Use of Pressure Cycling Technology (PCT) for the Release of DNA from Plants

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Introduction

Sample preparation is often a major bottleneck limiting rapid discoveries in such diverse fields as agriculture, environment, genetics, and drug development. To this end, a Pressure Cycling Technology-based sample preparation system (PCT SPS) was developed by Boston Biomedica, Inc. and introduced to the market in September 2002 (Garret et al., 2002). This System uses an instrument (Barocycler™ NEP2017) and single-use disposable processing tubes (PULSE™ Tubes). The PCT SPS applies cyclic ambient to high hydrostatic pressure to disrupt tissues, cells and cellular structures, releasing their contents into buffers or other solutions contained in the PULSE Tube. The instrument is either manually or computer controlled, is capable of cycling pressure between ambient and 40,000 PSI, and offers a working temperature range of 4°-37°C.

Figure 1. Barocycler NEP2017 and a schematic drawing PULSE Tube

Extraction of nucleic acids and proteins from plants is a time consuming and labor intensive process. Two common methods are grinding individual samples with mortar and pestle and mechanical mincing. These methods are prone to contamination as the same tools are often reused for multiple preparations. Losing material during sample transfer is another problem. When breeding selections and Intellectual Property protection issues are at stake, risk of contamination is unacceptable. Scientists therefore continue to search for more versatile and less complex methods for sample preparation, particularly those less prone to contamination. We compared cell disruption by mortar and pestle and mechanical mincing to Pressure Cycling Technology (PCT) for the extraction of DNA from leaves and seeds. Leaves and seeds of rice, and seeds from tomato, soy bean, tobacco, maize, wheat and cells of *Taxus* were processed by the PCT SPS. In all cases, the PCT SPS produced DNA of sufficient quality and quantity to provide a useful template for further molecular experimentation. The PCT SPS also obviated the need for labor intensive mechanical disruption of plant tissues, and offered the additional advantage of extraction in a closed, single-use container.

Materials and Methods

Plant Materials

- Rice leaf tissue and paddy rice seeds (harvested hulls intact) were used in dose-response curves
- Paddy rice seeds, milled rice grains and seeds of maize, tomato, wheat, apple, tobacco and soybean were used in DNA release studies
- DNA was extracted from two different cell lines of *Taxus*

Extraction Protocol

- Seed samples were processed using either the PCT SPS or mechanical disruption and were compared to an untreated control
- Mechanically disrupted samples were minced on a clean surface with a clean razor blade (seeds) or ground gently (cells)
- Untreated control samples were not processed in any way. Both were placed in centrifuge tubes
- Samples processed by the PCT SPS were placed in the PULSE Tubes with supplemental metal grinding disks.
  - 1.1 mL of extraction buffer (100 mM Tris HCl pH8.0, 50 mM EDTA, 500 mM NaCl, 1.25% SDS, 36.5 mM sodium bisulfite) was added to each sample
  - The samples were processed at cycles of 35 kpsi, 20 sec high pressure, 20 sec low pressure
  - The supernatants were then transferred from the PULSE Tube to centrifuge tubes
  - A standard chloroform extraction protocol was used to purify DNA
  - Each sample was treated with RNase A

PCR Amplification

- Polymerase chain reaction (PCR) was performed in a PTC100 thermal cycler (MJ Research) using the following profile for 35 cycles: 5 min at 94°C; 1 min at 94°C; 2 min at 55°C (38°C for tobacco); 1 min at 72°C; with a final 5 minute extension at 72°C.
- 20 ng of template DNA was mixed with 10 pmol of primer (listed in Table 1), 1X PCR buffer [50 mM KCl, 10 mM Tris-Cl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% gelatin], 40 nmol of dNTP mixture, and 5 units Taq polymerase for a total reaction volume of 50 μL.

Table 1: Primer sequences used to amplify DNA released via PCT.

crop	Primer name	marker type	annealing temp	forward sequence	reverse sequence
rice	RM470	SSR	55	TCCTCATCGGCTTCTTCTTC	AGAACCCGTTCTACGTCACG
soybean	Satt653	SSR	55	GCG AAA CTC AAA TTT GAC ACA ACT G	GCG CAA GGT TAA CTA CTA ACA AGA CAC A
maize	UMC1066	SSR	55	AGAACCCGTTCTACGTCACG	AGCAGCAGCAACGTCTATGACACT
apple	GD96	SSR	55	CGG CGG AAA GCA ATC ACC T	GCC AGC CCT CTA TGG TTC CAG A
wheat	WMS448	SSR	55	ACATCGCTCTTCACAAACCC	AGTTCCGGTCATGGCTAGG
tomato	SSR27	SSR	55	CCCAAATCAAGGTTTGTGGT	TCAGATGCCACCACTCTCAG
tobacco	rpz7	RAPD	38	CCAGGAGGAC	-----
Taxus	AJ320538	SSR	55	ACACCGTTTCGTGAGGTAGG	CATCCTGGAGCTGGTTGTTT

Results

In a series of experiments, the PCT SPS was compared to a mincing process for release of DNA seeds of from several crops, which have research and economic importance.

Several extraction buffers and extraction protocols were tested and produced adequate results, indicating that the PCT SPS is a good tool for buffer and protocol discovery.

As shown in Figure 2, the release of DNA correlates with the number of pressure cycles.

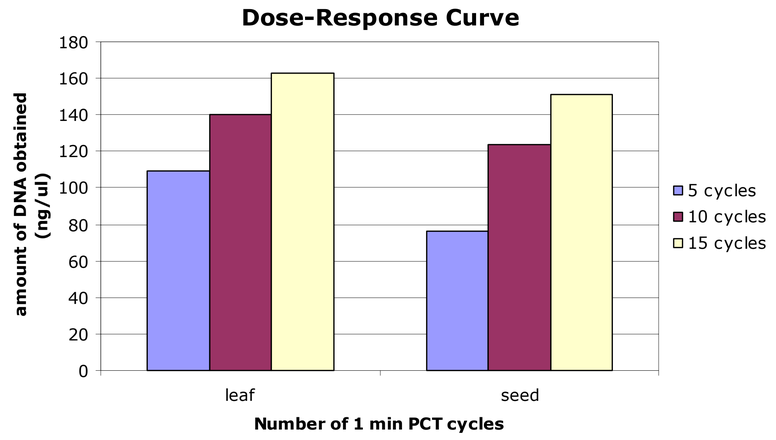


Figure 2. Dose-response curve showing the effect of using 5, 10 and 15 one-minute PCT cycles on equal amounts of rice seed and leaf tissue.

The PCT process was applied to seeds from maize, tomato, soybean, tobacco and apple as well as paddy rice and milled rice. *Taxus* cell samples were also processed using the PCT SPS. Table 2 describes the treatment and starting amounts for each sample type.

Table 2. List of all samples included in DNA release experiment, weight of starting samples and the treatments applied to each.

sample number	sample description	treatment for disruption	additional accessory added	weight of starting sample(g)	sample number	sample description	treatment for disruption	additional accessory added	weight of starting sample(g)
1	Jasmine milled rice	PCT	2 disks	0.11	26	Cayuga wheat	PCT	2 disks	0.08
2	Mochi milled rice	PCT	2 disks	0.13	27	Caledonia wheat	PCT	2 disks	0.07
3	Basmati milled rice	PCT	2 disks	0.13	28	Geneva wheat	PCT	2 disks	0.08
4	Jasmine milled rice	minced		0.11	29	Geneva wheat	minced		0.07
5	Jasmine milled rice	none		0.11	30	Geneva wheat	none		0.06
6	IR64 fresh rice	PCT	2 disks	0.09	31	TA209 tomato	PCT	2 disks	0.05
7	Kasalath fresh rice	PCT	2 disks	0.07	32	LI589 tomato	PCT	2 disks	0.05
8	Nipponbarre fresh rice	PCT	2 disks	0.10	33	LAI708 tomato	PCT	2 disks	0.05
9	Nipponbarre fresh rice	minced		0.10	34	LAI589 tomato	minced		0.05
10	Nipponbarre fresh rice	none		0.10	35	TA209 tomato	none		0.05
11	Noir soybean	PCT	2 disks	0.14	36	Hicks tobacco	PCT		0.02
12	Archer soybean	PCT	2 disks	0.15	37	C371G tobacco	PCT		0.02
13	Minsoy soybean	PCT	2 disks	0.12	38	RGH51 tobacco[1]	PCT		0.14
14	Noir soybean	minced		0.12	39	Hicks tobacco	minced		0.03
15	Noir soybean	none		0.16	40	C371G tobacco	none		0.01
16	MO17 Maize	PCT	2 disks	0.11	41	*Taxus* cells line D[2]	PCT	2 disks	0.10
17	B73 Maize	PCT	2 disks	0.11	42	*Taxus* cells line P[3]	PCT	2 disks	0.04
18	A632 Maize	PCT	2 disks	0.13	43	*Taxus cells line D*[2]	grinding		0.10
19	MO17 Maize	minced		0.13	44	*Taxus* cells line D[2]	none		0.10
20	MO17 Maize	none		0.17					
21	Golden Delicious Apple	PCT	2 disks	0.11					
22	Jonamac Apple	PCT	2 disks	0.10					
23	Cameo Apple	PCT	2 disks	0.12		[1]pelleting material			
24	Golden Delicious Apple	minced		0.10		[2]freeze-dried sample			
25	Golden Delicious Apple	none		0.11		[3] freshly harvested sample			

In most cases, yields of DNA obtained by BBI's PCT SPS were comparable to yields from mechanically disrupted cells. Data are summarized in Figure 3.

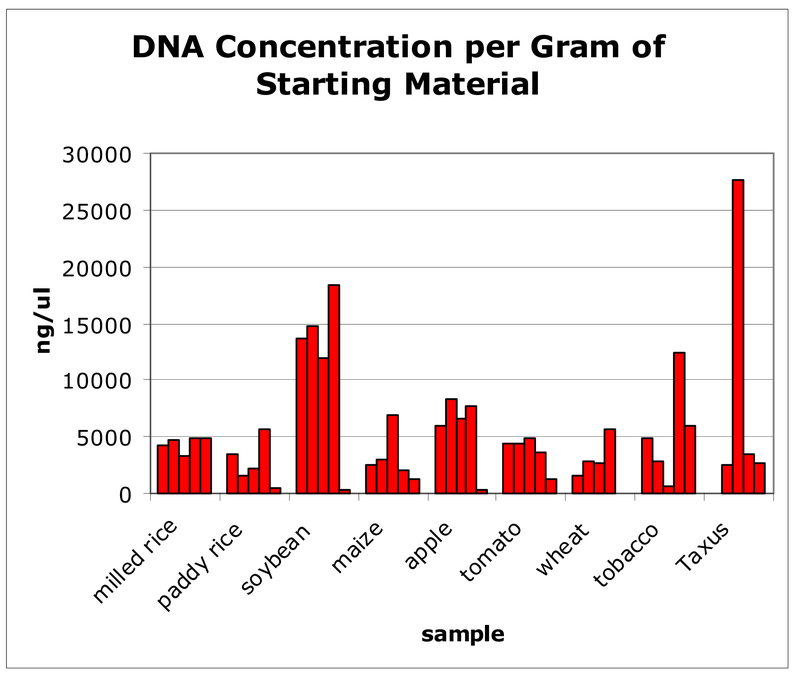
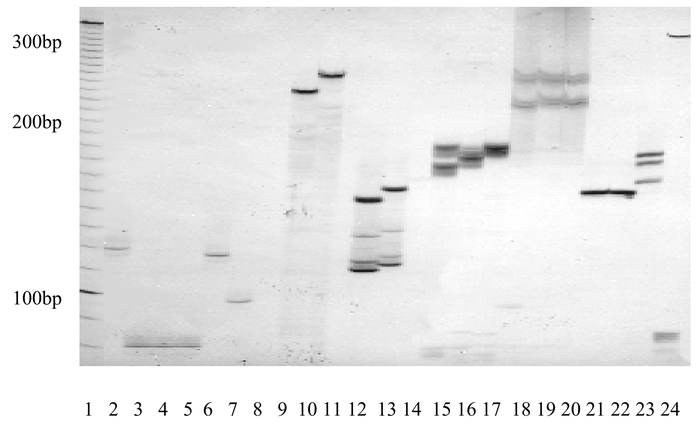


Figure 3. Resulting DNA concentrations obtained per gram of starting material when using PCT vs. mechanical grinding methods. Lanes are displayed (left to right) in the same order shown in Table 2.

As shown in Figure 3, relatively high yields were obtained from all varieties of soy bean and apple tested, while maize and tobacco showed the greatest differences in yield of DNA between varieties. Higher yields of DNA were obtained from milled rice than paddy rice.

The DNA samples released by PCT were amplified via PCR using the primers described in Table 1. The amplified products were visualized on 4% denaturing polyacrylamide gels and detected by silver stain as described by Panaud et al., 1996. Figure 4 shows the amplified fragments that distinguish between varieties within a species.



Lane 1: 10bp size standard; **Lane 2**: milled rice variety Jasmine; **Lane 3**: milled rice variety Mochi; **Lane 4**: milled rice variety Basmati; **Lane 5**: paddy rice variety IR64; **Lane 6**: paddy rice variety Kasalath; **Lane 7**: paddy rice variety Nipponbare; **Lane 8**: blank lane; **Lane 9**: soybean variety Archer; **Lane 10**: soybean variety Minsoy; **Lane 11**: blank lane; **Lane 12**: maize variety B73; **Lane 13**: maize variety A632; **Lane 14**: blank; **Lane 15**: apple variety Cameo; **Lane 16**: apple variety Golden delicious; **Lane 17**: apple variety Jonamac; **Lane 18**: wheat variety Cayuga; **Lane 19**: wheat variety Geneva; **Lane 20**: wheat variety Caledonia; **Lane 21**: tomato variety TA209; **Lane 22**: tomato variety L1589; **Lane 23**: tomato variety L1708; **Lane 24**: *Taxus* cell line CO93P.

Figure 4. DNA fragments obtained from PCR amplification of DNA released by the PCT SPS method, visualized with silver stain on 4% denaturing polyacrylamide gel.

Discussion

As the cost of labor surpasses that of technology and as Intellectual Property (IP) protection issues come to the forefront, scientists search for more efficient, reliable methods to perform routine but important tasks such as that of pulverizing plant tissues for release of DNA. Common methods of mechanical grinding are not only labor intensive, but also raise concerns over issues of cross-contamination and loss of sample volume. When issues such as IP protection rights and breeding selections are critical, these risks are unacceptable.

PCT Sample Preparation System commercialized by BBI proved to be an effective device for the release of DNA and is extremely adaptable to the specificities of species, crop, and sample condition. Furthermore, the quality and quantity of DNA isolated from PCT-treated samples is sufficient for PCR amplification using SSR and RAPD primers. Release of RNA from plant samples also proved effective using the PCT SPS method (data not shown).

The system works well for the release of nucleic acids from plant tissue and eliminates the need for mechanical disruption. The PCT SPS is suitable for many research or testing applications.

The PCT SPS is simple to use and to program making optimization for specific applications possible. The versatility of the System makes it useful for release of nucleic acids as well as a wide range of other purposes, including buffer discovery and protocol development.

- The PULSE Tube also serves as a convenient collection, transport, and storage device for use in the field because tissue samples may be placed directly in the tubes until brought to the laboratory for processing.
- Since it is not necessary to transfer the tissue to a new tube for processing, the likelihood of cross contamination or confusing one sample with another is greatly reduced.
- The System would be particularly useful if precious samples, such as diseased tissue or archeological samples were to be processed, or if limited amounts of starting material were available.
- The PCT SPS would be most useful in laboratories processing many different specimens for evaluation.

References

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