Gene Expression Analysis by Microarray Using RNA Extracted by Pressure Cycling Technology (PCT)

Genes that code for protein are transcribed into messenger RNA (mRNA). This process is generally referred to as gene expression. The mRNA is subsequently translated (with or without additional processing *in vivo*) into protein. The presence and relative amount of transcribed mRNA may have important implications in the diagnosis of infectious and genetic diseases, drug discovery, drug therapy, and numerous other applications. Accurate determination of both the presence and amount of mRNA can be one of the essential components in proper identification of a disease state or determination of the efficacy of drug treatment when comparing populations of cells. However, it is often difficult to work with mRNA because it is prone to degradation by ribonucleases, heat, or extreme pH. In molecular tests, mRNA is often converted using reverse transcriptase (RT) *in vitro* to form complementary DNA (cDNA). The resulting cDNA can then be amplified in a polymerase chain reaction (RT-PCR), where it is less prone to degradation and therefore more suitable for analysis. The resulting cDNAs can then be evaluated in hybridization reactions such as in microarrays. However, the first critical step in the preparation of cDNA is the release of mRNA from the cell with minimal perturbation. Unless the mRNA is introduced into the RT reaction in sufficient copy number and high quality, there may be a bias in both the resulting representation and quantification, thus making interpretation by a microarray more difficult. To reduce the likelihood of bias being introduced during sample preparation, PBI has developed techniques based on pressure cycling technology (PCT) to release mRNA from cells and tissues.

Pressure Cycling Technology (PCT)

PCT uses alternating cycles of high and ambient pressures to induce cell lysis. Cell suspensions or tissues, such as rat brain, 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 [1]. The PCT SPS offers a safer, more efficient method for RNA extraction than other methods in use today.

Methods

To demonstrate the PCT SPS for release of RNA, 200 mg fresh frozen rat brain was extracted in 1.1 mL TRI Reagent (MRC) using standard PCT conditions, i.e., 35 kpsi, five 1 min cycles at 4°C. A positive control sample was obtained using mortar-pestle grinding on dry ice. Crude lysate was clarified by centrifugation. Total RNA was purified using the TRI Reagent protocol (Ambion, Austin, TX). The purified RNA was quantified at OD_{260} and an aliquot was examined in a formaldehyde agarose gel to assess the quality of the RNA (See Figure 1). A cDNA library was prepared by rtPCR from 1.0 μg of each purified total RNA. The resulting cDNA was examined using the SuperArray microarray GEArray™ Q-series protocol on a MM-013N membrane (Neuroscience-1 Ion Channel & Transporter series, SuperArray Bioscience Corp, Frederick, MD). Chemiluminescent signals were detected by CCD camera.

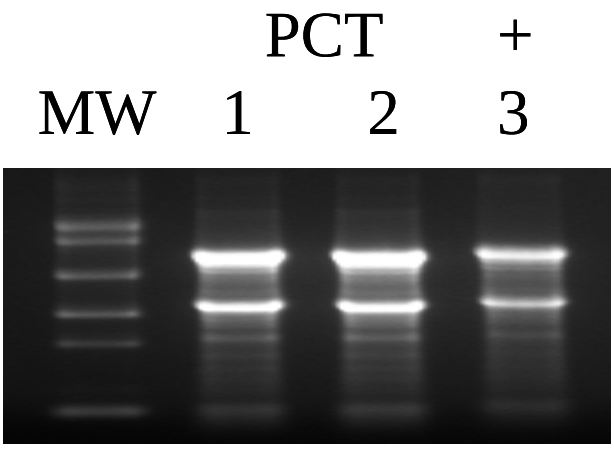

Fig. 1. *RNA extracted from rat brain examined by formaldehyde agarose gel electrophoresis. MW depicts molecular weight marker, Lanes 1 and 2 are duplicates of rat brain extracted by PCT, and Lane 3 is rat brain extracted by mortar and pestle*

Results and Discussion

Data show that the PCT SPS yields quality and quantities of RNA equal to or better than conventional extraction procedures, such as mortar and pestle grinding. High quality, reproducible total RNA was extracted using the PCT SPS as indicated by the rRNA bands in the formaldehyde agarose gel (See Fig. 1).

Total RNA prepared by the PCT SPS and subsequently purified by the Tri Reagent Protocol was successfully used to prepare a cDNA library. The resulting cDNA was analyzed on microarray assays [2]. In this experiment, cDNA derived from PCT extracted RNA produced a high quality microarray with spots of greater intensity than those produced from RNA extracted by mortar and pestle (See Fig. 2A and 2B).

2A 2B

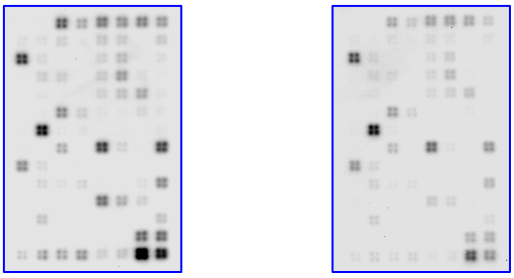

Fig. 2 *(A) cDNA microarray analysis derived from RNA extracted by the PCT SPS (B) cDNA microarray analysis derived from RNA extracted by mortar and pestle*

In comparison to the mortar/pestle extracted RNA sample (positive control), the PCT extracted RNA was of better quality and produced a superior microarray, as indicated by the relative intensity of spots on the microarray.

The PCT SPS provides a standardized method for preparing mRNA suitable for use with sophisticated analyses, such as microarrays. Data show that the integrity of fragile molecules, such as mRNA, is maintained during extraction by the PCT SPS.

Furthermore, the PCT SPS extraction method is compatible with standard downstream purification processes. RNA extracted by the PCT SPS is suitable for rtPCR and may be coupled with other techniques, such as amplification.

Acknowledgements

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References

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

[2] www.ncbi.nlm.nih.gov/About/primer/microarrays.html

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