Rapid purification of ribosomal RNAs from neutral agarose gels

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DNA fragments can be purified from agarose slices by dissolving the agarose in chaotropic agents like NaJ or $NaClO_4$, followed by subsequent binding of the DNA to silica or glass particles (1, 2). We have recently reported (3) on a one-hour method for the purification of nucleic acids (DNA and RNA, both double-stranded and single-stranded) from clinical specimens like serum and urine. This method is based on the very strong nuclease-inactivating properties of the chaotropic agent Guanidiniumthiocyanate (GuSCN), together with the NA binding properties of silica particles or diatoms in the presence of this agent. Here we describe an adaptation of the method for the purification of ribosomal RNAs from neutral agarose gel slices.

Total nucleic acid (NA) was purified from 5 samples (400 µl each) of a culture of *Escherichia coli* HB101 (carrying a high copy plasmid) using diatoms as NA- carrier by Protocol Y/D as described previously (3). Two NA samples were used as markers in the gel shown (lanes 2 and 9); the other 3 NA samples were electrophoresed (8−10 V/cm) through a separate neutral 1% agarose (Sigma Chemical Company, type II, Medium EEO) gel containing ethidiumbromide. Gel slices (approximately 50 µl each) containing either the 23S or the 16S rRNA species were cut from this gel with a flamed razor blade. The slices were dissolved in preassembled Eppendorf tubes containing 900 µl lysisbuffer L_6 (containing GuSCN) and 40 µl of a diatom suspension (D) under continuous rotation (Mixer CM-9, Sarstedt, Rummelsdorff; 1600 r.p.m.) for 30 min at room temperature. The tubes were centrifuged for 15 sec at 12,000 ≈ × g, the supernatant was discarded and the diatom/RNA complexes were washed twice with washing buffer L_2 (containing GuSCN), twice with ethanol 70% (vol/vol), once with acetone, dried at 56°C (10 min) and rRNAs were subsequently eluted in TE-buffer (10 mM Tris.Cl, 1 mM EDTA, pH = 8) as described (3) and electrophoresed through a neutral gel (lanes 3−8). Ribosomal RNAs were recovered essentially undegraded at approximately 30−50% efficiency (lanes 2 and 9 represent the amount of rRNA that would be observed at 100% efficiency). The whole experiment (from bacteria to gel-purified rRNAs) took about 3 hrs; no RNAse inhibitors like RNAsin, vanadylribosyl complexes or di-ethylpyrocarbonate were used. The preparation of the diatom suspension (D), buffers L_6 and L_2 and electrophoresis conditions were as described previously (3).

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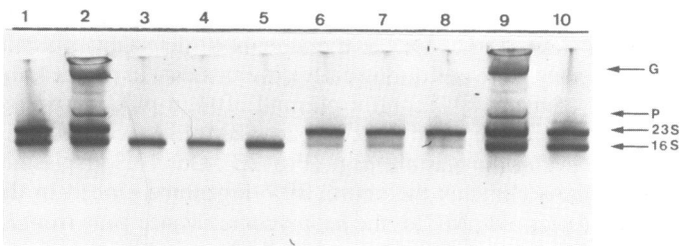

Figure 1. Ethidiumbromide-stained neutral agarose gel, photographed under UV trans-illumination. Lanes 1 and 10, 2 µg marker *E. coli* 23S and 16S rRNAs (Boehringer Mannheim, GmbH); lanes 2 and 9 total NA from *E. coli*; lanes 3−5 and 6−8, gel-purified 16S and 23S rRNA, respectively; G = genomic DNA, p = plasmid DNA.