

## Improved DNA Recovery from Spinach Leaves Using The PCT Shredder<sup>™</sup> and Pressure Cycling Technology (PCT)

### Introduction

The high content of fibrous material in many plant samples as well as the presence of rigid cell walls complicates extraction of DNA from plant tissues. To release target analytes, plant samples often require extensive and time consuming sample disruption by grinding with a mortar and pestle [1, 2] or by homogenizing with glass or metal beads [3, 4]. Such methods are often inefficient and may even be deleterious to the DNA. Here we describe a system for the efficient extraction of DNA from spinach leaves using The PCT Shredder and the Pressure Cycling Technology Sample Preparation System (PCT SPS). Initial disruption of plant tissue with The PCT Shredder followed by DNA extraction by pressure cycling technology (PCT) are carried out in the same processing container (Shredder PULSE Tube). This method of extraction is safe, convenient and efficient. Furthermore, the extracted DNA was far less sheared as compared to DNA extracted by bead beating.

### Pressure Cycling Technology (PCT)

In the Pressure Cycling Technology Sample Preparation System (PCT SPS) hydrostatic pressure is rapidly cycled between ambient and ultra high levels (45,000 psi) to control biomolecular interactions [5]. High hydrostatic pressure acts preferentially on the compressible components of the sample, such cell membranes, resulting in cell lysis and the release of intracellular contents. The PCT SPS can be used to disrupt plant and animal tissues, cells, cellular structures and microbes to extract nucleic acids, proteins and lipids. The system is comprised of a small, semi-automated bench-top instrument (Barocycler NEP3229 or the NEP2320) used in combination with single-use sample processing containers (PULSE Tubes). PCT in the presence of suitable extraction reagents, such as DNAzol<sup>®</sup>, results in isolation of intact DNA for genomic analysis and other applications.

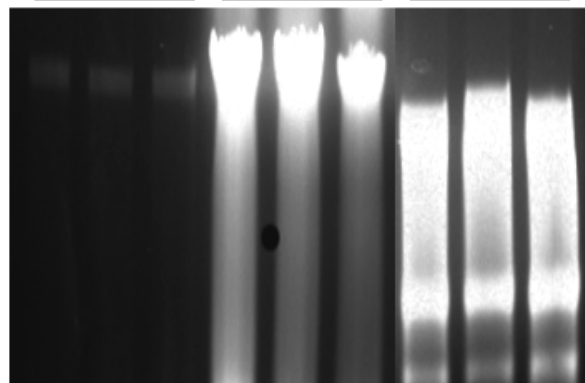
### The PCT Shredder

The PCT Shredder is designed to physically disrupt and enhance extraction of tough, fibrous and other difficult-to-disrupt biological materials such as certain plant and animal tissues [6]. The PCT Shredder is used to rapidly grind the sample directly in a specially designed Shredder PULSE Tube to increase the tissue surface area and to improve cell lysis prior to treatment by PCT for extraction of nucleic acids, proteins, lipids and other cellular contents. Since shredding and PCT are done in the same tube, loss of sample or the likelihood of cross contamination is significantly reduced as compared to other processing methods.

### Materials and Methods

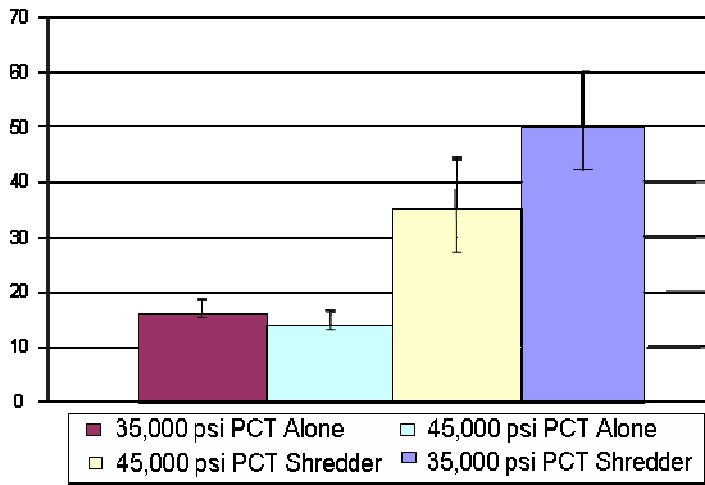
A comparison was made of samples processed by The PCT Shredder followed by PCT, PCT alone, or bead beating. For each condition, approximately 200 mg of fresh baby spinach leaves were chopped or torn into pieces (excluding midveins). One set of samples was processed with The PCT Shredder in the presence of 0.7 mL of DNAzol<sup>®</sup> (Invitrogen) for 20 seconds at ambient temperature according to instructions in The PCT Shredder Product insert. After shredding, the sample volume was brought up to 1.4 mL with additional DNAzol<sup>®</sup> reagent. The Shredder Pulse Tube was then capped with a high pressure Shredder PULSE Tube cap provided with The PCT Shredder Kit and subjected to PCT (35,000 or 45,000 psi held for 20 seconds, followed by atmospheric pressure held for 10 seconds and repeated for 30 cycles) at ambient temperature. A second set of samples was processed by PCT alone without pre-processing with The PCT Shredder. These samples were loaded into standard FT500 PULSE Tubes with 1.4 mL of DNAzol<sup>®</sup> and subjected to PCT as described above. A third set of samples was subjected to bead beating in a Mini-beadbeater-1 (BioSpec Products) using 1 mL of 1.0 mm Zirconia beads in a 2 mL centrifuge tube. Samples were disrupted by bead beating at full power using ten 10 second bursts. Because of the heat generated during bead beating, samples were cooled on ice between bursts. After extraction by PCT or bead beating, DNA was purified using the DNAzol<sup>®</sup> isolation protocol for plants according to manufacturer's instructions. DNA recovery was measured by Qubit assay using the Quant-iT dsDNA BR kit (Invitrogen). DNA was visualized by agarose gel electrophoresis using the Reliant FastLane Gel System (Lonza).

PCT Alone	Shredder + PCT	Bead Beating
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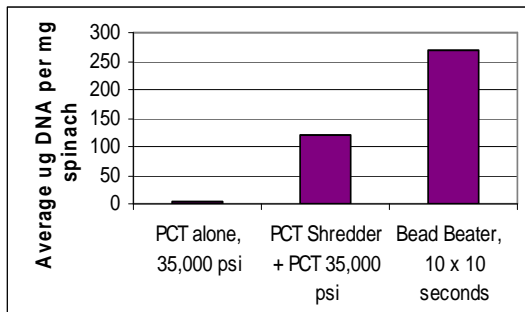
**Figure 1.** DNA Visualization by Agarose Gel Electrophoresis. Lanes 1-3: PCT Alone at 35 kpsi; Lanes 4-6: PCT Shredder + PCT at 35,000 psi; Lanes 7-9: Bead Beating, ten 10 second bursts at full power.

## Results and Discussion



**Figure 2.** A Comparison of DNA Recovery from Spinach Leaf at 35,000 and 45,000 psi with and without Shredder (n=3 per group). Results are expressed in micrograms of recovered DNA per gram of leaf tissue.

spinach from the combination of The PCT shredder and PCT treatment at 35,000 psi, the DNA recovered from the bead beating process was significantly more sheared and fragmented than DNA obtained from the combination of The PCT Shredder and PCT (See Figure 1).



**Figure 3.** A Comparison of DNA Recovery from Spinach Leaf by Bead Beating versus PCT at 35,000 psi with and without Shredder (n=3 per group). Results are expressed as micrograms of recovered DNA per gram of leaf tissue.

Three methods of DNA extraction of DNA from spinach leaves were compared in this study. Specifically, yield and quality of DNA were evaluated for samples processed by The PCT Shredder in combination with PCT, PCT alone, and bead beating. Data show that DNA recovery from samples processed by the combination of The PCT Shredder followed by PCT was significantly higher than from samples processed by PCT alone (See Figures 2 and 3). Interestingly, in these experiments, more DNA was recovered from samples processed at 35,000 psi than from samples processed at 45,000 psi (Figure 2), indicating that higher pressure is not necessary to release high quality DNA from this sample. However, we have not rigorously excluded the possibility that 45,000 psi may actually be detrimental to extraction of DNA from spinach. Therefore, in all subsequent experiments, PCT was performed at 35,000 psi. Extraction by The PCT Shredder followed by PCT was also compared to bead beating, a common method for the extraction of DNA from plants. Although samples disrupted by bead beating averaged ~270 µg of DNA per gram of spinach as compared to ~120 µg of DNA per gram of

## Conclusions

The combination of The PCT Shredder followed by pressure cycling technology (PCT) is an effective and safe method for extraction of DNA from samples such as spinach. Efficient recovery of high quality DNA can be obtained for sequencing, cloning or other experiments. Since disruption and extraction are performed in the same container (Shredder Pulse Tube), the possibility of sample loss due to multiple transfers is reduced. In addition, the likelihood of cross-contamination is minimized. Although more total DNA was obtained using bead beating, the quality of the DNA, as assessed by average length and degree of fragmentation, was significantly lower than the DNA obtained by using the combination of The PCT Shredder and the Pressure Cycling Technology Sample Preparation System.

## References

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