

Rapid mini-preparations of total RNA from bacteria

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While a wide variety of methods exist for RNA preparation from different cell types/organisms, we describe a small scale preparative method that to date has worked for over 10 different bacterial genera. This method is a modification of a large scale method described for *B.subtilis* (1). We developed this method so that it would fullfil several criteria required for work in this laboratory on detection of gene expression of bacterial pathogens in environmental samples. These criteria are: simplicity, small scale (start off volumes of less than 1.5 ml and 10^8 cells), no requirement for physical contact of the sample with equipment which could become a source of cross-contamination on multi-sample analysis and finally the production of nuclease-free intact total RNA suitable for analysis by gel electrophoresis, Northern blotting and RNA-targeted amplification reactions. The protocol is: pellet cells in an Eppendorf microfuge at 8000 g for 2 min. Resuspend cell pellet in 20 µl of DEPC treated H_2O (2) and add 3 µl 0.5% v/v DEPC (diluted in H_2O). Add 200 µl ice-cold acetone, mix by hand and centrifuge at 10,000 g for 2 min. Remove supernatent with care, resuspend pellet in 30 µl of DEPC-H_2O and add 1 µl 100 µg/ml Proteinase K. Incubate on ice for 10 min. Add 3.5 µl DEPC (0.5% v/v), 200 µl phenol (preheated to 70°C), 150 µl chloroform, mix by hand, add a further 120 µl DEPC-H_2O and centrifuge at 12,000 g for 5 min. Remove the aqueous phase (top layer) and add to 1 ml ethanol. Allow RNA to precipitate for 1 hr at −20°C. Pellet RNA at 12,000 g for 15 min, resuspend in 50−100 µl DEPC-H_2O and store at −20°C. Figure 1 shows the RNA products prepared from 10^6 cells of different bacterial strains electrophoresed on 1.2% TBE agarose gels. Intact 16S and 23S rRNA bands can be visualised. Figure 2 shows the result of a Northern blot on total RNA prepared from *E.coli* and hybridised with oligodeoxynucleotides derived from the *E.coli* 16S rRNA and 4.5S RNA genes. Discreet bands show that the prepared RNA is intact and as such a suitable template for Northern blot analysis. Contaminating genomic DNA has been found in some preparations, particularly from Gram + bacteria, but due to its large molecular weight has no deleterious effect on Northern blot analysis. A lithidium chloride treatment can be added to remove this DNA (2). For RNA specific amplification using RT-PCR on the prepared RNA, we use a DNase I pretreatment of sample as described by Dilworth and McCarrey (3) before amplification.

We have determined that the RNA prepared is stable and nuclease-free by performing incubations at 37°C for 48 hr. RNA yields for 10^8 cells, calculated by OD-260 spectrophotometry, are *B.subtilis* — 7 µg, *P.aeruginosa* — 17 µg, *E.coli* — 14 µg, *S.aureus* — 5 µg, *A.salmonicida* — 20 µg, *S.marcescens* — 4 µg, *C.glutamicum* — 6 µg and *V.anguillarum* — 17 µg. This is the equivalent of approximately 100 fg of RNA per cell.

We are using this method to study gene expression of fish pathogens in sea water microcosms where cell numbers/sample are below 10^6. The general nature of this method demonstrated by its effectiveness with several bacterial genera means that it may be a useful tool for analysing microbial gene expression in environmental, food or clinical samples where cell numbers are low and without the need to culture these microorganisms to larger numbers for analysis.

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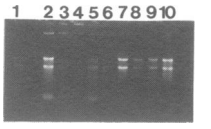

Figure 1. Agarose gel electrophoresis of RNA products prepared from different bacteria. Lane 1—prokaryotic 16S/23S rRNA molecular weight markers. 2—*A.salmonicida*, 3—*B.subtilis*, 4—*S.marcescens*, 5—*S.aureus*, 6—*C.glutamicum*, 7—*V.anguillarum*, 8—*A.hydrophila*, 9—*E.coli*, 10—*P.aeruginosa*.

Figure 2. Northern blot analysis of a combined probing of prepared *E.coli* total RNA with 16S rRNA and 4.5S RNA specific oligonucleotides. Arrow A = 16S rRNA and arrow B = 4.5 S RNA.

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