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Silicone lubricant enhances recovery of nucleic acids after phenol–chloroform extraction

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Phenol–chloroform extraction is a major step in the purification of nucleic acids. Phenol, a strong deproteinizing agent, with chloroform removes most of the proteins from the nucleic acid. Residual proteins are often removed by enzymatic digestion using sodium dodecyl sulfate (SDS)/proteinase K, but the physical separation of the proteins from the nucleic acids is accomplished by centrifugation of a mixture of the nucleic acid solution and phenol and chloroform. During this process these organic compounds make complexes with the proteins; these complexes partition phenol–chloroform in the bottom and nucleic acids in aqueous phase on the top. To avoid protein contamination, the supernatant on the top is carefully removed without disturbing the interface. Total recovery of the aqueous phase is difficult to achieve, since some interphase proteins may be collected with the upper layer. However, because only very small quantities are produced at a time, maximum sample recovery is of utmost importance. Such extractions are often required in molecular biology work to inactivate and remove enzymes used in one step before proceeding to the next. Isolation of RNA requires even more precautions since only a very minor contamination of the interface containing the RNase may degrade the RNA.

We found a simple and inexpensive method of improving the recovery of the nucleic acid after phenol–chloroform extraction. Addition of a little silicone lubricant (vacuum grease, DOW Corning Corp., Midland, MI) to the phenol–chloroform extraction mixture resulted in a tight interface that facilitated total recovery of the aqueous phase without interfering with the lower protein and organic phases. Silicone grease is insoluble in water and can be autoclaved. It is nontoxic and does not interfere in the phenol–chloroform extraction procedure. Moreover, the proteins remain below the silicone partition, leaving the clear aqueous phase on the top. In our practice, we store autoclaved silicone lubricant in a 10 ml syringe. During the extraction procedure we dispense approximately 30 to 50 μl of the lubricant into the tube containing the DNA/RNA solution by 18-gauge needle. The phenol–chloroform extraction is performed by vortexing followed by centrifugation at 12,000 rpm for 15 minutes at either 4°C or room temperature. The aqueous phase may then be recovered without any loss.

We have used this procedure successfully during extraction of small samples of RNA (by the guanidine isothiocyanate–phenol–chloroform method [Chomczynski and Sacchi, 1987]) and DNA. When RNA was extracted from 10^5 human non-small-cell lung cancer cells, recovery of total RNA was higher in the presence of silicone lubricant (Figure 1A). Total RNA was separated using 1.4% formaldehyde/MOPS agarose gel, and 28S rRNA was quantitated after ethidium bromide staining using a densitometer (Molecular Dynamics, CA). Results showed about

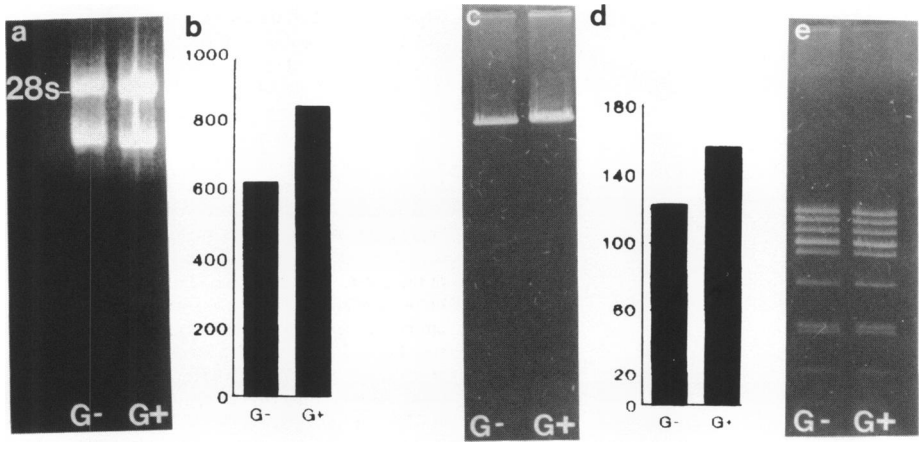

Figure 1. **(A)** RNA was extracted with (G+) or without (G–) silicone lubricant from 10^5 cells and electrophoresed in 1.4% gel. **(B)** 28S rRNA bands were quantitated and recovery of the samples were compared densitometrically. **(C)** Total plasmid DNA was extracted and digested with *Eco*RI, DNA was separated in the gel. **(D)** Band intensity was compared. **(E)** Plasmid DNA extracted with or without silicone was double-digested with two different enzymes; silicone extraction did not interfere with enzyme digestion.

20–30% increased yield of RNA with silicone lubricant extraction (Figure 1B). Similarly, 14 kb plasmid DNA was isolated using alkaline lysis mini preparation method with or without silicone lubricant. The total plasmid DNA was digested with *Eco*RI and run in 1% agarose gel (Figure 1C). Quantitation of the ethidium bromide-stained band of linearized plasmid DNA showed more than 20% increased yield using silicone extraction (Figure 1D).

To determine the effect of adding silicone on further enzyme reactions, 4 μg of plasmid DNA extracted with or without grease was digested with *Eco*RI and *Pst*I together; after 3 hours of digestion at 37°C, DNA sampales were run on 1% agarose gel in TBE (Tris–boric acid–EDTA) buffer (Figure 1E). DNA isolated using both extraction prodcedures was digested completely, indicating that use of grease in the extraction method does not interfere with enzyme digestion. Thus, this method is useful for isolating RNA from a minimal number of cells for making cDNA and PCR amplification. Moreover, an increased recovery of the nucleic acids using phenol–chloroform silicone extraction would be widely useful, particularly while dealing with small samples.

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