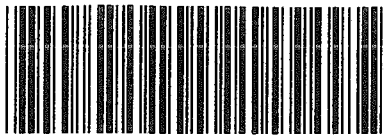

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A New Method for DNA and RNA Purification

John Watson, PhD Judith Schanke, PhD Haiying Grunenwald, PhD Ronald Meis, PhD
Les Hoffman, PhD Marlena Lewandowska-Skarbek, PhD Erich Moan, BS

While nucleic acid-based methodologies have become fully integrated into basic research laboratories, clinical laboratories have found much of the technology difficult to utilize for rapid, routine diagnostic procedures. In particular, current extraction protocols for DNA and RNA remain a cumbersome and time consuming obstacle to integrating molecular technology into the clinical laboratory. We describe a unique, rapid, and rigorous method for obtaining highly purified RNA and DNA from eukaryotic, bacterial, and viral samples. The procedure incorporates: 1) ionic detergent-based cellular disruption, 2) salt precipitation of the proteins, and 3) precipitation of the nucleic acids. Where desired, treatment with RNase-free DNase I eliminates DNA from RNA preparations, while treatment with RNase A provides DNA free of intact RNA. DNA or RNA can be purified in less than 60 minutes. This procedure was used to extract nucleic acids from hepatitis C virus (HCV) positive human plasma, *Saccharomyces cerevisiae*, *Staphylococcus aureus*, a mouse tail snip, tissue culture cells, and from human serum mock-infected with *E. coli*. The nucleic acids obtained were free of contaminating protein as determined by UV spectrophotometry. The functional integrity of the extracted nucleic acids was confirmed using polymerase chain reaction (PCR) of a tetranucleotide repeat marker from human DNA and the gene encoding the 16S ribosomal RNA from *E. coli*. The quality of the RNA prepared using this method was assayed using reverse transcription-PCR (RT-PCR) to amplify HCV RNA from human serum samples. The nucleic acid purification protocol described here is commercially available and provides research, clinical, forensic, and pharmaceutical laboratories with a user friendly, non-hazardous method for extracting DNA or RNA from a variety of sources.
Keywords: DNA; RNA; Purification; Extraction; Isolation; Forensics; Molecular; Diagnostics.
Journal of Clinical Ligand Assay 1998;21:394–403.

Introduction

The extraction of nucleic acids is a crucial but poorly studied technology in molecular biology laboratories. Any purification technology must optimize: lysis of the nucleic acid-containing sample; removal of proteins, lipids, and carbohydrates; removal of contaminating small solutes; and concentration of the nucleic acid. While adequate protocols for performing DNA and RNA purifications have existed for some time (*1*,*2*), the post-genomics era requires improved methodologies. In particular, methods are needed that have wide applicability, reduce hazardous material production, involve fewer manipulations, and can be performed by less rigorously trained personnel.

A number of emerging fields are driving the development of rapid nucleic acid purification protocols. All of them share the need to take molecular methods that were originally developed by highly trained researchers and adapt them for routine use. For example, hospital laboratories are beginning to offer probe and amplification technologies. These laboratories require purification methods that can extract eukaryotic, bacterial, fungal, and viral nucleic acids. Forensic laboratories require highly reliable, reproducible, and extremely sensitive extraction protocols (*3*). Pharmaceutical companies have begun using microbial pathogen genotyping and patient genotyping to predict pharmaceutical outcomes (*4*). Epidemiology and clinical genetics investigators often have minimal training in molecular techniques. Researchers in these fields would benefit from a rapid, facile method for purifying nucleic acids.

Most currently available methodologies for extracting nucleic acids are not sufficiently versatile to be useful for the wide variety of applications that require pure DNA or RNA. Those methods that are suitable for extracting nucleic acids from a wide variety of samples typically require the use of hazardous organic solvents such as phenol and/or chloroform (*1*,*2*). Other methods, based on columns, require multiple manipulations between microcentrifuge tubes and columns that can lead to cross-contamination of samples. The simplest methods currently available are based on using an ionic detergent to disrupt cellular membranes followed by the elimination of proteins by precipitation with a high salt buffer (*5*). The primary limitation of this method is that it is not useful for extracting RNA or DNA from limited

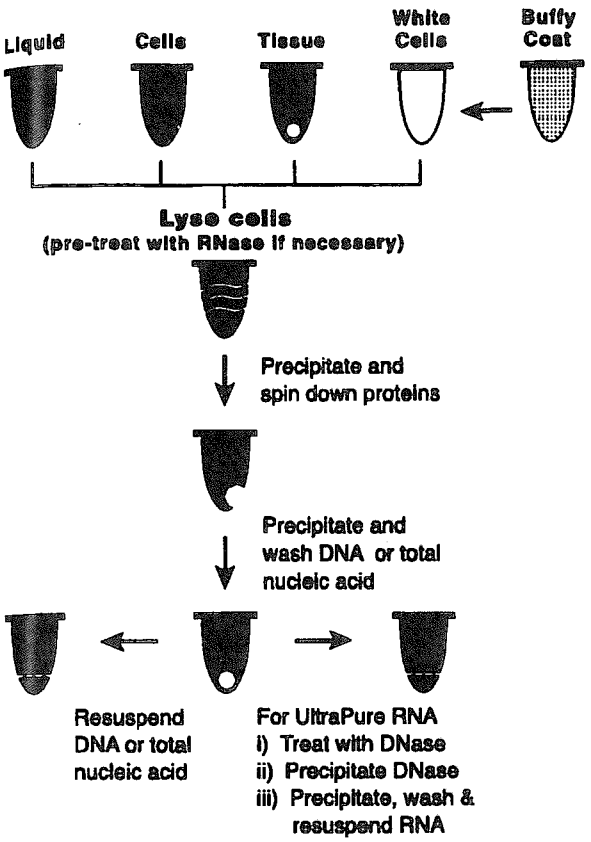

Fig. 1. *Schematic diagram of the DNA, RNA, and total nucleic acid (TNA) purification protocols.*

amounts of starting material.

The objective of this study was to adapt a salt precipitation method so that it would be useful for samples containing small amounts of DNA. A co-precipitant polymer was incorporated into our method that quantitatively precipitates DNA and RNA. For the applications tested, the co-precipitant does not interfere with subsequent use of the nucleic acids. By including either an RNase or DNase treatment, we have developed a rapid method for the extraction of total nucleic acid (TNA), pure DNA, or pure RNA from a large variety of starting materials, including those with only a trace amount of nucleic acid (Fig. 1).

Materials

Nucleic Acid Extractions: High-performance liquid chromatography (HPLC) grade isopropanol and ethanol were purchased from Fisher Scientific. All other components for the DNA extraction kit were from the MasterPure™ Complete DNA and RNA Purification Kit (Epicentre Technologies, Madison, WI).

Polymerase Chain Reaction (PCR) Amplifications: An MJ PTC-200 thermocycler was used for all PCR amplifications. Primers were obtained from Operon (Alameda, CA). All components for PCR including MasterAmp™ 2X PCR Optimization Buffer, MasterAmp Reverse Transcription-PCR (RT-PCR) Kit, MasterAmp Taq, MasterAmp Amplitherm™ and RetroAmp™ RT-DNA Polymerase were obtained from Epicentre Technologies. Agarose gels were stained with ethidium bromide (Sigma, St. Louis, MO) or SYBR Gold (Molecular Probes, Eugene, OR).

DNA Sequencing: DNA sequencing was performed on a LI-COR™ Model 4000 Automated DNA Sequencer (LI-COR Incorporated, Lincoln, NE) using Long Ranger™ acrylamide (FMC, Rockland, ME). Sequencing reactions were performed using the SequiTherm EXCEL™ II Long-Read™ DNA Sequencing Kit-LC (Epicentre Technologies).

Methods

Purification of TNA— Part A: Cell Lysis (Fig. 1):

E. coli Liquid Culture: One hundred µL of an overnight culture were centrifuged at 13,500 x*g* for 2 minutes in a microcentrifuge at 4°C . The supernatant was removed by decanting. Pelleted cells were vortex mixed briefly and resuspended in 300 µL of tissue and cell lysis buffer containing 50 µg proteinase K. The sample was incubated at 65°C for 15 minutes with vortex mixing every 5 minutes and then processed as described in Part B below.

S. aureus from Culture Plates: A sterile toothpick was used to remove *S. aureus* cells from the appropriate culture plate. The toothpick was dipped into 300 µL of tissue and cell lysis buffer containing 50 µg proteinase K. The sample was incubated at 65°C for 15 minutes with vortex mixing every 5 minutes, then processed as described in Part B below.

HL-60 Tissue Culture Cells: One million cells of the HL-60 human tissue culture cell line were pelleted by centrifugation at 13,500 x*g* for 5 minutes. The cell pellet was resuspended in 300 µL of tissue and cell lysis buffer. Proteinase K treatment was not necessary for the lysis of HL-60 cells. Lysed cells were processed as described in Part B below.

Liver Tissue and Maize: Three hundred µL of tissue and cell lysis buffer containing 50 µg of proteinase K were added to 2 mg samples of bovine liver tissue or maize leaf and homogenized for 30 seconds at 4°C. The sample was incubated at 65°C for 30 minutes with vortex mixing every 5 minutes and then processed as described in Part B below.

Buccal (Cheek) Cells: Buccal cells were isolated from volunteer subjects using a cytology brush. The brush was rotated 20 times, with pressure, on the inside cheek of the volunteers. The brush was then dipped into 500 µL of 10 mM Tris, 1 mM EDTA (TE) buffer in a microcentrifuge tube and rotated while pressing against the side of the tube to release the buccal cells into the buffer. The cells were pelleted by centrifu-

gation at 13,500 x*g* for 5 minutes. The supernatant was removed by decanting and the buccal cells were vortex mixed briefly and resuspended in 300 µL of tissue and cell lysis buffer containing 50 µg proteinase K. The sample was incubated at 65°C for 30 minutes with vortex mixing every 5 minutes and then processed as described in Part B below.

MOUSE TAIL: A 10 µg sample of mouse tail tissue was sliced finely using a razor blade and resuspended in 300 µL of tissue and cell lysis buffer containing 50 µg of proteinase K, and homogenized for 30 seconds at 4°C. The sample was incubated at 65°C for 30 minutes with vortex mixing every 5 minutes and then processed as described in Part B below.

YEAST: Yeast RNA was isolated by removing a single 2 mm colony of *S. cerevisiae* from a culture plate and mixing into 300 µL of tissue and cell lysis buffer containing 50 µg proteinase K. The sample was incubated at 65°C for 30 minutes with vortex mixing every 5 minutes and then processed as described in Part B below. Yeast total nucleic acid was isolated by centrifuging 1.5 mL of an overnight culture. The pellet was resuspended in 300 µL of 2X tissue and cell lysis buffer containing 50 µg proteinase K. The sample was incubated at 65°C for 30 minutes with vortex mixing every 5 minutes and then processed as described in Part B below.

SALIVA: Saliva (150 µL) was diluted with 150 µL 2X tissue and cell lysis buffer containing 50 µg proteinase K. The sample was incubated at 65°C for 30 minutes with vortex mixing every 5 minutes and then processed as described in Part B below.

PLASMA: A sterile toothpick was used to pick a single colony of *E. coli* from a culture plate used to mock-infect 1 mL of plasma. Forty µL of plasma were then added to 300 µL of tissue and cell lysis buffer containing 50 µg proteinase K. The sample was incubated at 65°C for 15 minutes with vortex mixing every 5 minutes and then processed as described in Part B below, except 170 µL of protein precipitation reagent and 600 µL of isopropanol were used in the nucleic acid extraction steps.

WHOLE BLOOD-RAPID MICRO PROTOCOL: For extraction of whole blood without prior lysis of the red cells, the following protocol was used:

Plasma containing hepatitis C virus (HCV) was introduced into whole blood using the following protocol: Two hundred microliters of whole blood were centrifuged and 75 µL of plasma were removed. The plasma was replaced with 75 µL of plasma containing HCV RNA (obtained from Dr. Yury Khudyakov and Dr. Howard Fields, Hepatitis Branch, Centers for Disease Control and Prevention, Atlanta, GA). The resulting mock-infected whole blood sample was vortex mixed to resuspend the pelleted cells. Nucleic acids were extracted by transferring 9.5 µL of whole blood to 300 µL of tissue and cell lysis buffer containing 50 µg proteinase K per sample. The sample was incubated at 65°C for 15 minutes with vortex mixing every 5 minutes and then processed as described in Part B below except that 155 µL of protein precipitation reagent was used in the nucleic acid extraction steps.

WHOLE BLOOD MACRO PROTOCOL-FROM BUFFY COATS: For isolation of large amounts of genomic DNA from blood the following protocol was used:

Blood was drawn into EDTA tubes. Five milliliters of blood were centrifuged at 4200 x*g* for 10 minutes in a swinging bucket rotor to generate a white cell-containing buffy coat interface between the plasma and the red cell layers (buffy coats could also be generated by leaving the tubes overnight at room temperature). Six hundred µL of buffy coat were selectively drawn by pipetting at the interface between the white and red cell layers. If the buffy coat layer was ambiguous, sample was drawn from the uppermost part of the red cell layer. Three hundred µL of white cell sample were added to 1,200 µL red cell lysis buffer, the sample was mixed by vortexing, and then incubated at room temperature for 10 minutes. The red cell lysate was removed by centrifuging the sample at 13,500 x*g* for 2 minutes and aspirating the supernatant. The white cell pellet was resuspended in 600 µL of cell and tissue lysis buffer (without proteinase K treatment). Lysed cells were processed as described in Part B, except that 300 µL of protein precipitation reagent and 750 µL of isopropanol were used in the nucleic acid extraction steps.

PARAFFIN-EMBEDDED TISSUE: DNA was isolated from 20 mg of a 35 micron thick paraffin-embedded sample. (Thin paraffin sections allow the best recovery and quickest extraction times). Five milliliters of xylene were added and the mixture was incubated for 10 minutes to extract the paraffin. The xylene was decanted and the extraction was repeated. Five milliliters of 100% ethanol were added and incubated with the tissue for 10 minutes. The ethanol was decanted and the extraction repeated. The remaining traces of ethanol were removed by aspiration. The tissue was resuspended in 300 µL of tissue and cell lysis buffer containing 2 µL of 50 µg/µL proteinase K. The tissue was homogenized and incubated at 37°C for 30 minutes and then processed as described in Part B.

PCR AMPLICON PURIFICATION: Fifty µL of a PCR amplification product from the lambda cII gene were diluted to 150 µL (final volume) with water. After addition of 150 µL of 2X tissue and cell lysis buffer, the sample was vortex mixed and processed as described in Part B.

Part B: Protein and Nucleic Acid Precipitation (for all samples except where noted above): After cell lysis (Part A), all samples were placed on ice for 3

minutes. One-half volume (typically 150 µL) of protein precipitation reagent was then added, the samples were vortex mixed, and the proteins were pelleted by centrifugation at 13,500 x*g* for 7–10 minutes at 4°C. The nucleic acid-containing supernatant was decanted into a new microcentrifuge tube, and 500 µL of isopropanol were added. Samples were inverted 30–40 times and then centrifuged at 13,500 x*g* for 10 minutes. After centrifugation, the isopropanol was decanted.

Part C: Washing and Resuspension of Nucleic Acids: The nucleic acid pellet (containing DNA and/or RNA, depending on the desired product) was washed gently twice with 70% ethanol. All residual ethanol was removed by aspiration and the pellet was resuspended in 50 µL of TE buffer.

Purification of DNA Without Contaminating RNA: To eliminate RNA, the protocol was performed identically to the TNA protocol with the following modification. After Part A of the TNA protocol, the sample was equilibrated to 37°C prior to the addition of 1.0 µL of 5 µg/µL RNase A per 300 µL of tissue and cell lysis buffer. The sample was incubated for 10–30 minutes at 37°C. Sample was then processed as described in Parts B and C of the TNA protocol.

Purification of RNA Without Contaminating DNA: To eliminate DNA from RNA preparations, the protocol was performed identically to the TNA protocol with the following modification. After Part B of the above protocol, the tube was centrifuged briefly and the last traces of isopropanol were removed by aspiration. The nucleic acid-containing pellet was resuspended in 200 µL of 25 units/mL RNase-free DNase I and incubated for 10–30 minutes at 37°C. After incubation, 200 µL of 2X tissue and cell lysis buffer were added to the samples. The samples were then vortex mixed and placed on ice for 3 minutes. Two hundred microliters of protein precipitation reagent was then added and the proteins were pelleted by centrifugation at 13,500 x*g* for 7–10 minutes at 4°C. The nucleic acid-containing supernatant was decanted into a fresh microcentrifuge tube and 500 µL of isopropanol was added. Samples were then inverted 30–40 times and centrifuged at 13,500 x*g* for 10 minutes. After centrifugation, the isopropanol was decanted. The sample was then processed as described in Part C of the TNA protocol.

PCR Amplification: 16S RIBOSOMAL DNA AMPLIFICATION: TNA from the *E. coli* mock-infected human plasma sample was used as the template for PCR amplification of a 350 bp region of the 16S ribosomal gene. One nanogram of *E. coli* DNA was used as a positive control for PCR amplification. The amplification reactions included 1X PCR buffer, 3 mM $MgCl_2$, 1X MasterAmp PCR Enhancer (with betaine), 200 µM dNTPs, 50 pmoles each *E. coli*-specific 16S rDNA primer (5'AGAGTTTGATCCTGGCTCAG3', 5'CTGCTGCCTCCCGTAGGAGT3' [6]), 1.25 units MasterAmp Taq DNA polymerase, and 1 µL isolated sample or 1 ng *E. coli* DNA control template. A reaction with no DNA template (a negative control reaction to check for *E. coli* DNA contamination) was also performed. Reactions were denatured for 4 minutes at 94°C and then cycled 25 times using the following conditions: 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute. Five microliters of the sample were separated by agarose gel electrophoresis, stained with ethidium bromide, and visualized by transillumination.

CHLC STR MARKER AMPLIFICATION: Three short tandem repeat (STR) markers from the Cooperative Human Linkage Center (CHLC) family of markers (*7*) were amplified using the following protocol. The 50 µL reactions contained 1 µL of DNA, 25 pmoles of the six primers, 25 µL 2X MasterAmp PCR PreMix G, and 1.25 units of MasterAmp Taq DNA polymerase. Reactions were denatured for 2 minutes at 94°C and were cycled 30 times with the following conditions: 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute; followed by a single step of 72°C for 4 minutes. Five microliters of the sample (10%) were separated by agarose gel electrophoresis, stained with ethidium bromide, and visualized by transillumination.

HEMOCHROMATOSIS AMPLIFICATION: A common mutation leading to hemochromatosis can be detected by PCR-restriction fragment length polymorphism analysis (RFLP) (*8*). One µL of DNA from a saliva sample was added to a 50 µL PCR reaction containing 50 pmoles of each primer, 25 µL 2X MasterAmp PCR PreMix E, and 1.0 unit of MasterAmp Taq DNA polymerase. Reactions were denatured for 5 minutes at 95°C and were cycled 38 times using the following conditions: 96°C for 0.5 minute, 55°C for 0.5 minute, and 72°C for 1 minute; followed by a single step of 72°C for 7 minutes. Five microliters of the sample (10%) were separated by agarose gel electrophoresis, stained with ethidium bromide, and visualized by UV transillumination.

FACTOR V DNA AMPLIFICATION FROM BREAST CANCER TISSUE PARAFFIN SECTIONS: One microliter of the TNA sample was used as template to amplify a 267 bp region of the Factor V gene. The reaction contained 1X MasterAmp PreMix B, 50 pmoles each Factor V primer (5'TGTTATCACTGGTGCTAA3', 5'TGCCCAAGTGCTTAACAAGACCA3') and 1.25 units of MasterAmp Amplitherm DNA polymerase. Forty cycles of amplification were performed with the following profile: 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 45 seconds. Five microliters of the sample (10%) were separated on a 2% agarose gel, stained with ethidium bromide, and vi-

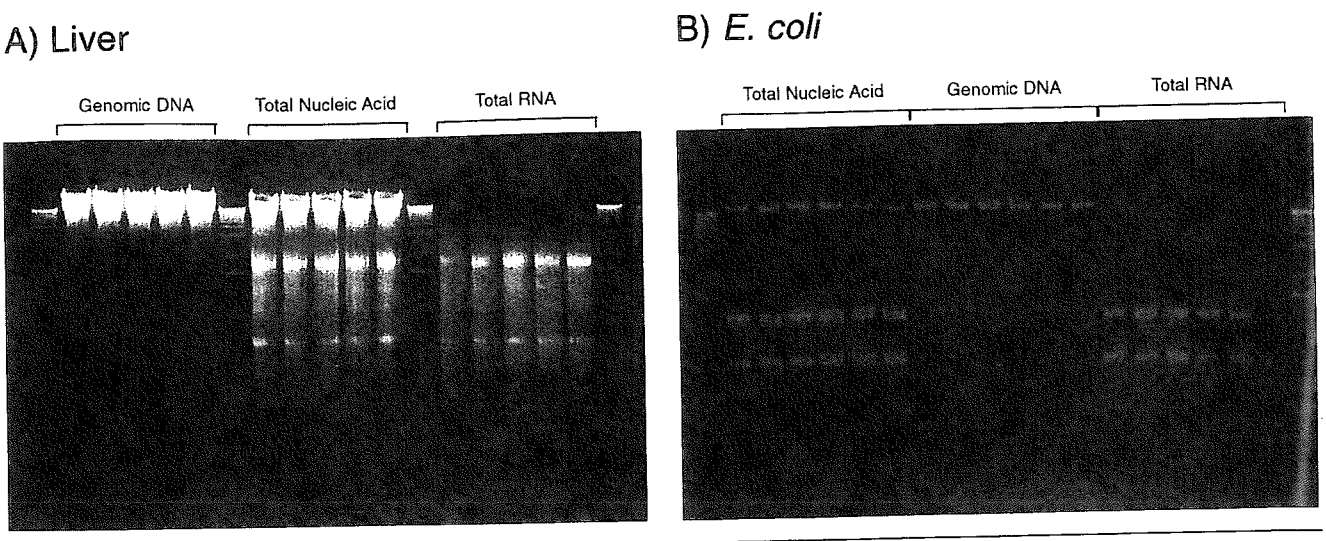

Fig. 2. *Purification of DNA, RNA, and TNA from bovine liver and* E. coli. *DNA , RNA, and TNA were isolated from bovine liver samples using the protocol described in the text. The purified liver (**A**), and* E. coli *(**B**) nucleic acids were resolved by electrophoresis on a 2% agarose gel and stained with ethidium bromide. Unlabeled lanes are DNA size markers.*

sualized by transillumination.

LAMBDA cII AMPLIFICATION: One nanogram of lambda DNA was used as template to amplify a 466 bp region of the cII gene. Samples were heated to 94°C and MasterAmp AmpliTherm DNA polymerase was added. Samples were then incubated at 94°C for 4 minutes, followed by 35 cycles of 1 minute at 94°C, 1 minute at 55°C, and 1 minute at 73°C, followed by 4 minutes at 73°C. The reaction contained 1X MasterAmp PCR PreMix B, 50 pmoles each Factor V primer (5'AAAAAGGGCATCAAATTAAACC3', 5'CCGAAGTTGAGTATTTTTGCTGT3'), and 1.25 units MasterAmp AmpliTherm DNA polymerase. Five microliters of sample were separated on a 2% agarose gel, stained with ethidium bromide, and visualized by transillumination.

RT-PCR Amplification: A 296 bp region of the highly conserved 5' noncoding region of HCV was used to detect the presence of HCV virus in the plasma sample. The RT-PCR reaction contained 5 µL of the isolated RNA (containing 300–400 copies of HCV), 1X RT-PCR Buffer, 3 mM $MgCl_2$, 0.5 mM $MnSO_4$, 2X MasterAmp PCR Enhancer (with betaine), 12.5 pmoles each of the HCV RT-F (5'CTGTGAGGAACTACTGTCTTC3') and HCV RT-R (5'GGTGCACGGTCTACGAGACCT3') primers, 400 µM each dNTP, and 2.5 units RetroAmp RT DNA Polymerase. Reverse transcription was performed at 60°C for 20 minutes followed by 40 cycles of 92°C for 30 seconds, 55°C for 1 minute, and 72°C for 1 minute. Ten microliters (20%) of the RT-PCR product were separated on a 2% agarose gel, stained with SYBR Gold, and visualized by transillumination.

DNA Sequencing: 6.8 µL of the purified lambda cII PCR product (see above) was added to a standard SequiTherm EXCEL II cycle sequencing reaction. An IRD41 labeled primer to the cII gene of lambda (5'AAAAAGGGCATCAAATTAAACC3') was used for priming of the sequencing reactions on a LI-COR Automated DNA Sequencer. Cycling parameters included a template denaturation step of 95°C for 5 minutes, followed by 30 cycles of: 95°C for 30 seconds, 50°C for 15 seconds, and 70°C for 1 minute. 1.2 µL of the sequencing reaction were resolved by electrophoresis on a 6% Long Ranger gel.

Results

Analysis of Sample-to-Sample Extraction Variability: One of the greatest concerns when performing nucleic acid extractions is variability in performing the extraction method. This is of particular concern when attempting quantitative detection of microorganisms. To determine if this method would result in consistent nucleic acid recovery, we performed multiple extractions from five liver samples and six *E. coli* samples for TNA, DNA, and RNA as described in the methods section. Purified samples were visualized on agarose gels (Fig. 2). Both the liver and *E. coli* samples showed distinct ribosomal RNA bands (TNA and RNA samples) and genomic DNA bands (TNA and DNA samples) by ethidium bromide staining. Samples were assayed for the presence of contaminating proteins by assaying the $OD_{260/280}$ ratios spectrophotometrically. In a typical experiment $OD_{260/280}$ ratios were 2.05, 2.08 for TNA, 1.86, 2.07 for DNA and 1.89, 1.89 for RNA. The only time that OD ratios less than 1.8 were observed was when an excess of starting

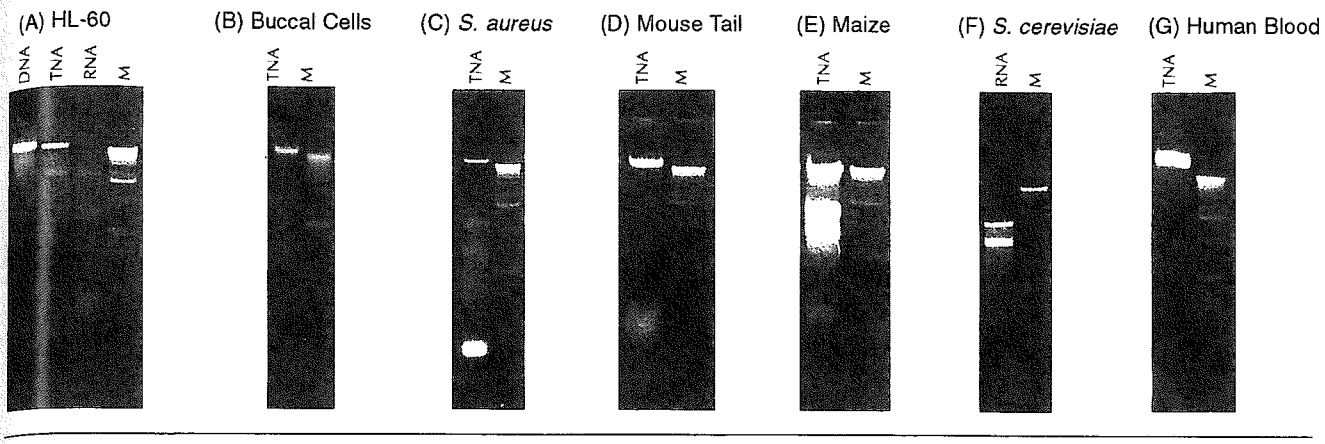

Fig. 3. *Purification of nucleic acids from multiple sample types. TNA, DNA, and RNA were purified from HL-60 human tissue culture cells (A), TNA was purified from human buccal (cheek) cells (B),* S. aureus *(C), mouse tail (D), maize (E),* S. cerevisiae *(F, note only RNA was visible—see text), and whole blood (G). Fifteen µL samples were run on 2% agarose gels with DNA size markers (M, shown for each gel) and stained with ethidium bromide.*

material was used in the extraction (data not shown). In all cases, lowering the amount of starting material resulted in extraction of nucleic acids without protein contamination.

Extraction of Nucleic Acids from Multiple Sample Types: Molecular laboratories often need to use a number of extraction methods for isolating nucleic acids from the various materials they analyze. To determine the applicability of our method to various sample matrices, we extracted nucleic acids from HL-60 human tissue culture cells, *S. aureus*, mouse tail, maize, human buccal cells, *S. cerevisiae*, and whole blood (Fig. 3). The standard protocol performed well for purification of total nucleic acids from all samples except yeast; a slight modification of our standard protocol was required to efficiently recover yeast DNA (see Methods).

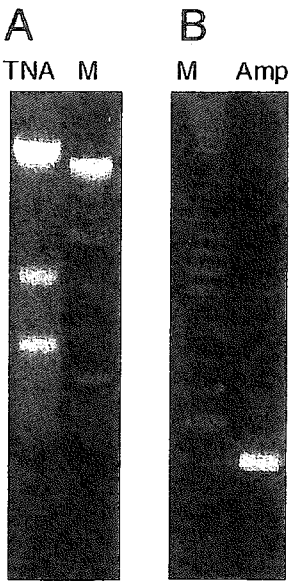

Fig. 4. *Purification of nucleic acids directly from liquid samples. TNA was purified from 150 µL of saliva as described in the text. Fifteen µL of TNA sample (TNA) or 10 µL of DNA size markers (M, Kb ladder, Gibco) were resolved by electrophoresis onto a 2% agarose gel prior to staining with ethidium bromide (A). Five microliters of the saliva TNA sample was used to amplify a region of the Factor V gene, 10 µL of DNA size markers (M, 100 bp ladder, Bayou Labs) (B).*

Direct Isolation of Nucleic Acids from Liquid Samples: In many cases it is difficult, or impossible, to pellet the material of interest from a liquid sample prior to the extraction of nucleic acids. To accommodate liquid samples, we made the tissue and cell lysis buffer at twice the usual concentration and diluted the liquid sample with an equal volume of the 2X lysis buffer. Following this modification, liquid samples could be treated identically to pelleted samples (see Methods). Total nucleic acid recovered from saliva was visualized on an ethidium bromide stained gel (Fig. 4A) and used for PCR amplification of the hemachromatosis gene (Fig. 4B; HFE gene, forward primer 5'TGGCAAGGGTAAACAGATCC, reverse primer 5'CTCAGGCACTCCTCTCAACC). It should be noted that for samples with high protein concentration (such as plasma) the capacity of the protein precipitation step of the protocol will be exceeded when using a one-to-one dilution with the 2X tissue and cell lysis buffer. In this case, a smaller amount of starting sample should be used in the extraction (see plasma protocol).

Restriction Endonuclease Digestion of Total Nucleic Acids: To determine if the isolated DNA could be digested by a restriction enzyme, 500 ng of a yeast DNA sample were digested with 1 unit each of *Kpn* I and *Hind* III restriction enzyme for 60 minutes at 37°C in 15 µL total volume. The entire sample was

Fig. 5. *Fifteen μL of yeast TNA isolated using a modified protocol (see methods) was digested with 1 unit each of* Hind *III or* Kpn *I for 1 hour. The TNA was resolved by electrophoresis on a 1% agarose gel and visualized by ethidium bromide staining (R). DNA size markers are indicated (M).*

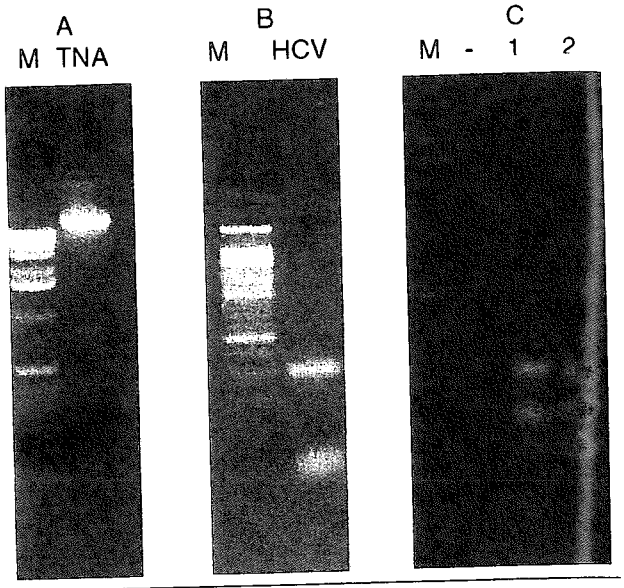

Fig. 6. *Amplification of DNA and RNA isolated from hepatitis C virus (HCV) positive whole blood. TNA was purified directly from whole blood without prior lysis of red blood cells and visualized by ethidium bromide staining of an agarose gel (**A**). The nucleic acid sample was then amplified to detect the presence of HCV RNA (**B**, HCV) or Cooperative Human Linkage Center (CHLC) short tandem repeat (STR) markers of human genomic DNA (**C**,2). A positive control of the human DNA was also amplified (**C**,1). DNA size markers are indicated (M).*

then resolved by electrophoresis on a 1% agarose gel and the DNA was visualized by ethidium bromide staining (Fig. 5).

Use of a Single TNA Sample For RNA or DNA Amplification: Most salt precipitation-based nucleic acid purification methods require a red cell lysis step prior to nucleic acid extraction from the white blood cells (*5*). Elimination of the red cell lysis step is advantageous for processing large numbers of samples in which purification of small amounts of nucleic acids are required for analysis, as in pathogen screening of the blood supply. To determine if the method could be modified to extract nucleic acid from whole blood without eliminating red blood cells, decreasing amounts of whole blood were extracted using this protocol. The maximum amount of whole blood that could be added without saturating the protein precipitation step of the protocol was 9.5 μL (data not shown). Whole blood was doped with HCV-containing plasma prior to extraction of the total nucleic acid (Fig. 6A). PCR amplification was performed using the same total nucleic acid sample to detect the presence of the HCV genomic RNA (Fig. 6B), and for molecular typing of the blood donor by CHLC STR size marker analysis (Fig. 6C) (*7*).

Detection of Bacteremia in Plasma: Most samples arriving in a clinical laboratory, and many archived samples, exist as serum or plasma. To determine whether microbial nucleic acids could be extracted directly from a plasma sample, a single colony of *E. coli* was mock-infected into plasma. The total nucleic acid was extracted and visualized on an ethidium bromide stained agarose gel (Fig. 7A). PCR amplification using primers to *E. coli* ribosomal DNA was performed and confirmed that the nucleic acid con-

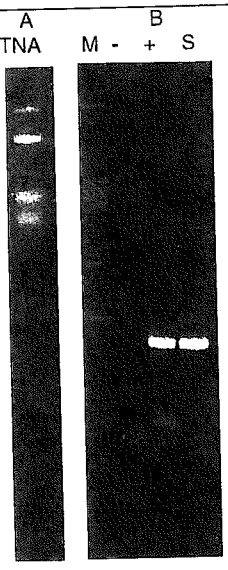

Fig. 7. *Detection of* E. coli *in human plasma. A single colony of* E. coli *was mock-infected into human plasma and TNA was isolated from the serum and visualized by ethidium bromide staining of an agarose gel (**A**). The nucleic acid sample was then amplified using* E. coli *specific ribosomal primers (**B**, S). A positive control of* E. coli *DNA was also amplified (**B**,+). A negative control containing no DNA did not amplify (**B**,-). DNA size markers are indicated (M).*

Fig. 8. *Amplification of the Factor V gene from a paraffin-embedded tissue sample. TNA was isolated from a paraffin-embedded specimen of a human breast tumor. The purified nucleic acid was then used to amplify a region of the Factor V gene (FV). DNA size markers are indicated (M).*

tained microbial DNA (Fig. 7B).

Extraction of DNA from Paraffin-embedded Tissue: Paraffin-embedded tissues are among the most challenging materials from which to extract nucleic acids (*9*). Crosslinking appears to substantially reduce the average size of the extractable nucleic acid to less than 100 bp. However, since PCR requires only a few copies of intact DNA in the sample, it is often feasible to amplify from even very degraded samples. After nucleic acid extraction of a paraffin-embedded breast tissue sample, a 287 bp region of the Factor V gene could be amplified (Fig. 8). It should be cautioned that larger fragments may be more difficult to amplify due to the reduced probability of extracting long nucleic acids from paraffin-embedded tissues.

Purification of PCR Product Prior to Cycle Sequencing: The presence of unincorporated deoxynucleotides after PCR amplification interferes with the direct use of the PCR products for cycle sequencing. To determine whether our method would separate a PCR amplicon away from free nucleotides, we purified a PCR product from the cII region of lambda (Fig. 9A). We then compared the ability of the purified vs unpurified amplicon to function as a template in a cycle sequencing reaction (Fig. 9B). Clearly, our method was able to eliminate inhibitory substances (most likely nucleotides) from the PCR product.

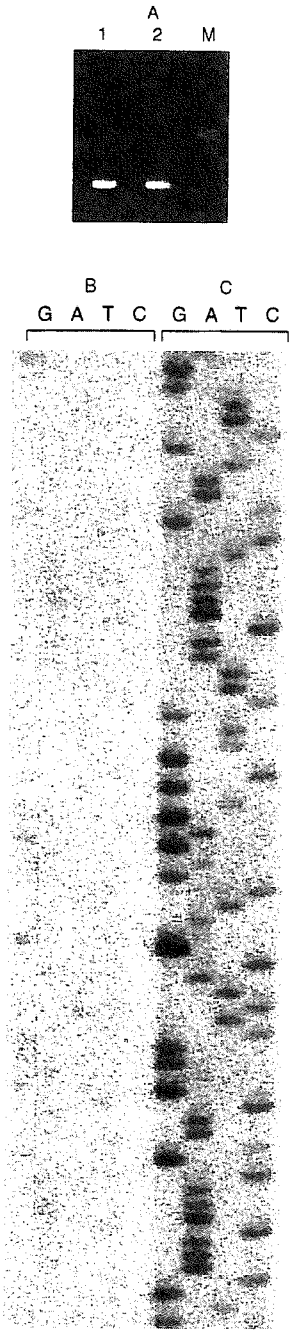

Fig. 9. *Purification of a PCR product prior to cycle sequencing. Fifty microliters of an amplification reaction of the lambda cII gene were diluted to 150 µL total volume and then extracted as a liquid sample. Fifteen µL of the starting sample (**A**, 2) and 15 µL of the purified sample (**A**, 1) were resolved by electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining. DNA size markers are indicated (**A**, M). One microliter of the starting sample (**B**) and the purified sample (**C**) were used as a template for a cycle sequencing reaction. DNA size markers are indicated (M).*

Discussion

One of the challenges in the post-genomics era is to bring the methods of molecular biology to laboratories that may not have the resources or personnel necessary to perform the techniques as they currently exist. In particular, hospital, forensic, public health,

and pharmaceutical laboratories have only recently begun to routinely incorporate molecular methods into their standard protocols. Although the extraction of nucleic acids has been a routine method in biomedical research laboratories for decades, it is still a laborious and variable technique. The purpose of this investigation was to develop a simple, rapid, widely applicable method for nucleic acid extraction.

The most significant variable in any nucleic acid purification protocol is the matrix of the starting material. It is often necessary to optimize the initial disruption of the material to be extracted. In this study, most of the tissues investigated were disrupted by treatment at 37°C for 30 minutes with 0.17 µg /mL of proteinase K in the presence of a detergent. However, some samples required modification to the initial steps of the protocol. For example: paraffin-embedded tissues required elimination of the paraffin using xylene; tissue samples required homogenization for maximum yield; processing of large amounts of blood required the elimination of the red cells; and extraction from plasma required reducing the volume of the starting material. We have found that the standard protocol can be used for extracting nucleic acid from virtually any biological sample if the amount of starting material is sufficiently reduced. Since the method gives nearly quantitative recovery from even very small samples, reducing the sample size will typically still produce enough DNA or RNA for amplification. If larger amounts of nucleic acid are needed the investigator can easily scale up the protocol. All that is necessary is to keep the ratios of buffer to starting material consistent.

One of the advantages of this new method is that after the initial disruption step, all materials can be processed using essentially the same protocol. This minimizes personnel training time and reduces costs associated with purchasing separate reagents for multiple protocols. All of the starting biological materials we have tested have been tractable, in some cases with slight modification, to extraction of their nucleic acids. Although we tested a limited variety of samples, the full range of biological materials that can be used for nucleic acid extraction with this technology can only be determined over time. An unexpected finding was that nucleic acids could be purified from Gram-positive bacteria and yeast, since the literature suggests that these organisms typically require a lytic enzyme for disruption of the cell wall. We were able to purify TNA from both of these types of microorganisms by treatment with proteinase K in the presence of a detergent. Although no attempt was made to determine nucleic acid yields in this study, preliminary data with yeast suggest that our method is more efficient than other commercially available kits in extracting DNA from yeast (Eric Moan, unpublished data).

The quality of the nucleic acid purified using our new method was assayed by spectrophotometry, ethidium bromide-staining of agarose gels, restriction enzyme digestion, PCR amplification, and cycle sequencing. The nucleic acid obtained was free of contaminating protein based on measured $OD_{260/280}$ ratios greater than 1.8. While the RNase-treated DNA was free of intact RNA, small fragments of RNA could be seen in some preparations (Fig. 2). The contaminating RNA does not appear to interfere with any functional applications of the DNA tested. Previously, DNase treatment has been shown to be a highly effective method for removing contaminating DNA from RNA preparations (*10*). With the new method, a brief DNase treatment was typically sufficient to remove all intact DNA as determined by ethidium bromide staining of agarose gels. It should not be assumed that quantitative removal of the DNA fragments was effected by the brief DNase treatment. In situations where DNA contamination must be minimized, the amount of DNase used, or the incubation time, can be increased. It should be noted that for many applications, TNA can be substituted for either purified RNA or purified DNA. One advantage of using TNA is that the purification protocol is shorter.

A number of methods have been described for the extraction of nucleic acids. One of the most common methods involves the use of proteinase K and SDS to facilitate cell lysis followed by phenol:chloroform extractions for eliminating proteins (*1,11*). Another method, typically used for RNA purifications, involves the use of guanidinium thiocyanate for cell lysis and differential partitioning of proteins and nucleic acids in phenol:chloroform at low pH (*2*). Boom and coworkers have described a method that combines the use of guanidinium thiocyanate with selective adsorption of nucleic acids to silica particles (*12*). A number of other protocols have been described including methods that use lithium chloride (*13*), polyethylene glycol (*14*), CTAB (*15*), sodium perchlorate (*16*), and caprylic acid (*17*).

The method described in this report is based on the salting-out protocol described by Miller and coworkers which uses SDS to lyse cells followed by the addition of high salt to precipitate SDS:protein complexes (*5*). The nucleic acid is then precipitated in the presence of isopropanol. Advantages of the original method include that it is simple to perform, rapid, requires no hazardous materials, and generates very pure nucleic acids. Commercial kits based on the Miller protocol have become a standard method for extracting DNA from human blood in the clinical laboratory. The salting-out method has found only

limited use in other applications, primarily due to its limited applicability to samples containing very small amounts of nucleic acid (*18*). By incorporating a co-precipitant we were able to purify very small amounts of either DNA or RNA. This method (patent pending) combines the speed, ease, and safety of a salt precipitation method with the ability to extract DNA or RNA from the smallest of samples. It should be of particular use to clinical, forensic, and environmental laboratories that require a technically simple, sensitive method that can be used on a wide variety of samples.

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From Epicentre Technologies, Madison, WI.

Correspondence and reprint requests to: John Watson, PhD, Epicentre Technologies, 1202 Ann St., Madison, WI 53713.