Extraction of Nucleic Acids from Challenging Biological Samples Using Pressure Cycling Technology

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Abstract

The widespread of genomic research and rising attention to micro-RNA function in living systems emphasis the need for reproducible and quantitative recovery of nucleic acids form biological samples. We found that, during an exposure of cells and tissues to cycles of alternating hydrostatic pressure, biomolecules, such as nucleic acids, proteins, lipids and small molecules can be efficiently solubilized into a suitable lysis buffer. Barocycler NEP3229, an instrument designed to facilitate sample processing using Pressure Cycling Technology (PCT), cycles hydrostatic pressure between ambient and 235 MPa within a 3 second rise time and millisecond pressure drop time. Sample extraction is performed in single-use dedicated containers called PULSE Tubes. Up to 0.5 g solid or 1.5 mL liquid samples can be loaded in a single PULSE Tube, while up to three tubes can be processed simultaneously. The composition of the lysis buffer, pressure cycle, and process temperature (4-37 ºC) conditions can be adjusted and optimized for specific applications.

Here, recent discoveries in the extraction of nucleic acids from important yet challenging biological samples are presented. First, PCT can be beneficial for the extraction of low abundance nucleic acids from hard-to-break materials, such as bone fragments, teeth, hair and skin. Suitable amounts of DNA have been extracted from extremely small quantities of samples, such as single hair or small bloodstain spot on a single cotton fiber. In the absence of pulverization and substantial de-calcification, DNA molecules were successfully extracted from exhumed ancient bone fragments for sequence analysis. PCT extracted DNA retains their integrity and results in little shearing due to the procedure. Secondly, a key benefit of PCT extraction is that suspicious infectious samples can be safely processed in a closed system. For example, in suspected bioterror attacks, it is critical to rapidly identify the terror agent to both treat exposed victims and to respond to the perpetrators of the attack. Using a model system for *B. anthracis*, the causative agent of anthrax, the PCT SPS was used to release DNA from *Bacillus subtilis* spores and vegetative cells. The PCT SPS effectively released high quality, amplifiable, and detectable DNA from these specimens. Third, we demonstrated that DNA released by PCT was amplified directly by PCR without prior purification. Amplified DNA prepared by the PCT SPS was suitable for a variety of downstream methods and analyses. Finally, PCT sample preparation method has also been shown capable of extracting RNA in high quality and preserving RNA from degradation by endogenous RNase activity for genetic expression microarray and miRNA analyses.

Application of PCT in Nucleic Acid Extraction

Fig. 1. The PCT Sample Preparation System includes an instrument, the Barocycler NEP3229, and specially designed PULSE Tubes. An illustration of the PCT extraction process is shown. → points to the lysis disk.

The Barocycler NEP3229 can raise pressure from ambient to 35 kpsi (235 MPa) in less than 5 seconds and ramp down pressure from its maximum to ambient instantaneously. Three PULSE Tubes can be processed simultaneously. The process temperature can be controlled using an external circulating water bath, allowing experiments to be performed between 4 and 37°C.

PULSE Tubes FT500 can hold 20 – 500 mg of solid sample or 1.2 – 1.5 mL of liquid sample. The sealed PULSE Tubes are immersed in pressurization fluid in the two reaction chambers of the Barocycler NEP3229 and are then subjected to cycled pressure. The recovered volume of PCT prepared samples is between 0.8 and 1.4 mL.

Bone Fragments

To optimize the release of DNA from bone fragments by PCT, a series of time course experiments were conducted by incubating bone fragments in various acids for 0 min to 12 hours at ambient pressure prior to PCT treatment. Optimum DNA release was obtained with a 60 min incubation in 1% acetic acid (pH 4.8), followed by PCT treatment (see Figure 2). Negative controls were incubated in buffer, but not exposed to PCT.

DNA released from PCT-processed bone was compared to DNA released from bone that had been first chilled in liquid nitrogen and subsequently pulverized using a standard tissue pulverizer (BioSpect Products, Bartlesville, OK). Each extract was purified using a QIAGEN DNeasy Kit (Germantown, MD) according to the manufacturer's instructions. The resulting DNA was amplified by PCR using primers for pig β-actin DNA. PCR products were analyzed using a 2100 BioAnalyzer (Agilent, Palo Alto, CA). Yield of DNA was determined semi-quantitatively by integrating the DNA PCR products obtained using the BioAnalyzer.

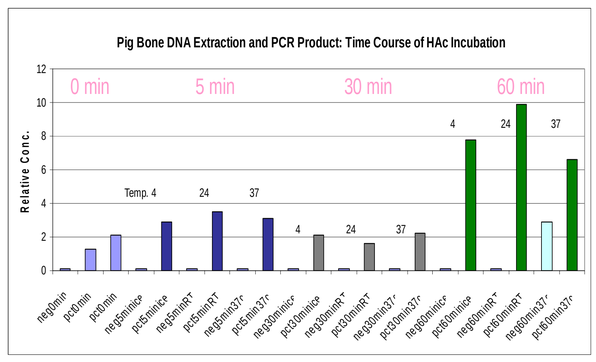

Figure 2. Optimization of the release of DNA from bone by PCT. Incubation ranged from 0, 5, 30 and 60 min. Temperatures were set at 4°, 24° or 37°C. Relative concentration was obtained based on the PCR products analyzed using the Agilent BioAnalyzer.

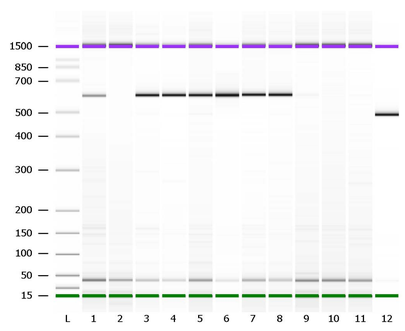

Figure 3. PCR products examined using the Agilent BioAnalyzer. Lanes 1-8 were from samples incubated with acetic acid (pH 4.9) and saturated EDTA for 60 min and followed by 10 cycles of PCT at 4°C. Lanes 9-11 were incubated with acetic acid for 1 hr but no PCT. Lanes 12 was a PCR positive control.

Step 1. Wash bone samples, break large piece to a size that can fit in the PULSE Tube

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Step 2. Load bone fragment in PULSE Tube, add acetic acid solution (saturated EDTA, pH 4.8 adjusted with acetic acid) and incubate for 1 hour at room temperature

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Step 3. PCT Treatment. 10 cycles between 35 kpsi for 20 sec. and ambient for 20 sec. at room temperature

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Step 4. Purify DNA using DNeasy tissue kit

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Step 5. DNA amplification and sequence analysis

Figure 4. Work flow of PCT Extraction of bone fragments

Single Hair

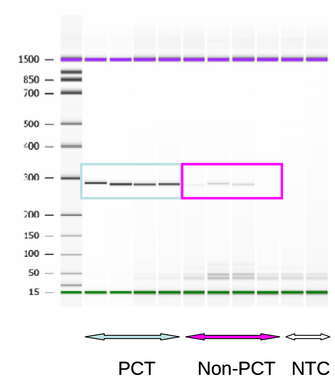

Figure 5. mtDNA PCR products from single human hair samples with PCT (10 cycles) or Non-PCT treated control samples and PCR no-template-controls (NTC).

Skin Tape

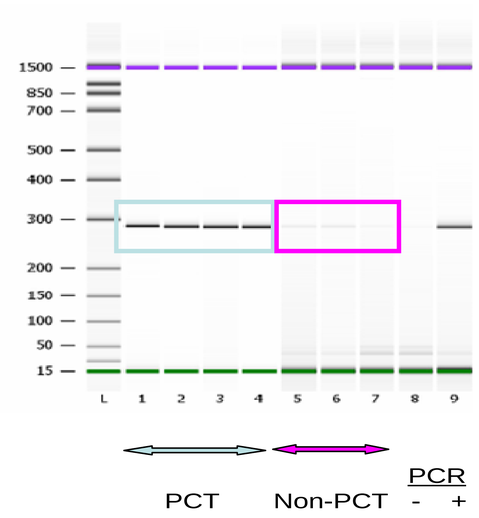

Figure 6. mtDNA PCR products from epidermal cells collected on Scotch Tape. PCT (10 cycles, Lanes 1-4) or Non-PCT (Lanes 5-7) treated control samples, no-template (Lane 8) and positive (Lane 9) PCR controls. Note: it is not necessary to remove cells from the tape prior to PCT processing.

Blood on Cloth

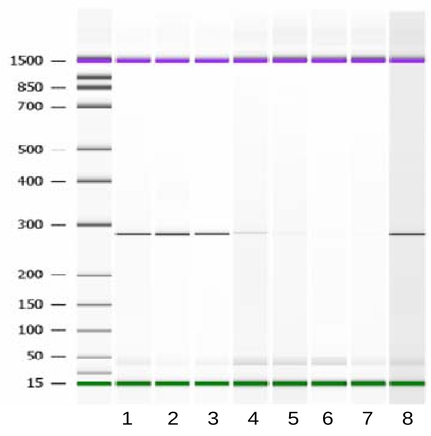

Figure 7. mtDNA PCR products from whole blood stained on single fiber. Lanes 1-2, PCT treated (5 cycles); Lane 3, bead beating control using Mini-cell Disrupter (BioSpec Products, Bartlesville, OK); Lane 4, non-PCT or non-BB treated blood-stained fiber control sample; Lane 5, no blood stained cloth control (no mechanical extraction); Lanes 6-7, no-template PCR control; and Lane 8, PCR positive control. Note: it is not necessary to isolate the blood stain from cloth or fiber prior to PCT processing.

Figures 5-7 show the release of mtDNA from a single human hair, skin tape, and blood stained fiber, respectively. In all experiments, mtDNA was amplified in a PCR reaction without carrying out additional purification. These experiments show that after only 5-10 minutes of sample processing by PCT, mtDNA is ready for downstream applications, thus significantly reducing the time to result. Control experiment results from these experiments show that little or no mtDNA is released from any of these samples without the application of PCT.

Antibioterrorism

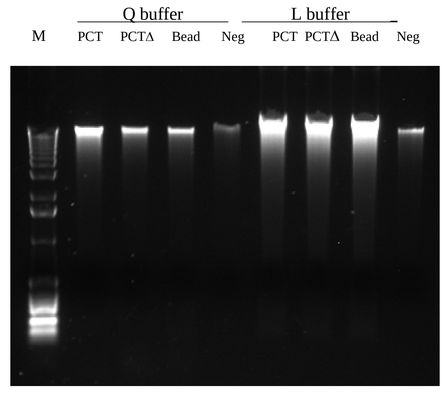

Figure 8. Agarose gel shows the genomic DNA extracted from *Bacillus subtilis* spores using two different extraction buffer (Q and L). Extractions using PCT and bead beating methods were compared.

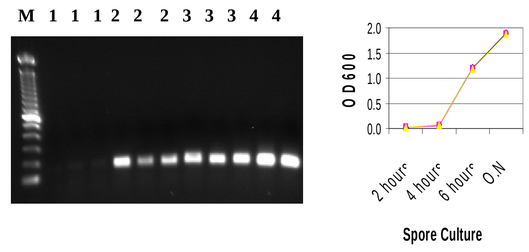

Figure 9. The sensitivity of DNA detection was improved by pre-incubation of *B. subtilis* spore culture in PULSE Tubes and by PCT extraction and PCR amplification. Lanes labeled 1-4 correspond to PCR products from pre-incubated spores for 0, 2, 4 and 6 hours at room temperature.

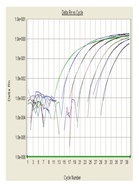

Figure 10. A real-time PCR assay is shown using serial diluted *B. subtilis* DNA in the effort of establishment of a real-time PCR quantification assay to determine the PCT extraction efficiency.

Summary

A series of studies were carried out in extracting DNA from B. subtilis spores as a model for detection of bioterrorism agents in our laboratory. These include pre-extraction of spore in media cultures in PULSE Tube, followed by direct extraction and amplification. We are currently optimizing PCT conditions to rapidly detect low copy number of sporal DNA on various matrices, such as filter and dust, along with a real-time PCR quantification assay.

The PCT Sample Preparation System Extraction Method:

- employs a new approach for the extraction of biomolecules from cells and tissues, including difficult-to-lyse materials, such as whole pieces of bone fragment.
- functions by different mechanisms to release biomolecules compared to other commonly used mechanical and chemical extraction methods
- is rapid, safe and reproducible
- is fully contained to minimize cross contamination and the exposure of operators to toxic or infectious agents
- is capable of releasing small molecules, nucleic acids, and proteins into buffers suitable for downstream purification or direct analysis

References

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