Extraction of DNA from Plant Tissue Using Pressure Cycling Technology (PCT)

Extraction of nucleic acids from plants is a time consuming and labor intensive process. Two common methods of extraction are (i) grinding individual samples with mortar and pestle and (ii) mechanical mincing. These methods are prone to contamination, as the same tools are often reused sequentially for multiple preparations. This risk of contamination is often unacceptable, especially in studies using rare or valuable specimens, when breeding selection is critical (particularly with the advent of genetically engineered crops), or when Intellectual Property protection is involved. Here we compare cell disruption by mortar and pestle and mechanical mincing to Pressure Cycling Technology (PCT) for the extraction of DNA from a wide variety of plants and plant tissues.

Pressure Cycling Technology (PCT)

PCT uses alternating cycles of high and low pressures to induce cell lysis. Cell suspensions or tissues, such as bone, are placed in specially designed, single-use processing containers (PULSE Tubes) and are then subjected to alternating cycles of high (up to 35,000 PSI) and ambient pressures in a pressure-generating instrument (Barocycler). Maximum and minimum pressures, the time at each pressure level, and the number of cycles is defined using a programmable logic controller. The reaction chamber of the Barocycler instrument is temperature controlled using a peripheral circulating water bath. Safety features in the PCT System design significantly reduce risk of exposure to the researcher to pathogens [2].

Methods

To demonstrate PCT performance, approximately 200 mg of fresh corn sprouts were processed at room temperature in buffer containing saturated Guanidinium HCl/1% CHAPS, using five 1 minute cycles (30 seconds at 35 kpsi, or 236 MPs, followed by 30 seconds at ambient pressure) in either the floor model Barocycler (NEP2017) or the bench top model (NEP3229). The performance of both Barocyclers was compared to extraction by mortar and pestle. Released DNA was purified using a QIAGEN DNeasy kit and the genomic DNA was visualized in an agarose gel (See Figure 1).

In other experiments, frozen corn leaves, frozen roots, and dry corn leaves were processed in a similar fashion as described above and compared to extraction by mortar and pestle. Purified DNA was then amplified in a PCR reaction using a primer set of chloroplast sequences. DNA was visualized in an agarose gel (See Figure 2).

PCT was further evaluated in experiments conducted at Cornell University. In these experiments, PCT conditions similar to those described above were used to process a wide variety of plants and plant tissue. A list of the plants and tissues studied is shown in Table 1. A comparison was made to plant tissues processed by mortar and pestle or mechanical mincing to PCT. In these experiments, released DNA was amplified using PCR (data not shown) [2].

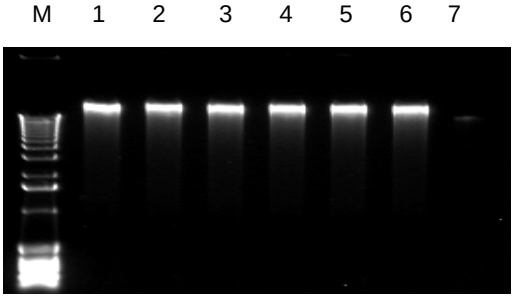

Figure 1. DNA extracted from corn sprouts comparing the Barocycler Model NEP2017, the Barocycler Model NEP3229, and mortar and pestle. Lane M is a 1 kb marker, Lanes 1 and 2 corn sprout processed by PCT (Barocycler NEP2017), Lanes 3 and 4 corn sprout processed by PCT (Barocycler NEP3229), Lanes 5 and 6 corn sprout processed by mortar and pestle, and Lane 7 is a Negative Control.

Results and Discussion

The Pressure Cycling Technology Sample Preparation System (PCT SPS) is an effective method for the release of DNA and RNA from a wide range of plants and plant tissues. Figure 1 shows total DNA released from fresh corn sprouts using the PCT SPS (with either the floor model Barocycler NEP2017 or the bench top model NEP3229) compared to mortar and pestle grinding. In all cases, similar amounts of high-quality DNA were released. However, the PCT SPS offered the additional advantages of processing multiple samples simultaneously while eliminating the likelihood of cross contamination. Sample preparation by the PCT SPS also added a significant safety feature, as processing took place entirely in a single-use, sealed vial (the PULSE Tube).

Figure 2 shows DNA released from frozen corn leaves, frozen corn roots, and dried corn leaves using the PCT SPS, compared to mortar and pestle grinding. Released DNA was amplified using a primer for a chloroplast sequence. These data show that DNA can be released from plant tissue in both frozen and dried states using the PCT SPS. The PULSE Tube offers the additional advantages of safety and convenience, as it can be used to store samples after collection in the field, for transporting the samples for analysis, for sample processing, and for post-processing storage.

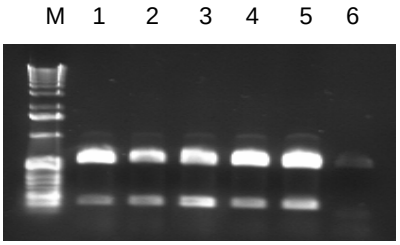

Figure 2. DNA extracted from frozen or dried plant tissue using the PCT SPS compared to manual grinding by mortar and pestle. Lane M is a 1 kb marker, Lane 1 frozen leaf processed by PCT, Lane 2 frozen root processed by PCT, Lane 3 frozen leaf processed by mortar and pestle, Lane 4 dried leaf processed by PCT, Lane 5 dried leaf processed by mortar and pestle, and Lane 6 is a Negative Control.

Table 1. A list of plants and plant tissues processed by the PCT SPS to release DNA

Plant	***Tissue***
Rice	Leaf
Paddy Rice	Seeds (hulls intact)
Paddy Rice	Seeds (no hulls)
Rice	Grains (milled)
Maize	Seeds
Tomato	Seeds
Wheat	Seeds
Apple	Seeds
Tobacco	Seeds

An extensive evaluation of the PCT SPS was conducted in Dr. Susan McCouch's laboratory at Cornell University. In these experiments, the PCT System was used to extract DNA from a wide variety of plants and plant tissues. Table 1 lists the plants and tissues processed by PCT. In all cases, sufficient DNA was released for amplification by PCR, resulting in specific DNA products (data not shown) [2]. However, these samples were processed simultaneously without the risk of cross contamination, yet they provide a standardized and rapid processing method through automation.

The PCT Sample Preparation System is an effective method for the rapid, safe, reproducible, and versatile for release of DNA from a variety of plants and plant tissues. It is extremely adaptable for different species, crops, and sample conditions. Furthermore, the quality and quantity of DNA isolated from PCT-treated samples is sufficient for PCR amplification and other downstream applications. The versatility of the PCT System makes it useful for the release of nucleic acids as well as a wide range of other purposes, including buffer discovery and protocol development. The PCT SPS obviates the need for labor-intensive mechanical disruption of plant tissues, and offers the additional advantage of extraction in a closed, single-use container. The PULSE Tube is a safe and convenient field collection/storage device, a transportation container, a processing vial, and a post-processing storage tube. And since it is not necessary to transfer the tissue to a new container when it is received by the laboratory for processing, the likelihood of cross contamination or sample mix-up is greatly reduced. The PCT Sample Preparation System is particularly useful with precious samples, such as diseased tissue or archeological samples, and in cases when limited quantities of starting material are available.

Acknowledgements

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References

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[2] Harrington, S., McCouch, S., Tao, F., Lawrence, N., Schumacher, R.T. (2004) "Use of Pressure Cycling Technology (PCT) for the Release of DNA from Plants" Annual Plant, Animal and Microbes Genomics Conference, San Diego, CA

Pressure BioSciences, Inc.
321 Manley Street • West Bridgewater • MA 02379
TEL 508-580-1818 • FAX 508-580-1822
www.pressurebiosciences.com