© Published in R. Winter ed. *Advances in High Pressure Bioscience and Biotechnology II*, Springer, New York, page 413-417

Biological Sample Preparation System Using Pressure Cycling Technology (PCT)

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Abstract. Boston Biomedica Inc., (BBI) has developed a proprietary Pressure Cycling Technology (PCT) based Sample Preparation System (PCT SPS) that utilizes a specially designed instrument (Barocycler™) and reaction containers (PULSE™ Tubes) to apply cycles of hydrostatic pressure to samples. The initial application of the PCT SPS is to liberate molecules from a variety of biological samples, including animal and plant tissues, microbes, and cultured cells. The typical extraction process is accomplished in five minutes or less with as few as five cycles of pressure ranging between atmospheric and 235 MPa. The lysate can then be withdrawn from the PULSE Tubes for downstream analysis, further processing or storage for future use. This System is suitable for genomic and proteomic applications.

1 Introduction

Effective release of biomolecules from tissues and cells is of critical importance in modern biotechnology, not only because it is often the first step in nearly all multi-step processes, but also because the quantity and quality of released biomolecules can profoundly affect the success of downstream applications [1,2].

Current mechanisms for extractions in analytical and preparative applications are either mechanically or chemically-based, or a combination of both. However, there can be a number of problems associated with the use of these processes, e.g. sample containment, cross-contamination, shearing of molecules-of-interest, and limited applicability to different sample types. Thus, new and improved extraction methods are needed to advance discoveries more rapidly.

BBI scientists and engineers have developed a simple, fast, safe, effective, and reproducible process to release cellular contents from biological samples using a patented system. This process employs cycles of pressure between ambient and high levels, e.g. 235MPa. It is capable of processing both solid tissues and liquid cultured cells, including a variety of hard-to-lyse materials. Five one-minute cycles have been found to be sufficent for processing most sample types. Materials

extracted by the PCT SPS are then ready to be used with many nucleic acids and protein purification methods and analytical applications.

2 Materials and Methods

The PCT SPS applies cyclic hydrostatic pressure (ambient to high) to samples contained in single-use reaction containers, i.e., PULSE (Pressure Utilized to Lyse Samples for Extraction) Tubes (Figure 1), using a Barocycler instrument. Both the PULSE Tubes and Barocycler are designed and manufactured by BBI Source Scientific, Inc., Garden Grove, California. The results shown here were performed using a Barocycler Model NEP2017, which has two pressure chambers, each holding up to three FT500 PULSE Tubes.

PCT extraction was compared to common processing procedures, such as mortar-pestle grinding, bead beating, and enzymatic digestion. In each experiment, non-PCT treated negative controls were also run. To estimate the extraction efficiency or relative yields, several downstream purification or analytical procedures were carried out with the samples obtained by the PCT SPS, as well as with the controls. A schematic diagram of the tests is shown in Scheme 1.

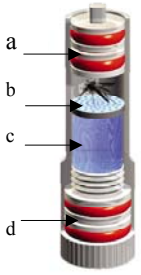
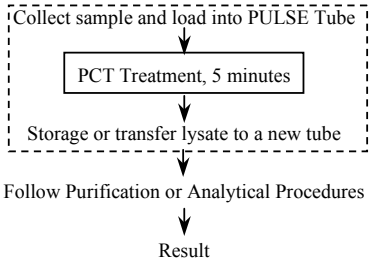


Fig. 1. Diagram of a PULSE Tube: a. ram, b. lysis disk (with multiple holes), c. collection chamber, d. cap



Scheme 1. Flow chart of PCT SPS process

An example of a typical pressure profile for the PCT SPS process is presented here. Fresh corn sprouts were collected and kept at –70°C prior to experimentation. Samples (0.20 ± 0.03g) were processed in FT500 PULSE Tubes with 1.1 mL lysis buffer (saturated guanidinium HCl and 1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate or CHAPS, pH not adjusted). Both pressure chambers were filled with hydrolic fluid, and equilibrated to 4°C (± 1) using a circulating waterbath. Pressure was cycled five times between ambient and various high levels. Pressure was kept constant at both ambient and high levels for 20 sec. each. Pressure rise and drop times were within 5 and 1 sec. respectively.

PCT treated samples were transferred to a microcentrifuge tube and particulates were clarified by centrifugation at 13,000×g, 4°C for 2 min. Supernatants (400 µL) were mixed with 7 µL RNase A stock (supplied in QIAGEN DNeasy Plant mini

kit) incubated at 65 ºC for 10 min. 130 µL buffer AP2 was then added. The mixture was purified with QIAGEN columns using the procedure described in the kit. DNA was eluted in 100 µL AE buffer and electrophoresed in a 1% agarose gel to indicate quality. Yield of DNA was determined by measurement at OD_{260}.

3 Results

Table 1 provides a list of examples of biological materials and downstream applications for which the PCT SPS process has been proven successful. Data generated from the experiments summarized in the list indicate that PCT can be used to process a wide variety of samples, such as animal and plant tissues, insects, blood, and microbes. The quality and quantity of molecules released from these biological materials by the PCT SPS were comparable or better than those obtained using conventional methods (due to page limits, detailed data are not shown).

Table 1. Biological samples that have been extracted using PCT SPS

Sample Group	Examples	Materials Extracted	Applications
Animal tissues	Soft: liver, brain, pancreas, spleen, kidney, lung	DNA, RNA[1], Proteins[2]	PCR, RT-PCR, ELISA, SDS-PAGE, Western Blot
	Hard: tail, heart, intestine, skeletal muscle, breast tumor, aorta	DNA, RNA[1]	PCR, RT-PCR
Plant tissues	Soft: corn sprout, leaves (corn, tomato, Juniper, wheat), grape skin	DNA, RNA[1], Proteins[2]	PCR, RT-PCR, SDS-PAGE
	Hard: stem, pine needle, grape seed	DNA	PCR
Insects, small organisms	mosquito, fruit fly, mealworm, tick	DNA, RNA[1]	PCR, RT-PCR
Blood	Blood, blood spot	DNA	PCR
Microbes	Yeast, *C. elegans*	DNA, RNA, Proteins	PCR, RT-PCR, SDS-PAGE
	mycobacteria, bacteria/spores, soil	DNA, RNA[1]	PCR

[1, 2] Not every example has been tested for RNA or proteins and subsequent RT-PCR or WB, ELISA.

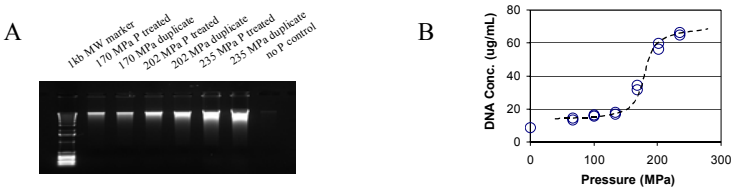


Fig. 2. Pressure profile from corn sprouts processed by the PCT SPS. A. 1% agarose gel showing purified genomic DNA from the PCT SPS extracted samples and a negative control sample. B. Pressure profile of the extracted and purified DNA

Shown in Fig. 2 is the pressure profile for processing corn sprouts. DNA yields obtained at 235 MPa (4 °C, 5 cycles) are comparable to those obtained by the mortar-pestle extraction method. This profile for corn sprouts is similar to other sample types tested to date. None of those required pressures in excess of 235 MPa for the efficient release of nucleic acids or proteins. However, higher pressures may be required for some samples or applications not yet evaluated. Optimum extraction efficiency for samples may be achieved at different pressure levels or with different cycle patterns. In addition, the choice of the processing buffer was found to significantly impact the release of biomolecules, particularly from certain hard-to-lyse samples. For some applications, processes at higher or lower temperatures were found to be even more efficient in releasing cellular contents.

4 Summary

The PCT SPS is a powerful, enabling platform technology that can be applied to a number of biotechnology applications, including the safe and efficient extraction of nucleic acids and proteins from a variety of biological materials.

Several possible mechanisms-of-action may be involved in the release by PCT of biomolecules from samples: 1) pressing tissues against the lysis disk, 2) tissue maceration as the sample passes through the lysis disk, thus increasing the surface area of tissues, 3) fast pressure changes resulting in rapid expansion of dissolved gases inside cells, causing disruption of membranes or cell walls, 4) pressure driven dissociation of macromolecular complexes and protein denaturation [3], 5) freeze-thaw and/or salt crystallization-dissolution cycles disrupting cells, if the process is controlled at appropriate temperature and pressure cycling rates, 6) asymmetric pressure effects on different parts of the sample, 7) the combination use of organic solvents and pressure to dissolve water-insoluble components, 8) increasing solubility due to the PCT process, especially at higher temperatures, and 9) local cavitation due to increased dissolution with pressure pulses.

The PCT SPS is ideal for centralized service and research laboratories, since it can be shared by scientists studying widely different subjects. Some of the technical advantages of the PCT SPS include: 1) versatility – this system is suitable for both solid and liquid samples, including hard-to-lyse materials; 2) rapidity – five 1 minute cycles are sufficient for most sample types; 3) safety – samples are sealed in individual PULSE Tubes, free from cross-contamination, aerosolization, and leakage; 4) efficiency – up to six samples can be processed simultaneously; 5) flexibility – custom selection of lysis buffers can be available for compatibility with downstream purification methods and analyses; 6) scalability – a wide range of weight or volume of biological material can be processed; 7) gentleness – little or no shearing of nucleic acids is observed and protein function can be maintained; 8) precision and reproducibility – the process is completely computer-controlled; and 9) temperature control – useful for preseving biomolecular activity.

Acknowledgements: The authors wish to thank the National Institutes of Health for their support of this work through their SBIR Program.

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