensic samples

David J Broemeling, BSc, Joel Pel, BSc, Dylan C Gunn, BSc, Laura Mai, BSc, Jason D Thompson, BSc, Hiron Poon, MSc, BSc, C/M, and Andre Marziali, Ph.D.

Abstract

Forensic crime scene sample analysis, by its nature, often deals with samples in which there are low amounts of nucleic acids, on substrates that often lead to inhibition of subsequent enzymatic reactions such as PCR amplification for STR profiling. Common substrates include denim from blue jeans, which yields indigo dye as a PCR inhibitor, and soil, which yields humic substances as inhibitors. These inhibitors frequently co-extract with nucleic acids in standard column or bead-based preps, leading to frequent failure of STR profiling. We present a novel instrument for DNA purification of forensic samples that is capable of highly effective concentration of nucleic acids from soil particulates, fabric, and other complex samples including solid components. The novel concentration process, known as SCODA, is inherently selective for long charged polymers such as DNA, and therefore is able to effectively reject known contaminants. We present an automated sample preparation instrument based on this process, and preliminary results based on mock forensic samples.

Keywords

DNA purification; extraction; forensic; sample preparation

Introduction

Forensic sample analysis is one of the fastest growing application areas of nucleic acid technologies. Many countries are maintaining large databases of DNA Short Tandem Repeat (STR) profiles for offenders, allowing police to link crime scene samples to suspects, offenders previously convicted of even minor crimes, or to other crime scenes. A 2003 UK study (1) documented that 77,814 crime scene samples were analyzed for DNA profiles in 2002. Unfortunately, not all analyzed samples are capable of producing full DNA profiles. The presence of contaminants (2-3), such as humic acids from soil (4), textile dyes such as indigo from denim (5-6), melanin from hair samples (7), and hematin from blood (8) pose a problem for DNA extraction and analysis, particularly in cases where the amount of DNA in the sample is small. Many of these contaminants both co-extract with DNA in routine extraction procedures, and inhibit downstream enzymatic reactions such as PCR. We estimate that 10% or more of crime scene forensic samples fail from insufficient DNA and/or co-extraction of PCR-inhibiting contaminants.

Dr. Andre Marziali, Ph.D., University of British Columbia.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Part of the reason for these failures lies in the nature of commonly used DNA extraction and purification methods. Since these often rely on chemical affinity between DNA and magnetic beads or filtration membranes, it is not surprising that some chemical species co-extract by binding to the same sites. It is even possible that in some cases, abundant contaminants bind to and saturate the extraction beads (or matrix) preventing binding of DNA and reducing yield. To address these problems, DNA extraction kit manufacturers are attempting to develop contaminant rejection methods for specific contaminants, though this requires prior knowledge of the contaminant type to be expected in a sample, and leads to more complex protocols.

This paper presents an instrument for automated DNA purification from complex and forensic samples, employing a novel purification method that may provide improved rejection of contaminants. The process is based on SCODA - Synchronous Coefficient of Drag Alteration - a multi-dimensional electrophoretic method for nucleic acid manipulation in a gel matrix (9). The electrophoretic nature of the process allows direct extraction from samples containing solid materials, with addition of lysis reagents to the sample as the only step to be performed prior to automated extraction. Though in prototype form, we have demonstrated that the instrument is capable of efficient extraction of nucleic acids from mock forensic samples, and promises to be an excellent platform for the development of a high-throughput, fully automated platform for forensic sample analysis.

We provide a brief background of the methods, present the instrument prototype, and present preliminary performance results on test samples.

SCODA

Nucleic acids are composed of many repeating nucleotide units, each with a single negative charge on the phosphate group. The resulting polymer has an exceptionally high linear charge density, and a relatively short persistence length (length scale below which it behaves as a rigid rod) compared to its typical contour length. For double stranded DNA (dsDNA) typical genomic fragments are 10,000's of nucleotide pairs (bp) long, while the persistence length of dsDNA is on the order of 200 bp. The combination of high conformational entropy of these molecules (available due to the large number of degrees of freedom of such a flexible molecule), and their high charge density, results in the conformation of these molecules having a large susceptibility to electric field particularly when reptating through a sieving gel. The influence of electric field on molecule shape leads to a field-dependent mobility for the molecule, such that the velocity of the molecule depends non-linearly on applied field. This property enables the application of non-linear AC electrophoretic methods to induce motion of DNA while maintaining other charged molecules essentially stationary (9).

We previously documented induced drift of DNA under a rotating electric field pattern intended to select DNA for motion as a result of the unique physical properties described above (9). The method is termed SCODA due to the drag or mobility alteration induced by rotating quadrupole fields which stretch the DNA synchronously with applied rotating driving fields. Evidence from numerous experiments (data not shown) indicates that this method of inducing DNA to migrate in a gel is highly selective and efficient for nucleic acids based on their non-linear response to electric field when migrating in a gel (9). A rigorous documentation of this selectivity is beyond the scope of this paper and is the subject of another manuscript in preparation, but preliminary results indicate that, while we have quantified DNA concentration enhancements of up to 10,000 fold using this method, visually detectable contaminants such as humic acids are not concentrated by SCODA. This method has therefore been used successfully to process exceptionally difficult samples, including samples containing humic acids from soil, providing high quality DNA that can be amplified and profiled.

We refer the reader to earlier work for details of the electrophoretic method and DNA selection mechanism (9). Here follows a description of the combined general protocol for SCODA extraction of nucleic acids.

SCODA purification protocol

The SCODA method uses rotating electric fields to concentrate nucleic acids in the center of an electrophoresis gel, without the need for electrodes in the concentration region, and therefore with decreased risk of chemical damage to DNA. Figure 1 illustrates the general method. The concentration gel is a 1.5 cm x 1.5 cm gel in the center of a cross-shaped gel boat. Briefly, the sample to be analyzed is lysed, placed in the long arm of the cross, electrophoretically injected into the gel, and concentrated by the rotating fields present in the gel. Following electrophoretic concentration, the clean DNA sample can be extracted in a gel plug.

To prepare the system, a gel is cast by the user in the gel boat as shown in Figure 1, and a gel dam is cast at the reservoir end of the long arm of the cross. This dam, and the concentration gel, separates the sample from the buffer reservoir, forming the injection chamber.

Lysis is performed directly on either liquid or solid samples. There is no need to remove solid objects from the sample, or separate out particulates by filtration or centrifugation. Samples are re-suspended in lysis buffer, incubated, and poured, including any particulates, directly into the buffer chamber.

Buffer is added to the four chambers and an electrode plate is lowered into the buffer by the user. A DC electric field, generated by the instrument and applied along the injection chamber, drives negatively charged molecules, including contaminants and nucleic acids, into the gel. Rotating fields are then applied in the gel to specifically concentrate the nucleic acids at the center of the gel (Figure 1).

Once concentration is complete, a central column of gel can be extracted to recover the DNA with minimal contamination. Active control of electric fields is used to ensure that the focus location is reproducibly located in the center of the gel. Consequently, staining of the DNA is not required for regular processing.

Though the DNA is presently extracted in a gel, we find that this gel can be added directly to PCR mix for amplification and profiling. The first denaturing step of the PCR amplification melts the gel and releases the DNA, after which the sample remains liquid.

Instrument Design

System Overview

We have developed a prototype instrument that is capable of single-step DNA purification and concentration based on the SCODA method described above. The prototype and a functional schematic are shown in Figure 2. The instrument consists of a custom electrophoresis gel boat, a custom electrode plate to generate both injection and concentration electric fields in the gel boat, an evaporative temperature control system, electrophoresis power supplies, and a computer control interface. As sample handling steps in the SCODA protocol are limited to fluid transfer and easily automatable, this procedure and next-generation instrument would be well suited to a high throughput, fully automated platform.

Gel Boat

The custom fabricated gel boats consist of a 5 mL injection chamber, a concentration gel casting region, and four electrophoretic buffer reservoirs. Small samples can be diluted to 5 mL while

larger samples can be divided into 5 mL aliquots for multiple injections. Due to the inherent focusing process employed in SCODA, DNA entering the gel from multiple injections of different aliquots accumulates in the same focal region.

Due to the electrophoretic nature of the SCODA process, the DNA focuses at the geometric centre of the electrodes, rather than at the centre of the gel. Proper DNA extraction thus requires repeatable positioning between the gel boat and the electrodes. To enable this, the gel boat is designed with locating holes so the boat can be referenced to the electrodes in the same way each run.

Electrodes/Electrode Plate

Generation of rotating fields in the gel requires four electrodes surrounding the gel as previously described (9). In addition, a DNA injection electrode must be mounted at the rear of the sample chamber to allow DC injection into the concentration gel. Platinum electrodes are mounted on a custom designed printed circuit board with locating pins that mate to the gel boat for accurate positioning. The printed circuit board is mounted to a vertical stage which is lowered manually into the prepared gel boat.

Power Supplies

Voltages are applied at each of the four electrodes by custom built high power amplifiers capable of delivering +/- 90 V at 150 mA.

User interface

A simple LabVIEW (National Instruments, Austin, TX) program allows a user to control the SCODA instrument via a National Instruments data acquisition card. The program allows access to the high level functions of the instrument, allowing the user to select injection or concentration modes, applied voltage, period, and total run time. Additionally, the program provides feedback to the user, including errors in protocol and parameter selection, the engagement of safety interlocks, and gel temperature.

DNA Extraction Mechanism

The final element of the instrument is an extraction mechanism which allows the user to remove a small plug of gel (~20 µL) containing the concentrated DNA. The mechanism works independently of the electrophoresis instrument by referencing to the gel boat using the same locating holes used for the electrode plate. A 2-axis micrometer stage is used to precisely control the location of a guide through which a stainless steel tube is moved to punch and extract the desired gel core. Calibration of the extraction mechanism is performed by means of a control run with nucleic acid staining dye (*i.e.* SYBR Green, Invitrogen, Carlsbad, CA). Following this calibration, the extraction mechanisms can be used to repeatably extract DNA that has not been stained.

Methods

Sample Preparation

Mocked forensic casework samples were prepared by spiking various substrates, some containing known PCR inhibitors, with human blood to investigate SCODA's ability to efficiently extract purified DNA from the sample matrix for forensic identification. Our intent in the experiments presented here was not to provide an extensive comparison of SCODA to other purification techniques but to demonstrate proof-of-concept sample purification for this novel technology. Subsequent work will provide detailed performance comparison to other

existing methods. An initial demonstration set of samples were prepared by combining 0.5 -5 μ L of whole human blood and spiking into the following substrates, as described in Table 1.

Samples were prepared at the RCMP Forensic Laboratory and placed into 2 mL microcentrifuge tubes for storage at -20°C ; each sample was thawed just prior to lysis.

General Laboratory Preparation

Great care was taken to avoid contamination of samples. Experiments were prepared in a designated area kept free of post-PCR amplified products and other DNA sources. All surfaces and tools, including pipettes, were washed with a combination of 10% bleach and DNAZap™ (Ambion, Austin, TX). Care was taken to use only new and sealed consumables, such as microcentrifuge tubes that were certified nucleotide and nuclease-free, as well as barrier pipette tips (Diamond® filter, Gilson, Middleton, WI). Fresh nuclease-free reagents including concentrated 50x TAE buffer (2 M Tris-Acetate, 0.05 M EDTA, pH 8.3, Eppendorf, Westbury, NY) and ultrapure water (Nanopure Infinity, Barnstead, Dubuque, IA) were combined with SeaKem® Genetic Technology Grade™ (GTG) agarose (Cambrex/Lonza, East Rutherford, NJ) for preparation of SCODA gels and running buffer. The actual SCODA gel boat and instrument surfaces, including electrode plate, were cleaned prior to the experiments with a 10% bleach solution and DNAZap™.

Sample Lysis

Lysis buffer was prepared according to standard RCMP protocols (10) with slight modifications to create a stain extraction buffer (SEB) containing 10 mM Tris-Cl, 10 mM EDTA, 0.6% w/v DTT (D9779, DL-Dithiothreitol, Sigma-Aldrich, St. Louis, MO), and 1% w/v sarkosyl detergent (L9150, N-Lauroylsarcosine sodium salt, Sigma-Aldrich). The SEB was adjusted to pH 8.0 with concentrated HCl and stored at room temperature. Proteinase K (P6556, from *Tritirachium album*, Sigma-Aldrich) was diluted in 50 mM Tris-Cl (pH 8.0) and 1.5 mM Calcium Acetate to 10 mg/mL and stored in individual use aliquots of 50 μ L at -20°C .

One distinct advantage of the SCODA protocol is that the entire sample can be treated with lysis buffer and be placed into the injection chamber for DNA extraction without any fractionation of the sample. This procedure may reduce the possibility that nucleic acids of interest are lost during centrifugation or other means of solid sample matrix separation that are required for common spin column purification techniques.

Samples were thawed and lysis reagents (400 μ L of SEB and 50 μ L of 10 mg/mL Proteinase K) were added prior to mixing on a mini-benchtop vortexer. Tubes were sealed with Parafilm® (Alcan, Montreal, QC, Canada) and samples placed in 56°C waterbath for overnight lysis (16-20 hours).

SCODA Gel

With SCODA gel casting dams in place, a 1% agarose gel (SeaKem GTG with 0.5x TAE buffer) was cast and allowed to solidify at room temperature for 30 minutes, after which the dams were removed.

SCODA Injection

The entire lysate including any solid sample matrix (soil and swab, filter paper, FTA paper, denim) were transferred to the SCODA injection chamber as shown in Figure 3. The injection chamber was filled to 5 mL with dH_2O to minimize the conductivity of the sample. Remaining buffer reservoirs were filled with 0.5x TAE running buffer.

An injection field of 10V/cm was applied for 20 minutes to force nucleic acids to migrate into the concentration gel. This injection period was chosen so that the sample chamber would be depleted of all nucleic acids, which stack within the agarose gel for subsequent concentration. To remove any residual contaminants in the sample chamber, the remaining sample substrate and lysate were removed and the injection chamber was rinsed with dH₂O. Finally, all buffer chambers were refilled with 0.5x TAE running buffer. We envision this process will be automated in future prototype instruments.

SCODA Concentration

Concentration of the nucleic acids injected into the gel was achieved through application of rotating electric fields with a magnitude of 35% (of max scale using this prototype instrument). Each SCODA voltage was applied for a period of 2 seconds (total SCODA period of 24 seconds) for a total run duration of 4.5 hours.

Sample Extraction

After concentration, the DNA was extracted from the gel boat by manually cutting an agarose plug from the pre-calibrated center of the focusing fields. The gel plug was placed into a clean microcentrifuge tube and covered with RT-PCR grade water (Ambion) for storage at 4°C until quantification.

Quantification

Extracted DNA was evaluated through quantitative real-time polymerase chain reaction (qRT-PCR) using Applied Biosystems Quantifiler® Human DNA quantification kit (4343895, ABI, Foster City, CA) to determine the amount of amplifiable material recovered from each sample with SCODA. Each quantification reaction was prepared according to recommended ABI Quantifiler® protocols using a 25 µL total reaction volume (10.5 µL Quantifiler® Human Primer mix, 12.5 µL Quantifiler® PCR Reaction Mix, and 2 µL template) in a 96-well optical reaction plate (4306737, ABI). Control samples with a pre-formulated Human DNA standard from the Quantifiler® kit were added in duplicate to create a standard curve of 8 concentrations spanning 50 µg/µL to 23 pg/µL, along with four no-template controls (NTC).

The SCODA purified agarose blocks and storage buffer were melted in an 80°C waterbath for 5 minutes to homogenize the samples. In separate experiments we have used low melting point agarose to facilitate the homogenization and melting of samples prior to RT-PCR analysis with positive results. 2 µL of the homogenized sample was added to the qRT-PCR master mix for the unknown DNA concentration quantification reactions. The reaction plate was sealed with optical adhesive covers (4311971, ABI) and centrifuged at 3000 rpm/1800 rcf (5810R, Eppendorf) for 1 minute prior to loading into an Applied Biosystems 7500 RT-PCR instrument. Standard Quantifiler® conditions (hot start at 95°C for 10 minutes, then 40 repetitions of thermal cycling between 95°C for 15 seconds and 60°C for 1 minute) were run for the qRT-PCR amplification. Quantification was calculated by standard curve analysis generated by ABI Sequence Detection Software (version 1.3.1).

STR Profiling

DNA amplification of the template DNA (0.5 to 1.0 ng) was performed using the Applied Biosystems AmpFℓSTR® Profiler Plus™ (4303326, ABI) kit following the RCMP Automated Protocols (10) using a 15 µL PCR reaction volume (6.0 µL of template DNA, 5.7 µL of AmpFℓSTR® PCR reaction mix, 3.0 µL of AmpFℓSTR® PCR Profiler Plus™ primer set, and 0.3 µL of AmpliTaq Gold® DNA Polymerase, 4311816, ABI) for 28 cycles (hot start at 95°C for 11 minutes, denature at 94°C for 60 seconds, anneal at 59°C for 90 seconds, extend at 72°C for 90 seconds, final non-template extension at 60°C for 75 minutes). Samples were amplified

in a 96-well amplification plate (Diamed, Mississauga, ON, Canada) in an MJ Thermal Cycler (PTC-200, MJ Research, Waltham, MA).

Electrophoresis of the amplified DNA fragments was performed on an Applied Biosystems Prism® 3100 Genetic Analyzer. Amplicons (0.25 µL), denatured in 20 µL of Hi-Di Formamide and 0.5 µL of ROX, were electrokinetically injected at 3 kV for 10 seconds using POP-4 as the carrier polymer. The ABI Prism® 3100 Data Collection Software (version 2.0) was used. Sample file information generated by the 3100 Genetic Analyzer was analyzed using ABI GeneMapper® ID Software (version 3.2).

Results

Initial results from forensic sample purifications indicate that sufficient DNA for STR profiling was obtained from all of the samples tested and defined in Table 1. Our quantification results indicate that SCODA was able to purify between 2 - 100 ng of DNA from each sample, satisfying a conservative requirement of 1 ng DNA required for standard STR profiling (10). To demonstrate that the DNA was of acceptable yield and purity for profiling we ran several of the purified and quantified templates through the STR profiling procedure. A typical STR profile obtained from this analysis is demonstrated in Figure 4.

Strong signal peaks indicate the ability of the SCODA process to obtain amplifiable DNA from even highly contaminated samples; however, a low level of inhibition was observed in some of the larger loci of several samples. Recent modifications to the SCODA protocols that we present in this paper have improved contaminant rejection by not only preventing focusing of the contaminant, which was already prevented by the fundamental SCODA method, but by actively electrophoresing contaminants away from the DNA focus. Though beyond the scope of this paper, we have evidence that such methods will further improve forensic sample purification results. Furthermore, in this study, no efforts were made to optimize delivery of the concentrated DNA from standard agarose plugs into the PCR reactions. More recent work has indicated that the use of low melting point agarose and a simple sample preparation prior to direct loading into a PCR reaction can improve the yield of amplifiable DNA as well as reduction in contaminants that may inhibit subsequent PCR-based detection methodologies (data not shown). These results, including a comparison of SCODA purification to common alternative forensic sample preparation methods, will be the subject of future presentations of this technology.

Conclusions

We have demonstrated application of a novel form of non-linear AC electrophoresis to extraction of DNA from contaminated samples similar to problematic crime scene samples which arise frequently in forensic investigations. The prototype instrument presented is capable of processing such samples with only minor human intervention, and provides rejection of contaminants based on physical rather than chemical properties. In preliminary data presented here, and in other work not shown, this appears to lead to exceptional selectivity of extraction for nucleic acids, irrespective of the type of contaminant present in the sample. Consequently, we feel this method is an excellent platform for future development of automated and highly parallelized sample preparation instruments for forensics, which would be capable of providing highly pure DNA irrespective of the sample source and make-up.

Acknowledgements

Funding for this work has been provided by the Canadian Institute for Health Research through the Proof of Principle program under grant PPP-77272 and the National Institutes of Health through 1R01HG003640-01A1. The authors would like to thank the RCMP Forensic Laboratory Biology Section for providing mock samples, Quantifiler®

reagents, protocols, STR profiling, and analysis. We also acknowledge other members of UBC Applied Biophysics Laboratory for contributions to SCODA research and development not presented here.

Literature Cited

1. Improving Service Delivery. The Forensic Science Service. Report by the Comptroller and Auditor General, House of Commons. Mar 28. 2003 Available: http://www.nao.gov.uk/publications/nao_reports/02-03/0203523.pdf
2. Kontanis EJ. Evaluation of real-time PCR amplification efficiencies to detect PCR inhibitors. *J. Forensic Sci* 2006;51:795–204. [PubMed: 16882221]
3. Wilson IG. Inhibition and facilitation of nucleic acid amplification. *Appl. Environ. Microbiol* 1997;63:3741–51. [PubMed: 9327537]
4. Lakay FM, Botha A, Prior BA. Comparative analysis of environmental DNA extraction and purification methods from different humic acid-rich soils. *J. Appl. Microbiol* 2007;102:265–273. [PubMed: 17184343]
5. Larkin A, Harbison SA. An improved method for STR analysis of bloodstained denim. *Int. J. Legal Med* 1999;112:388–390. [PubMed: 10550601]
6. Del Rio SA, Marino MA, Belgrader P. PCR-based human leukocyte antigen (HLA) DQa typing of bloodstained light and dark denim fabric. *J. Forensic Sci* 1996;41:490–492. [PubMed: 8656191]
7. Eckhart L, Bach J, Ban J, Tschachler E. Melanin binds reversibly to thermostable DNA polymerase and inhibits its activity. *Biochem Biophys Res Commun* 2000;271(3):726–30. [PubMed: 10814530]
8. Akane A, Matsubara K, Nakamura H, Takahashi S, Kimura K. Identification of the heme compound copurified with deoxyribonucleic acid (DNA) from bloodstains, a major inhibitor of polymerase chain reaction (PCR) amplification. *J Forensic Sci* 1994;39:362–72. [PubMed: 8195750]
9. Marziali A, Pel J, Bizotto D, Whitehead LA. Novel electrophoresis mechanism based on synchronous alternating drag perturbation. *Electrophoresis* 2005;26:82–90. [PubMed: 15624147]
10. Poon, Hiron. Personal Communication. Royal Canadian Mounted Police Forensic Laboratory Services, Biology Services; Nov 23. 2006

Figure 1. Process flowchart for SCODA purification of nucleic acids from various sample substrates

Figure 2. Schematic of SCODA prototype instrument

Shown in detail are the gel boat, electrode plate, and temperature control mechanism, housed in the front of the instrument. Power supplies and other electronics are in the rear of the instrument for easy connection to a control computer.

Figure 3. Demonstration of SCODA's ability to directly purify DNA from unprocessed sample substrate

In this experiment a soil swab spiked with whole blood (5 μ L) was lysed (*upper left*) and the entire lysate, swab and soil were transferred to SCODA boat injection chamber (*upper right*) prior to injection (*lower*). After injection and concentration, DNA is extracted from the central focus (similar to step 6 in Figure 1; however, these samples were processed without fluorescent stain) and analyzed by qRT-PCR and STR profiling.

Figure 4. Representative STR profile from DNA extracted with SCODA. STR profile obtained from DNA purified from 0.5 μ L whole blood on soil swab

Table 1

Mocked forensic samples prepared for demonstration of SCODA DNA extraction

Substrate	Sample Size	Whole blood added to substrate
Sterile cotton swab dipped into 50 mL sample tube filled with raw soil and filtered, autoclaved, distilled (FAD) water	~0.1 g soil on 5 mm dia. × 15 mm sterile cotton swab	0.5 µL, 1 µL, 5 µL
FAD water deposited onto filter paper and air dried overnight	100 µL of FAD water deposited onto 3 cm diameter filter	0.5 µL, 1 µL, 5 µL
Whatman FTA® (Florham Park, NJ) paper	1 cm × 1 cm	1 µL, 2.5 µL
Blue denim fabric	1 cm × 1 cm	1 µL, 2.5 µL