Preparation of crude cell extract suitable for amplification of RNA by the polymerase chain reaction

Francois Ferre and Fermin Garduno

Cytometrics Inc., Division of Specialty Laboratories Inc., 11575 Sorrento Valley Road, San Diego, CA 92121, USA

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The following is a simple and efficient method to obtain RNA from crude eukaryotic cell extract for use as starting material for amplification by the polymerase chain reaction. This method is an alternative to the published procedure for purified RNA (1).

Method: Pellet the cells (2×10^6), wash 2 times in PBS, resuspend with 100 μ l of freshly made diethyl pyrocarbonate (DEPC) in double distilled water (1/1000) and transfer into a 1.5ml microfuge tube. Boil for 5 minutes, centrifuge (12,000g) for 30 seconds and harvest supernatant. Add 5 μ l of the supernatant to a reverse transcription reaction in a final volume of 20 μ l [1x reverse transcription buffer (BRL), 20 units of RNASIN(Promega), 1.0mM of each dNTP's, 10 μ mol of the 3' primer, 200 units of cloned MuLV-1 reverse transcriptase (BRL)]. Incubate at 37°C for 45 minutes; stop the reaction on ice. Dilute the reaction mixture with 80 μ l of PCR buffer (50mM KCl, 10mM Tris-HCl, pH8.3, 1.5mM MgCl₂, 0.01%(w/v) gelatin) containing 40 μ mol of the 3' primer, 50 μ mol of the 5' primer and 2 units of Taq polymerase. Add 100 μ l mineral oil to prevent evaporation. Perform the PCR assay as previously described (1). Electrophorese 20 μ l of the amplified product on a 4% Nu-Sieve agarose gel. Stain with EtBr and photograph.

Results: Amplification of the chimeric bcr.abl mRNA from K562 cells (2) was performed on purified total RNA (3) and RNA from crude cell extract (prepared by the method above). Equivalent amounts of total RNA were used (10^5 cells consistently yields 1 μ g purified RNA). An equal or greater amount of amplified product (195 bp fragment) was obtained with crude RNA extract (fig. 1: lanes 5 and 9) as opposed to purified total RNA (fig. 1: lanes 3 and 7). Hela cells, which do not express the chimeric bcr.abl mRNA, served as a negative control (fig. 1: lanes 2,4,6 and 8). These data demonstrate that using crude cell extract is as sensitive and specific as using purified total RNA as starting material for PCR assays.

Comments: The total number of boiled cells can be increased up to $10\text{--}20 \times 10^6$ cells/100 μ l DEPC, H₂O. To minimize RNA degradation after boiling, the supernate should be transferred immediately into the reverse transcription reaction. This method has been successfully used with clinical samples from patients with chronic myelogenous leukemia (CML) expressing the chimeric bcr.abl mRNA. Finally, with this method, various mRNA templates have been successfully amplified using a number of different sets of primers.

Fig. 1: PCR amplified fragments electrophoresed on a 4% NuSieve gel. Lane 1. 123 bp ladder. Lanes 2,4,6 and 8, Hela cells: 2. 1 μ g of purified RNA, 4. 10^5 boiled cells, 6. 0.1 μ g of purified RNA, 8. 10^4 boiled cells. Lanes 3,5,7 and 9, K562 cells: 3. 1 μ g of purified RNA, 5. 10^5 boiled cells, 7. 0.1 μ g of purified RNA, 9. 10^4 boiled cells.

References: (1) Kawasaki, E.S. et al. (1988) Proc. Natl. Acad. Sci. USA 85: 5698-5702. (2) Shtivelman, E. et al. (1985) Nature (London) 315: 550-554. (3) Chomczynski, P. and Sacchi, N. (1987) Analytical Biochemistry 162: 156-159.