Extraction of RNA 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. Unfortunately, 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, such as when breeding selection is critical (particularly with the advent of genetically engineered crops), or when intellectual property discovery or protection is involved. Here we compare cell disruption by Qiagen RNeasy with mortar and pestle to pressure cycling technology (PCT) for the extraction of RNA from corn sprouts, grape seeds, and grape skin.

Pressure Cycling Technology (PCT)

PCT uses alternating cycles of high and ambient pressures to induce cell lysis. Cell suspensions or tissues, such as leaves or seeds, are placed in specially designed, single-use processing containers (PULSE Tubes) and are subsequently subjected to alternating cycles of high (up to 35,000 PSI) and ambient pressures in a pressure-generating instrument (Barocycler©) – together, the PCT Sample Preparation System (PCT SPS). Maximum and minimum pressures, the time at each pressure level, and the number of cycles are defined using a programmable logic controller. The reaction chamber of the Barocycler instrument can be temperature controlled using a peripheral circulating water bath. Safety features in the design of the PCT SPS significantly reduce risk of exposure to the researcher to pathogens and prevent cross-contamination of samples [2]. The PCT SPS offers a safer, more efficient method for RNA extraction than other methods in use today.

Methods

The PCT SPS was used to extract total RNA from approximately 200 mg of corn sprouts. The PCT SPS was evaluated with several buffers commonly used to extract total RNA from plants. Buffers included 4M Guanidinium Thiocyanate (GTC) with 1% NP-40, Tris with EDTA, and 6M Guanidinium thiocyanate with 1% NP40. Five 1 minute cycles (30 seconds at 35 kpsi, or 236 MPs, followed by 30 seconds at ambient pressure) was applied to the sample. The released RNA was purified using QIAGEN RNeasy kit and visualized on a denaturing agarose gel (See Figure 1).

In another series of experiments, RNA was extracted and purified from grape seeds and grape skin using PCT conditions as described above and AP1 Buffer (Qiagen). The resulting RNA was amplified by rtPCR using a plant-specific primer and visualized on an agarose gel (See Figure 2).

Results and Discussion

Figure 1 shows that high molecular weight ribosomal RNA can be extracted efficiently from plant tissue using the PCT SPS. The PCT SPS offers the additional advantage of being able to simultaneously compare different extraction buffers under identical processing conditions. In this experiment, the best results were obtained using the chaotropic salt guanidine thiocyanate. In contrast, RNA was degraded in the TE buffer as shown in Lane 5.

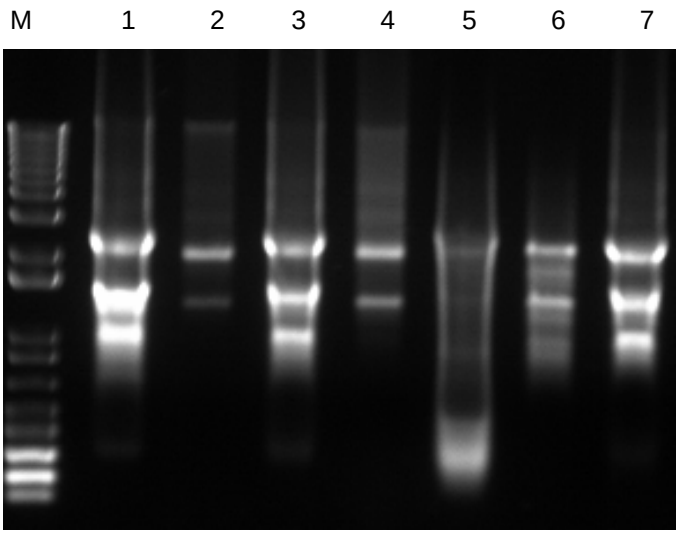

Figure 1. *RNA extracted from corn sprouts (CS) using the PCT SPS compared to extraction by Qiagen RNeasy. Lane M: 1kb MW marker, Lane 1: CS processed by PCT with saturated gdn/1% chaps, Lane 2: CS processed with saturated gdn/1% chaps/ no PCT, Lane 3: CS processed by PCT with 6 M GTC/1% NP 40, Lane 4: CS with 6 M GTC/1% NP 40 /no PCT, Lane 5: CS processed by PCT with TE Buffer, Lane 6: plant tissue with TE Buffer/no PCT and Lane 7: CS processed by QIAGEN RNeasy.*

Figure 2 shows that specific mRNA suitable for amplification in an rtPCR reaction can be extracted efficiently from corn sprout using the PCT SPS.

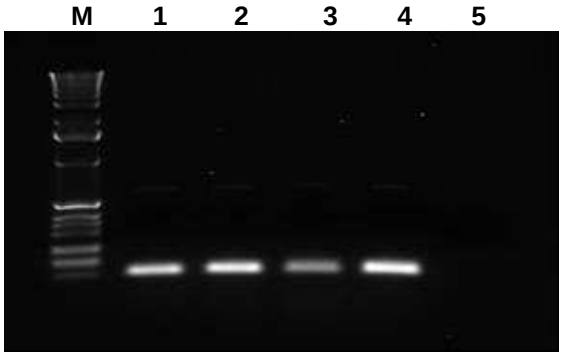

Figure 2. *RNA extracted from grape seeds and grape skins using the PCT SPS compared to extraction by mortar and pestle (M/P). Lane M: 1kb MW marker, Lane 1: grape seeds processed by the PCT SPS with saturated gdn/1% chaps, Lane 2: grape seeds processed by M/P, Lane 3 grape skin processed by the PCT SPS, Lane 5: grape skin processed by M/P, and Lane 5: rtPCR negative control*

The PCT Sample Preparation System is an effective method for the rapid, safe, reproducible, and versatile release of RNA from a variety of plant tissues. It is extremely adaptable for different species, crops, and sample conditions. Furthermore, the quality and quantity of RNA isolated from PCT-treated samples is sufficient for rt-PCR amplification and other downstream applications. The versatility of the PCT SPS makes it useful for the release of nucleic acids as well as a wide-range of other purposes, including buffer 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

[1] Schumacher, R.T., *et al.* (2002). Am. Laboratory 34, 38-43.

[2] Harrington, S., et al, (2004) “Use of Pressure Cycling Technology (PCT) for the Release of DNA from Plants” Annual Plant, Animal and Microbes Genomics Conference, San Diego, CA