Introduction of a Bench Top Instrument Employing Pressure Cycling Technology (PCT) for the Extraction of Nucleic Acids and Proteins

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Abstract

Sample preparation is a bottleneck limiting rapid discovery in genomic and proteomic research. To address this issue, a novel sample preparation system based on Pressure Cycling Technology (PCT) was developed by Boston Biomedica and introduced at PITTCON 2002. This enabling technology uses pulses of pressure to control biomolecular interactions.

The PCT Sample Preparation System (PCT SPS) is comprised of an instrument, a Barocycler™, and single-use sample processing containers (PULSE™ Tubes). The PCT SPS applies cyclic high and low hydrostatic pressure (between ambient and 35,000 psi) to samples - disrupting tissues, cells and cellular structures to release their contents. The floor model instrument, the NEP2017, is primarily an R&D tool for centralized laboratory use and has generated important discoveries in genomics and proteomics. This instrument is controlled manually or by computer; temperature is regulated by a water bath.

Here we introduce a new option to the PCT SPS – a portable Bench Top instrument. This instrument can process up to three samples simultaneously. Designed for researchers in their own laboratory, the "basic" instrument can be "enhanced" with features of the NEP2017 on request. Data are shown comparing the NEP2017 to the Bench Top instrument (the Barocycler NEP3229), and to current methods (mortar and pestle grinding and bead beating) for the release of DNA, RNA and proteins from animals and plants. The NEP3229 was evaluated for quantity and quality of released nucleic acids and proteins. Nucleic acids were assessed by gel electrophoresis, PCR amplification and microarray analysis. Protein preparations were evaluated using 1D and 2D gels and assessed for additional proteins and protein complexes. Data show that the NEP3229 is comparable to the NEP2017 in quality and quantity of released products, versatility, reproducibility, and safety.

PCT Sample Preparation System (PCT SPS)

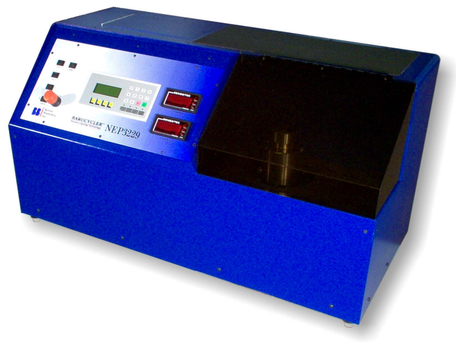
The PCT SPS uses either an automated, floor model instrument (the Barocycler NEP2017) or a semi-automated, Bench Top model (the Barocycler NEP3229), together with single-use sample processing containers (PULSE Tubes).

The Barocycler NEP2017 can ramp up pressure from atmospheric pressure to 35 kpsi (235 MPa) in about 3 seconds and ramp down pressure from its maximum to ambient in a fraction of a second. Up to six 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. The instrument is controlled manually or by computer.

Fig 1. Barocycler™ NEP2017

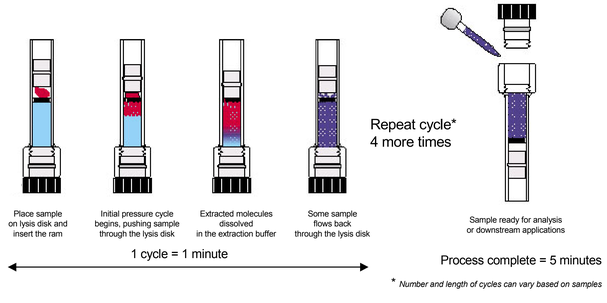
BBI now offers a Bench Top option (Barocycler NEP3229) to the PCT SPS. The NEP3229 is available in both "basic" and "enhanced" versions: the basic version has an external chiller hook-up and a key pad (Programmable Logic Controller, or PLC) to set various PCT parameters, while the enhanced version offers features such as computer control, automatic fill and dispense valves, and a chiller. The NEP3229 is a lightweight, portable, and affordable instrument designed to fit on a laboratory bench top, inside a biological safety cabinet, or on the shelf of a cold room. This instrument can also ramp up pressure from atmospheric pressure to 35 kpsi (235 MPa) in about 3 seconds and ramp down pressure from its maximum to ambient in a fraction of a second. Up to three PULSE Tubes can be processed simultaneously.

Fig. 2. Barocycler™ NEP3229



PULSE Tubes can hold up to 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 reaction chambers of the Barocycler NEP2017 or the Barocycler NEP3229 and are then subjected to cycled pressure.

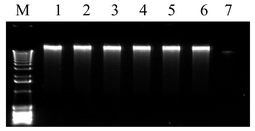
Fig. 3. Illustration of Sample Preparation in a PULSE Tube



Results

Here we show representative data from Corn Sprouts, Rat Liver, and Zebrafish comparing the Barocycler NEP2017 to the Barocycler NEP3229 and to conventional preparative methods for the release of DNA, RNA, or protein. The results are compared to those obtained from extraction methods using grinding by mortar and pestle or bead beating.

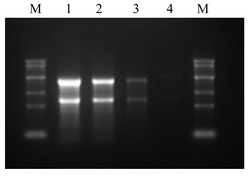
Fig. 4. DNA Released from Corn Sprouts



Lane	Method	Instrument	Ave. Yield (ug/g)
1, 2	PCT	NEP 2017	39
3, 4	PCT	NEP 3229	40
5, 6	Mortar & Pestle		60
7	Negative		1

200 mg samples were processed in 4M GTC, 1% NP40, RT
PCT: 5 cycles (0.5'↑, 0.5' ↓), 35 kpsi (235 MPa)

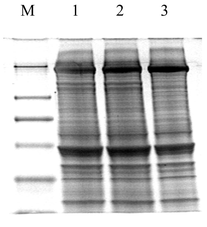
Fig. 5. RNA Released from Rat Liver



1	PCT	NEP 2017	98 ug
2	PCT	NEP 3229	49 ug
3	Bead Beating		28 ug
4	Negative		2 ug

200 mg samples were processed in 4M GTC, 1% NP40, RT
PCT: 5 cycles (0.5'↑, 0.5' ↓), 35 kpsi (235 MPa)

Fig. 6. Protein Released from Zebrafish



Lane	Method	Instrument	Yield (per g fish)
1	PCT	NEP 2017	83 mg
2	PCT	NEP 3229	80 mg
3	Mortar & Pestle		61 mg
	Negative		11 mg

Whole fish were processed in 0.1 M Tris, 0.15 M KCl, 1 nM PMSF, protease inhibitor cocktail, RT. PCT: 5 cycles (0.5'↑, 0.5' ↓), 35 kpsi (235 MPa)

Table 1 shows a comprehensive list of samples processed by PCT and various methods of downstream analyses applied to the released biomolecules.

Table 1. Samples Processed by PCT

Sample Group	Examples	Molecules Extracted[1,2]	Applications
Animal tissues	Soft: liver, brain, pancreas, kidney, lung, skin, zebra fish, shrimp	DNA, RNA, Proteins	PCR, RT-PCR, ELISA, SDS-PAGE, 2D gel, Western blot
	Hard: heart, skeletal muscle, breast tumor, aorta	DNA, RNA	PCR, RT-PCR
Plant tissues	Soft: corn sprout, leaves (corn, tomato, juniper, wheat), grape skin	DNA, RNA, Proteins	PCR, RT-PCR, SDS-PAGE
	Hard: stem, pine needle, seeds from wheat, grape	DNA	PCR
Insects, small organisms	Mosquito, *C. elegans*	DNA, RNA, protein	PCR, RT-PCR, SDS-PAGE
Blood	Blood, blood spot	DNA	PCR
Microbes	Yeast, Aspergillus	DNA, RNA, Proteins	PCR, RT-PCR, SDS-PAGE
	Mycobacteria sp., bacteria/spores, soil	DNA, RNA	PCR, 2D gel

[1]Not every example has been tested for RNA or proteins with RT-PCR, WB or ELISA.
[2]Comparable yields were generally achieved in these experiments by comparing to either one or two conventional methods.

Discussion

Boston Biomedica, Inc. has developed an automated Pressure Cycling Technology Sample Preparation System (PCT SPS) to alleviate the bottleneck of sample preparation. The PCT SPS uses cycles of pressure between ambient and up to 35,000 psi (235 MPa) to extract DNA, RNA, small molecules, and proteins from a wide range of solid tissues or cell suspensions. The PCT SPS consists of either a floor model instrument (the Barocycler NEP2017) or the soon-to-be-released Bench Top model (the Barocycler NEP3229). Both instruments use single-use PULSE Tubes for processing samples in a closed system.

The NEP2017 was designed to be used in a centralized laboratory and comes fully equipped with a circulating water bath and a computer to control PCT parameters. The NEP3229 was designed for use by scientists in their own laboratory and comes equipped with fixtures for a circulating water bath and with a Programmable Logic Controller (PLC) to set PCT parameters. However, the "basic" NEP3229 will also be offered in an "enhanced" version, which includes a water bath, computer, and auto-fill and purge, so that scientists may configure the PCT SPS to best meet their specific sample preparation requirements.

Our data indicate that whether configured with a Barocycler NEP2017 or NEP3229, the PCT SPS yields high quality and quantities of DNA, RNA, and protein from a wide variety of sample types. Furthermore, both configurations of the PCT SPS yield DNA, RNA and proteins comparable to conventional sample preparation methods. Regardless of which Barocycler model is used, the PCT SPS offers the additional, important features of increased safety, containment, reproducibility, and versatility, as well as reduced cross contamination, as compared to other current methods.

The PCT SPS is available with either a:

- Floor Model Barocycler NEP2017 Instrument
- Bench Top Model Barocycler NEP3229 Instrument

Comparable quality and yields of nucleic acids and proteins as released by conventional sample preparation methods may be obtained with either Barocycler model. Compared to these other methods, the PCT SPS is:

- Safer
- Faster
- Gentler
- More Powerful
- More Versatile
- More Reproducible

Please visit the Boston Biomedica Booth (#3854) during the PITTCON for additional product information.

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