TARGETS

Innovations in Genomics & Proteomics

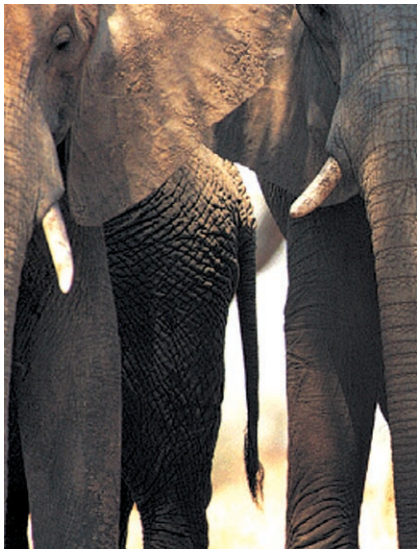
November 2002 Volume 1 No. 5 ISSN 1477-3627

- Powerful new extraction method for hard-to-lyse cells
- Data integration standards in model organisms
- Functional phosphoproteomics

Drug DISCOVERY today PUBLICATIONS

November 2002
Volume 1 · Number 5
pp. 147–176

TARGETS



Cover story
Elephants are known as being extremely powerful, as well as very gentle. Current methods for extracting biomolecules from cells tend to involve some harsh mechanical sheering protocols in conjunction with some fairly 'nasty' chemicals. On page 156, the authors review extraction methods currently in use, and compare them to a newly developed, automated process involving patented pressure cycling technology (PCT). PCT proves to be an extremely powerful but considerably 'gentler' approach to the extraction of biomolecules.
See page 156.
Cover design by Geraldine Woods.

***TARGETS** reviews advances in genomics and proteomics that will impact on drug and target discovery. Coverage includes new technologies, new applications of existing technologies, and current problem areas. All topics are reviewed by leaders in the field in the form of reviews, comment and analysis articles. Peer-reviewed for authoritative coverage, **TARGETS** will provide you with all the information you need to make informed tactical decisions for your drug discovery research.*

*Following from the outstanding success of **Drug Discovery Today**, this is a new series of publications dedicated to bringing you the latest news and reviews for the pharmaceutical and biotechnology industries. For more info visit* **www.drugdiscoverytoday.com**

REVIEWS

RESEARCH FOCUS

156 Tired of the same old grind in the new genomics and proteomics era?
by Patricia E. Garrett, Feng Tao, Nathan Lawrence, Jay Ji, Richard T. Schumacher and Mark M. Manak

163 Data integration standards in model organisms: from genotype to phenotype in the laboratory mouse
by Carol J. Bult

TECHNICAL FOCUS

169 Towards targeting signalling networks by functional phosphoproteomics
by Jasminka Godovac-Zimmermann and Larry R. Brown

FORTHCOMING ARTICLES

Integrating computer-based *de novo* drug design and multidimensional filtering for desirable drugs

The use of protein structure to characterise a protein family: designing selective inhibitors of protein kinases

Handling human samples for pharmacogenomics and drug discovery

GPCRs: structural approaches to drug discovery

Transgenic rat models for HIV-1

A critical analysis of chemical genomics using cell-based uHTS in the process of lead discovery

Functional genomics to understand variability of sepsis: from utopia to reality

TARGETS Vol. 1, No. 5 December 2002

Tired of the same old grind in the new genomics and proteomics era?

Patricia E. Garrett, Feng Tao, Nathan Lawrence, Jay Ji, Richard T. Schumacher and Mark M. Manak

New discoveries in life sciences depend on accurate analysis of biomolecules, which in turn depends on the extraction of high-quality molecules in high quantities from the tissues of plants, animals or microorganisms. The extraction process for hard-to-lyse cells and tissues has been a bottleneck in the path to discovery for many years. This review describes extraction methods currently in use, and compares them to a newly developed, automated process involving patented pressure cycling technology (PCT). The PCT sample preparation system (SPS) uses an instrument capable of rapid, temperature-controlled pressure cycling between ambient and high pressures, and single-use sample tubes containing a ram and a lysis disk. The quality and quantity of nucleic acid and protein prepared by the PCT SPS method are comparable to the older methods, whereas ease and safety of processing, reproducibility, speed and control are enhanced.

Patricia E. Garrett
Richard T. Schumacher
Boston Biomedica
375 West Street
West Bridgewater
MA 02379, USA
Jay Ji
Feng Tao
Nathan Lawrence
Mark Manak*
BBI Biotech Research
Laboratories
217 Perry Parkway
Gaithersburg
MD 20877, USA
*e-mail: mmanak@bbii.com

▼ Research that requires isolation of nucleic acids, proteins and small molecules from biological samples has increased dramatically in recent decades. This is due, in part, to innovations in analysis such as those by recent Nobel recipients Fenn, Tanaka and Wüthrich, whose work helped to create the field of proteomics [1,2]. Although analytical techniques have advanced rapidly, some bottlenecks still remain in the pathway between organism and new knowledge. One of these has been a persistent difficulty in releasing molecules intact from tissues and some cellular structures that are characterized as hard to lyse – a term that encompasses nearly every component of biological systems except some liquids and unattached cells, including, for example, fungi, insects, leaves, seeds, solid tumors, muscle, skin, other organs and many types of microorganisms.

Efficient release of biomolecules from cells and tissues is an extremely important first step in many analytical processes, regardless of whether studies involve human or animal physiology or medicine, agriculture, or the environment, because the quantity and quality of extracted material can profoundly affect the success of downstream applications. The analytical targets – DNA or RNA, proteins and small molecules – have different requirements on the basis of their chemical composition and stability, but some sample preparation methods can be adapted for use with several or all of these analytes.

To this day, efforts in many laboratories to pull cells and tissues apart and get their component molecules into solution, or into a form suitable for analysis, still involve manual grinding with a mortar and pestle, sometimes at liquid nitrogen temperature [3]. Other current mechanical or chemical methods include sonication, bead beating, French press, freezer milling, rotor–stator homogenization, enzymatic digestion and chemical dissolution, all of which have limitations related variously to process time, lack of automation capability, poor reproducibility, or applicability to only a narrow range of tissue types [4]. All sample preparation methods also carry the risk, to varying degrees, that they will perturb important naturally occurring complexes or relationships among the constituent molecules, making interpretation of the analytical data more difficult.

Recently a new approach to sample preparation using patented pressure cycling technology (PCT) has been developed and commercialized by Boston Biomedica [5]. In this sample preparation system (PCT SPS), an instrument (Barocycler™ NEP2017) capable of rapid, temperature-controlled pressure cycling between ambient and high pressure, applies up to 235 MPa (35 kpsi) hydrostatic pressure to samples contained in single-use

1477-3627/02/$ – see front matter ©2002 Elsevier Science Ltd. All rights reserved. PII: S1477-3627(02)02228-6

processing and storage tubes containing a ram and a lysis disk (PULSE™ Tubes). Cycle times vary with the material being processed, but five 1 min cycles at high pressure with 30 sec ambient intervals are sufficient to release nucleic acid and proteins from many cell suspensions and solid tissues. Chaotropic agents, buffers or other chemicals such as detergents can be used as lysis solutions.

This review compares the parameters of several methods for sample preparation from hard-to-lyse biological materials, as well as results of the application of different sample preparation methods to specific materials.

Currently available methods for sample preparation

Efficient disruption of cell walls and membranes is one of the most crucial steps affecting the yield and quality of isolated nucleic acids and proteins [6]. Combinations of mechanical or enzymatic and mechanical approaches are most commonly used for disruption of the difficult-to-lyse materials [3,4,7]. Mechanical disruption is effectively performed with a mortar and pestle, with the sample frozen in liquid nitrogen and ground manually to a fine powder. Grinding is often followed by a thorough homogenization with a Dounce (glass-to-glass grinder) or a mechanical homogenizer in a guanidium isothiocyanate (GITC) lysis buffer for nucleic acids, or in lysis buffers containing detergents for release of proteins. Although widely used for research applications, this approach is cumbersome, time consuming and prone to cross-contamination. As such, it is not practical for high-throughput screening or repetitive operations. In general, processing individual samples this way continues to be impractical for routine applications and a major bottleneck for more widespread use of molecular technology. A range of devices and instruments has been developed to facilitate mechanical homogenization of tissues [8]. In addition to the Dounce mentioned above, mechanical homogenizers (such as the Brinkman Polytron), sonicators, French presses, bead mills and other grinding instruments have been used widely. Homogenizers consist of a tube made of glass, inert plastic or stainless steel into which is inserted a tight-fitting pestle of the same material. The motion of the pestle up and down the tube causes the cells trapped against the wall to break open. In bead milling, cell disruption occurs by the crushing action of minute beads that are vigorously agitated by shaking or stirring the bead mill. Short pulses of shaking should be used to minimize the heat generated and to limit damage to some fragile molecules. For high-throughput operations, standard bead mills have been adapted to 96-microwell plates (Geno/grinder, SPEX CertiPrep, Metuchen, NJ; Mixer Mill, Qiagen, Valencia, CA [9]). In blade homogenizers or blenders, tissues are disrupted by very high speed turning of cutting blades, which are effective in rapidly homogenizing plant or animal tissues. Rotor-stator homogenizers are similar to blenders, but equipped with a rotor sited within a static tube to minimize the aeration and foaming that occur in conventional blenders. Ultrasonic disintegrators generate intense sonic pressure waves in liquid media that cause the formation of microbubbles that grow and collapse violently (cavitation). This causes an implosion within the cell membranes that leads to cell breakage. Ultrasonic disintegrators in either traditional or 96-well format (Misonix, Farmingdale, NY) generate considerable heat during operation, causing excessive foaming, or protein denaturation, if not carefully controlled. Mechanical approaches can be effective in extracting DNA, although shearing often occurs, which is a problem when high-molecular-weight DNA is required.

An alternative to stand-alone mechanical extraction of DNA from some cells and tissues is the use of enzymatic treatments, including digestion with proteases such as proteinase K to break open cells to release DNA. This approach works best with small pieces (<200 mg) of soft animal tissues, but may require an overnight digestion. For some tissue types, specific enzymes such as collagenase are also used before cell lysis. Other enzymes, such as lysozyme, zymolase or lysostaphin, are frequently used with bacteria and yeast to dissolve the coat, capsule, capsid or other structure not easily disrupted by mechanical methods alone [10]. Enzymatic treatment is usually part of a combination approach, as it is often followed by sonication, homogenization or vigorous vortexing in a GITC lysis buffer [11]. Enzymatic approaches are typically not suitable for RNA isolation because endogenous ribonucleases (RNases) will degrade the RNA during the incubation period. Ribonucleases are, in fact, the main source of failure in RNA isolation efforts [12,13]. Even when present in extremely low concentrations, these highly active yet very stable enzymes can rapidly degrade RNA before its isolation. To isolate high quality RNA, therefore, it is important to process the sample as soon as possible after harvest, or to snap freeze the sample in liquid nitrogen rapidly after harvest to minimize degradation. For processing, lysis must be made to occur very quickly in a potent protein denaturant, such as GITC lysis solution, because incomplete disruption will allow RNases trapped in intact cells to continue to degrade the RNA.

Applications of enzymatic approaches to extraction might also be limited, given that lysing enzymes are often crude preparations that contain proteases, in addition to RNases, that attack the molecules of interest as well as the cell wall. Protoplasts generated from enzymatic digestion usually require lysis with detergents that will also denature

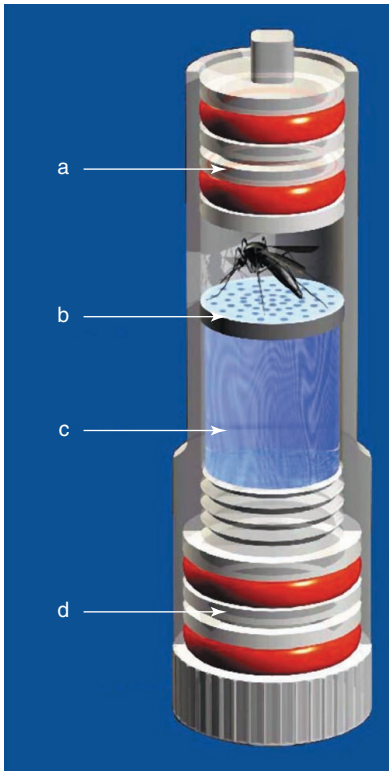


Figure 1. A PULSE Tube. **(a)** Ram, **(b)** lysis disk (with multiple holes), **(c)** collection chamber, **(d)** cap. (Reproduced with permission from Ref. 5.)

many proteins, diminishing or destroying their biological activity in the process.

A new approach to extracting molecules from hard-to-lyse cells and tissues

Boston Biomedica's PCT SPS uses a computer-controlled instrument (Barocycler NEP2017) to exert high hydrostatic pressure and expose samples to rapid pressure changes [5]. For solid samples, a container with a lysis disk (PULSE Tube; Fig. 1) allows pressure to be applied directly to the sample with a ram that compresses the solid and pushes it through the lysis disk into the lysis buffer in the compartment below. Subsequent cycles continue the disruption of the solid tissue and the release of molecular components into buffer or another lysis fluid, resulting in a solution or suspension that can be used in downstream applications either directly or following subsequent purification. This method features temperature control, process automation and user- or manufacturer-designed protocols that define pressure and cycle times. The Barocycler NEP2017 can ramp up pressure from atmospheric to 235 MPa (35 kpsi) in less than 5 sec and ramp down pressure from its maximum to ambient in a fraction of a second. Up to six PULSE Tubes can be processed simultaneously in two Barocycler reaction chambers that each accommodates up to three PULSE Tubes, allowing a potential throughput of 30–60 samples per hour when 5 min cycles are used. The process temperature can be controlled using an external circulating waterbath, allowing sample processing between 4 and 37°C. The cell and tissue disruption by cycled pressure application allows the requirement for lysing enzymes to be minimized or in some cases eliminated.

Currently available PULSE Tubes (Catalog #FT500) can hold 50–500 mg of solid sample or 1.2–1.5 ml of liquid sample. The loaded and sealed PULSE Tubes are placed in a reaction chamber in the Barocycler NEP2017, pressurization fluid is added to fill the chambers and the system is subjected to cycled pressure. Different types of samples can be processed simultaneously using the same pressure–time–temperature protocol. The recovered volume of PCT prepared samples is then available for downstream applications. Both the PULSE Tubes and Barocycler are designed and manufactured by BBI Source Scientific, Garden Grove, California, a Boston Biomedica company.

Table 1 lists examples of the sample types that have been processed by PCT SPS and used in various analytical procedures. Nucleic acids and proteins have been examined by gel electrophoresis before or after carrying out a purification procedure. Antigenic activities for some IgM and IgG antibodies, determined using ELISA (enzyme-linked immunosorbant assay) or western blot, were found to be unaffected by the PCT SPS processing at 35 kpsi. Early results indicate that the biological activity of enzymes and clotting factors tested (alkaline phosphatase, amylase, Factor VIII, HIV reverse transcriptase) is generally preserved at 35 kpsi with optimized buffer conditions, although the activity of some multimeric enzymes, such as Factor VIII, can be diminished.

The materials in question: classes of hard-to-lyse cells and tissues

Plant tissues

Efficient release of cellular contents from plant material typically requires mechanical processing before solvent extraction protocols are carried out. Plant cells from disrupted tissues can then be lysed with an ionic (N-Laurel sarcosine) or nonionic detergent (cetyltrimethyl ammonium bromide) solution followed by precipitation with isopropanol and ethanol [14,15]. Certain types of plant tissue, such as the soft-leaf tissues of *Nicotinia* and *Arabidopsis*, can be effectively disrupted by homogenization in lysis buffer alone. For harder plant tissues, polyvinylpyrrolidone added to the lysis buffer before organic extraction complexes with polysaccharide and phenol compounds commonly found in

Table 1. Molecules extracted from various biological samples using the PCT SPS

Sample Group	Examples	Molecules Extracted[a]	Applications
Animal tissues	Soft: liver, brain, pancreas, kidney, lung	DNA, RNA, protein	PCR, RT-PCR, ELISA, SDS-PAGE, 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, protein	PCR, RT-PCR, SDS-PAGE
	Hard: stem, pine needle, grape seed	DNA	PCR
Insects, small organisms	Mosquito, *C. elegans*	DNA, RNA, protein	PCR, RT-PCR, SDS-PAGE
Blood	Blood, blood spot	DNA	PCR
Microbes	Yeast	DNA, RNA, protein	PCR, RT-PCR, SDS-PAGE
	Mycobacteria sp., bacteria/spores, soil	DNA, RNA	PCR

[a]Not every example has been tested for RNA or proteins with RT-PCR, WB or ELISA.

plants, and facilitates tissue disruption [16]. Soaking seeds or other plant material overnight in water can facilitate homogenization. Some tissues such as grain or pine needles can be ground dry without liquid nitrogen before homogenization, whereas hard or woody plant materials might require freezing and grinding in liquid nitrogen, or milling.

An effective procedure in widespread use for processing plant tissue is grinding with a mortar and pestle to fine powder, usually in the presence of liquid nitrogen, followed by the addition of extraction solution, and homogenization in a Polytron for 2 min at moderate speed before proceeding to extraction with phenol/chloroform, GITC or one of the other available methods [17].

In contrast to these methods, the PCT SPS allows an automated combination of the grinding and homogenization steps for plant tissues and diminished use of lysing enzymes, leading directly in some cases to a solution or suspension ready for downstream processes. See Fig. 2 for an example of DNA isolation from grape seeds.

Microbes

Certain microbes, such as yeast and fungi, are extremely difficult to disrupt because their cell walls form capsules or spores, which are challenging to open. Some fungi can be processed by multiple rounds of freezing and thawing using a crushed dry ice/ethanol bath, followed by a 30 min boiling step, then grinding against the side of a microfuge tube with a sterile pipette tip, followed by extraction using the Qiagen DNeasy plant tissue kit [18]. Filamentous fungi, however, must be ground in liquid nitrogen to a fine powder with a mortar and pestle, and then thoroughly homogenized or sonicated in lysis buffer to be solubilized completely. Mechanical disruption of yeast has traditionally been accomplished by a press or bead mill, following enzymatic digestion. Complete disruption of microbes, such as *Mycobacteria* sp., can also be achieved by mechanical disruption using a bead mill – a process in which the bacterial pellet is suspended in lysis buffer, mixed with small glass beads (0.5–1.0 mm) containing magnetic particles for ease of removal from the suspension, and shaken at high velocity [19–21].

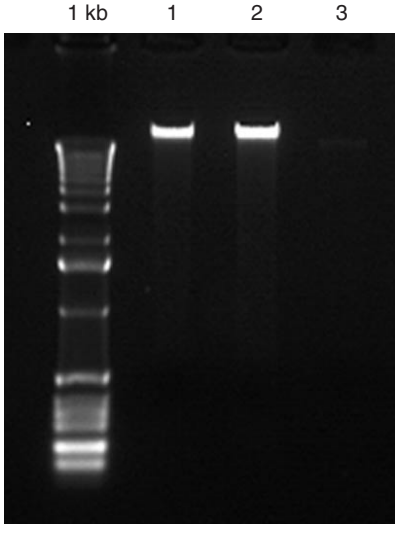


Figure 2. Genomic DNA released and purified from grape seeds. Lane 1 shows DNA in the lysate generated by pressure cycling technology (PCT) with 35 kpsi, 5 cycles at 4°C. The sample in lane 2 was processed with mortar and pestle grinding. Lane 3 was a 'negative control' – that is, lysate without PCT or mortar and pestle treatment. (Reproduced with permission from Ref. 5.)

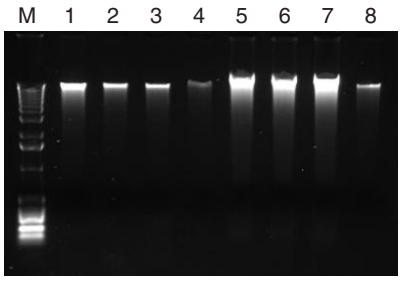


Figure 3. Genomic DNA extracted from Bacillus subtilis spores. PCT conditions were 5 × 1 min cycles, 35 kpsi at room temperature. Bead beating was at 3 × 30 sec pulsing at maximum setting followed by 30 sec cooling on ice. After PCT or bead beating treatment, DNA was purified from clarified supernatants using the Qiagen DNeasy minikit and run on agarose gels. Lanes 1-4 were lysed in Qiagen ATL Buffer supplemented with 1% Triton X-100. Lanes 5-8 were lysed in saturated guanidinium HCl and 1% CHAPS (3-[(3-chalamidopropyl) dimethylammonio] -1 propanesulfonate), 1% Triton and 1%SDS, pH not adjusted. Lanes 1 and 5 PCT treatment at room temp; Lanes 2 and 6 PCT treatment at 37°C; Lanes 3 and 7 bead beating; Lanes 4 and 8 treatment with lysis buffer only, no PCT or bead beating.

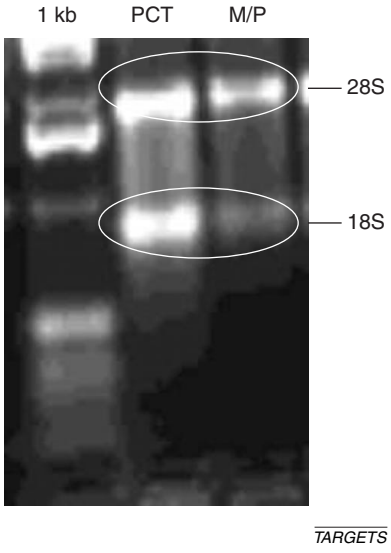


Figure 4. Pressure cycling technology extraction of RNA from mosquitoes. The mosquito samples (~50 mg) were either processed using the standard five-cycle pressure cycling technology protocol or homogenized with mortar and pestle. A guanidium isothiocyanate lysis buffer was used for both extractions. After the pressure or mortar-and-pestle (M/P) process, samples were briefly centrifuged; 200 µl of supernatants were processed using QIAGEN RNeasy kit without the homogenization treatment suggested in the kit manual. Final total RNA products were dissolved in 100 µl AE buffer. The circles show the 28S and 18S from the total RNA preparation. (Reproduced with permission from Ref. 5.)

Like plants, certain bacteria require digestion with enzymes to remove polysaccharides and other cell-wall material before nucleic acids are released [22]. Bacterial cell walls can be digested with proteinase K, lysozyme, zymolase, glucalase and/or lyticase to produce spheroplasts, which are readily lysed by vortexing in guanidinium-based lysis buffers. Gram-positive bacteria usually require more rigorous digestion than gram-negative organisms and their spheroplasts are then lysed with vigorous vortexing in GITC lysis buffer.

As with plants, the PCT SPS provides an automated, safe, process-controlled alternative to conventional methods of lysis that can combine the disruption and digestion steps and limit the requirement for lysing enzymes, producing a suspension prepared for extraction with minimal exposure of the analyst to the microorganism before and during processing. An example using *Bacillus subtilis* spores is described (Fig. 3).

Animal and human tissues

Many animal and human tissues, such as liver or soft tissue sarcomas, can be lysed simply by cutting them into small pieces (<100 mg) and incubating with proteinase K; a standard DNA solvent extraction process is then used. Fibrous tissues – such as heart or skeletal muscle – or tissues rich in protein – such as brain, thymus and spleen – are more difficult to process, and require a step to disrupt these tissues, followed by homogenization in dry ice or under liquid nitrogen. Pulverizing the tissue into powder while keeping the tissue completely frozen is the key to isolating intact total RNA. Additional challenges are posed when processing organs such as pancreas and spleen, which have very high endogenous levels of RNase and require special handling. Spleen and thymus are particularly high in nuclease and nucleic acids, and thus should be processed quickly by grinding in liquid nitrogen; this is followed by a quick homogenization in lysis solutions to inactivate nucleases [23]. Working with liver tissue requires that care be taken to separate it from the gall bladder, which contains high levels of degradative enzymes. In these cases, the sample should be quick-frozen in liquid nitrogen immediately following removal from the animal. It is necessary to grind the tissue quickly in guanidinium to prevent degradation of RNA. Two grams of tissue can be ground in 20 ml guanidinium solution in a tissue mixer with two or three 10 sec bursts for complete grinding. The debris is pelleted, and the supernatant treated with 2% Sarkosyl before solvent extraction is carried out [17].

Tissues rich in protein and/or lipid, such as brain tissue, can also can also complicate RNA processing, making clean separation from DNA and other cellular material difficult. For such materials, extraction with phenol:chloroform:isoamyl alcohol, followed by extraction with 1/10 vol chloroform: isoamyl alcohol helps to reduce flocculent material present

in the interface of the solvent layers. Hard tissues such as bone, teeth and some hard tumors require milling.

Sample preparation methods compared

Table 2 summarizes important features of each of the sample preparation methods discussed in previous sections, particularly the range of cell and tissue types most commonly treated using each method, and the advantages and shortcomings that further influence the choice of sample preparation method when a new study is planned. The PCT SPS provides a convenient, versatile format for processing a broad range of tissue and cell types, provides excellent yields of DNA, RNA and protein, and is well-suited for preservation of labile molecules such as RNA and certain proteins.

Sampling of applications

Bacteria: PCT SPS compared to enzymatic digestion for DNA isolation

B. subtilis spores were cultured, pelleted and resuspended in two different lysis buffers. As shown in Fig. 3, DNA was successfully isolated from *B. subtilis* spores using the PCT SPS. DNA yields were comparable by PCT and bead beating methods. The absolute yields of DNA obtained using the guanidinium buffer (buffer B) were higher than those with the ATL lysis buffer (buffer A).

Mosquitoes: PCT SPS compared to mortar and pestle for RNA isolation

In another experiment, RNA was extracted from mosquitoes using the PCT SPS, and compared to mortar-and-pestle processing (Fig. 4). The results show that 18S and 28S rRNA

Table 2. Method comparison summary

	Method	Operational Temperature	Application Scope – Analytes	Application Scope – Cells and Tissues	Advantages	Disadvantages
Mechanical or Physical	PCT SPS	Computer controlled, 4°C to 37°C	DNA, RNA, protein	Wide range of animal, plant & microorganism cells and tissue accommodated – range is still being extended	Rapid, easy to use, closed system for safety, automated, versatile, reproducible	Equipment cost, limited field experience, sample volume restrictions
	Mortar and pestle, tissue pulverizer	Liquid nitrogen, dry ice or ambient	DNA, RNA, protein	Solid tissues – most commonly used homogenization method in animal and plant laboratories	Low equipment cost, long experience in the field, broad applicability for solid tissues	Manual, slow, labor intensive, handling of LN2, open system, requires cleaning, poor reproducibility, difficult to automate
	Rotor-stator homogenizer; polytron; Dounce homogenizer	Room temperature or on ice	DNA, RNA, protein	Soft tissues – commonly used	Low equipment cost, long experience in the field, broad applicability in soft tissues	Manual, requires cleaning limits in sample sizes; open system; shearing or denaturation of macromolecules, not suitable for high throughput
	Bead milling with a bead beater or vortex	Room temperature, pre-chilling	DNA, RNA, protein	Animal and plant tissue, microbial cells, capable of parallel processing	Fast, automated; may process samples simultaneously	Difficult to control temperature, shearing of DNA
	Sonication; French press	On ice or low temperature	DNA, protein	Cell suspensions or microbe liquid samples, commonly used	Easy to control conditions, long experience in the field	Shearing of DNA, requires cleaning, not useful for solid tissues
Chemical	Enzymatic Digestion; chemical dissolution	Optimum temperature for enzyme activity, often elevated	DNA, RNA, protein	Soft tissue and liquid samples – commonly used, often in combination with mechanical methods	Suitable for automation, high throughput, no specific equipment requirement	Applicable to limited sample types and sizes, higher temperature, risk of loss of protein structure or function, time consuming

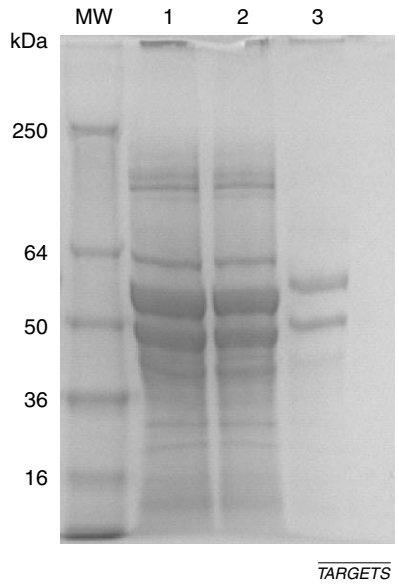


Figure 5. Protein released from breast tumor tissue. Lane 1 shows protein present in the lysate generated by pressure cycling technology (PCT) with 35 kpsi, five cycles at 4°C in PBS. The results in lane 2 were obtained from mortar and pestle grinding. Lane 3 was a 'negative control' – lysate without PCT or mortar and pestle treatment. No purification procedures were applied. (Reproduced with permission from Ref. 5.)

were recovered from the PCT SPS-treated sample with yields comparable to the mortar-and-pestle method, with significantly less labor.

Breast tumor: PCT SPS compared to mortar and pestle for protein isolation

Proteins from human breast tumor samples that had been frozen (–70°C) for up to 20 years were released using PCT SPS in 5 min. Results were compared to those obtained using a mortar and pestle method. Proteins were released in high yield, and no smearing was observed in the protein band processed with the PCT procedure. The yields were comparable between the two methods, indicating that excellent quality and quantity of protein can be obtained using PCT (Fig. 5).

Grape seeds: PCT SPS compared to bead beating for DNA isolation

The PCT SPS can be used to effectively release DNA from grape seeds. Yields of high-molecular-weight DNA comparable to those from mortar and pestle processing were obtained by the PCT extraction method, with less labor and potentially improved reproducibility (Fig. 2).

Conclusion

In an era of high-volume, high-throughput biochemistry and biology, sample preparation remains problematic, especially for hard-to-lyse materials. The shortcomings of traditional methods are well known, and these have not been amenable in general to automation, with its concomitant improvements in control, documentation and reproducibility. The application of cycled, high hydrostatic pressure using the PCT SPS is a new approach to the disruption of cells and tissues for extraction of nucleic acids and proteins. Cell and tissue applications to date include a wide variety of liquids, suspensions and solids. Results are comparable to those with older methods, whereas ease and safety of processing, reproducibility and control of conditions, and processing time are improved.

References

1 Pandey, A. and Mann, M. (2000) Proteomics to study genes and genomes. *Nature* 405, 837–846
2 Cho, A. and Normile, D. (2002) *Science* 298, 527–528
3 Johnson, B. (1998) Breaking up isn't hard to do: a cacophony of sonicators, cell bombs and grinders. *The Scientist* 12, 23
4 Salusbry, T. (1994) *Protein Purification Methods: A Practical Approach* (Harris, E.L.V. and Angal, S., eds), pp. 87–97, IRL Press
5 Schumacher, R.T. *et al.* (2002) An automated system for extraction of hard-to-lyse samples. *Am. Lab.* 34, 38–43
6 Logemann, J. *et al.* (1987) *Anal. Biochem.* 163, 16–20
7 Carnicini, P. *et al.* (2002) *Biotechniques* 33, 306–309
8 Hopkins, T. (1991) *Purification and Analysis of Recombinant Proteins* (Seetharam, R. and Sharma, S.K., eds), Marcel Dekker
9 Obenauf, R.H. *et al.* (2000) *Handbook of Sample Preparation and Handling* (7th edn), SPEX CertiPrep, Inc.
10 Poddar, S.K. (1991) A modified method of genomic DNA preparation in agarose inserts for pulse field gel electrophoresis. *Electrophoresis* 12, 674–675
11 Santos, L. *et al.* (1999) *Arch. Vet. Faculty* 27, 99–101
12 Boom, R. *et al.* (1990) Rapid and simple method for purification of nucleic acid. *J. Clin. Microbiol.* 28, 495–503
13 Farrell, R.E., Jr (1996) *RNA Methodologies* (2nd edn), Academic Press
14 Gross-Bellard, M. *et al.* (1973) Isolation of high-molecular-weight DNA from mammalian cells. *Eur. J. Biochem.* 36, 32–38
15 Dellaporta, S. *et al.* (1983) A Plant DNA Minipreparation: Version II. *Plant Mol. Biol. Rep.* 1, 19–21
16 Wilkins, T.A. and Smar, L.B. (1996) Isolation of RNA from Plant Tissue. In *A Laboratory Guide to RNA: Isolation, Analysis and Synthesis* (Krieg, P.A., ed.), Wiley Liss
17 Kingston, R. (1995) *Current Protocols in Molecular Biology*, p. 4.3.1, John Wiley
18 Griffin, D.W. *et al.* (2002) A rapid and efficient method for extracting DNA from fungi. *Lett. Appl. Microbiol.* 34, 210–214
19 Caldarelli-Stefano, R. *et al.* (1999) Use of magnetic beads for tissue DNA extraction and IS6110 mycobacterium tuberculosis PCR. *Mol. Pathol.* 52, 158–160
20 Hurley, S. *et al.* (1987) Rapid lysis technique for mycobacterial species. *J. Clin. Microbiol.* 25, 2227–2229
21 Greenfield, L. and White, T.J. (1993) *Diagnostic Molecular Microbiology: Principles and Applications* (Persing, D.H. *et al.*, eds.), American Society for Microbiology, Washington, DC
22 Wilson, K. (1994) *Current Protocols in Molecular Biology*, p. 2.4.1, John Wiley
23 Strauss, W. (1995) *Current Protocols in Molecular Biology*, p. 2.2.1, John Wiley