Simple and Rapid Method for Isolation of RNA from Gram-Negative Bacteria

*BioTechniques* 20:546-547 (April 1996)

Several molecular biological techniques require high-quality RNA, essentially free from DNA, in high yield. Several methods are widely used to isolate RNA from gram-negative bacteria including acid-phenol extraction, guanidine isothiocyanate or hydrochloride extraction (1) and cesium chloride precipitation (2). These methods are laborious, costly and time-consuming. Considering these limitations, we have developed a simple alkali lysis procedure for high-quality RNA isolation from gram-negative bacteria, which is rapid, inexpensive and does not involve the utilization of phenol, chloroform or diethyl pyrocarbonate (DEPC). In this report, we compared the efficiency of this quick and simple RNA isolation procedure with the recently developed TRIzol™ method (Life Technologies, Gaithersburg, MD, USA), which uses monophasic solutions of phenol and guanidine isothiocyanate.

*Porphyromonas gingivalis* ATCC 53977 was grown anaerobically in trypticase soy broth (TSB) containing hemin (10 µg/mL) and menadione (1 µg/mL) at 37°C. *E. coli* MV1184 was grown aerobically in Luria broth (LB) at 37°C. A 50-µL sample of *P. gingivalis* grown for 18 h was inoculated into 8 mL of TSB containing 125 µM 2,2'-dipyridyl to induce *hemR* (hemin receptor) expression, and grown to an *A₆₀₀* of 0.7, while 500 µL of *E. coli* cultured for 18 h were inoculated into 6 mL of LB and grown for 4 h. Bacterial cells were harvested in sterile 1.8-mL microcentrifuge tubes using a Tomy Model MTX-150 refrigerated microcentrifuge (Tomy Tech, Palo Alto, CA, USA) (7000 rpm for 5 min), the supernatant fluid discarded and the tubes placed on ice. Lysis solution (40 µL) containing 100 mM NaOH, 0.5 g of sodium dodecyl sulfate (SDS), 5 mM EDTA and 8 g sucrose per 100 mL was added to each of the tubes. Pellets were dissolved in lysis solution by pipetting up and down with a micropipet (avoiding air bubbles). The tubes were then incubated at 37°C for 15 min, placed on ice, and 15 µL of 3 M sodium acetate (pH 5.6) were added. After vortex mixing thoroughly, the tubes were centrifuged at 4°C (15 000 rpm for 15 min; TMA-11 rotor). The supernatants were then collected in fresh tubes to which 2 vol of ice-cold absolute ethanol were added and mixed well. The samples were centrifuged (12000 rpm for 10 min; TMA-11 rotor) again, and the pellet was washed once with ice-cold 70% ethanol. The pellets were next air-dried at room temperature under a vacuum for 30 min and stored at -70°C in 80 µL of DEPC-treated water or absolute ethanol.

**Figure 1.** RNA isolated from E. coli and P. gingivalis. Lane a, molecular weight markers; lane b, *P. gingivalis*; lane c, *E. coli* RNA isolated by the new method; lane d, *P. gingivalis* and lane e, *E. coli* RNA isolated by the TRIzol procedure and treated with DNase prior to loading on the gel. RNA samples were mixed with RNA dye containing formamide and loaded on the gel.

**Figure 2.** Northern blot analysis of the *P. gingivalis* *hemR* transcript. Lane a, molecular weight markers; lanes b and c, *P. gingivalis* RNA isolated by the new and TRIzol methods, respectively. 17.5 µg of RNA from the samples were resolved on a 1.2% formaldehyde-agarose gel and a Northern blot was prepared. The blot was hybridized with a 0.4-kb PstI-BamHI fragment from the *P. gingivalis hemR* gene.
ethanol. By this method, we could routinely isolate 80–100 μg of RNA per 8 mL of culture. RNA isolated by this method or with TRIzol (manufacturer’s recommended procedure) exhibited an A260–280 ratio of 1.6–1.8. When needed, RNA samples were supplemented with RNA loading dye [0.72 mL formamide, 0.16 mL 10x MOPS buffer, 0.26 mL 37% formaldehyde, 0.18 mL distilled water (DEPC-treated), 0.10 mL 80% glycerol, and 8.0 mg bromophenol blue] and resolved on 1.2% agarose gel. By this method, we could routinely isolate, bromophenol blue and resolved on 1.2% agarose gel. Alternatively, bromophenol blue (50 mg per 100 mL) could be added to the lysis buffer, and RNA, isolated as described above, could be directly applied to formaldehyde-agarose gels.

RNA isolated from P. gingivalis and E. coli using this new method was fractionated on formaldehyde-agarose gels along with preparations obtained by the TRIzol method. Figure 1 compares the quality of RNA isolated by this new protocol relative to the TRIzol method. No differences are seen in Figure 1, indicating no visible contamination in the new procedure. In the TRIzol method, chromosomal DNA contamination was detected for both organisms and was removed by DNase (RNase free) digestion prior to electrophoresis. RNA samples resolved on a formaldehyde-agarose gel were then transferred to a Hybond™-N+ nitrocellulose membrane (Amersham, Arlington Heights, IL, USA) by standard methods (3). This Northern blot was subjected to hybridization with a 0.4-kb EcoR-BamHI DNA fragment internal to the P. gingivalis hemR gene (Karunakaran and Kuramitsu, unpublished results) as a probe utilizing nonradioactive ECL™ direct nucleic acid labeling and detection systems (Amersham). Figure 2 shows the results obtained following exposure of the blot to Hyperfilm™-ECL (Amersham). The results clearly indicate a transcript size of 3.1 kb for the P. gingivalis hemR gene with RNA isolated by both methods.

These results indicate that the newly described method represents a rapid and simple method for isolating high-quality RNA from gram-negative bacteria. Northern blot analysis readily detects specific mRNA transcripts in these preparations.

**REFERENCES**


This study was supported in part by Grant DE08293 from the National Institutes of Health. Address correspondence to Howard Kuramitsu, Department of Oral Biology, Rm. 304 - Foster Hall, State University of New York at Buffalo, 3435 Main Street, Buffalo, NY 14214-3092, USA. Internet: karamits@ubvms.cc.buffalo.edu

Received 5 September 1995; accepted 6 November 1995.

T. Karunakaran and Howard Kuramitsu
State University of New York at Buffalo
Buffalo, NY, USA

**Improved Isolation of Genomic DNA from Mycobacteria in Agarose Plugs by Rapid Lysis with a Combination of N-Acetylglucosaminidase and Lysozyme**

BioTechniques 20:547-552 (April 1996)

Pulsed-field gel electrophoresis (PFGE) allows the separation of large DNA fragments. The technique, originally developed by Schwartz et al. (6) for the separation of yeast chromosomes, has been applied to the analysis of bacterial genomes. After digestion by low-frequency cleavage restriction endonucleases, bacterial chromosomes provide DNA patterns composed of a few, usually well separated fragments.

Isolation of genomic DNA from mycobacteria is a time-consuming and tedious process (1,6). Most pathogenic mycobacteria grow slowly in culture media and their cells are difficult to lyse. The tough mycobacterial cell walls contain thick layers of lipopolysaccharide-protein complexes that make them resistant to the standard protocols (1,6). Due to the resurgence of tuberculosis, the molecular fingerprinting of mycobacterial isolates by restriction fragment length polymorphism (RFLP) analysis (7,8) is gaining importance in mycobacterial research and epidemiology. This involves handling of a large number of strains at a time. The time-consuming procedure in the isolation of DNA from mycobacteria is the disruption of the thick, lipopolysaccharide-rich cell wall without causing damage to the genomic DNA. We report a rapid and gentle method to extract sufficient quantity of unsheared genomic DNA from mycobacterial cells. Until now, several groups had isolated mycobacterial genomic DNAs from *Mycobacterium tuberculosis*, *M. bovis* BCG strains, *M. paratuberculosis* and *M. avium* using the usual method by utilizing lysozyme or zymolase for preparation of intact genomic DNAs in agarose plugs and analyzing by PFGE (2–4,7,8). However, these suffered from impurity of