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Novel procedure of elution and concentration of nucleic acids with NACS Prepac™ minicolumns by electrophoresis

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The innumerable procedures for the elution of nucleic acids from agarose gel have mainly two pitfalls : first, many subsequently intended enzyme reactions are interfered with by "inhibitory" constitutents of agarose; second, they are too time consuming if larger numbers of samples are to be handled.

We were succesful in avoiding these complications when preparing large numbers of different size class nucleic acid samples for UV-spectrophotometry, hybridization, transcription (with SP6 RNA-Polymerase), and DNA recombination in combining elution from agarose and concentration in NACS Prepac™ minicolumns (BRL-Gibco) by electrophoresis.

Procedure : 1) Treat minicolumns as described in the manufacturer's booklet. 2) Equilibrate with electrophoresis buffer (2.5 mM Na-acetate, 1mM EDTA, 20 mM Tris-acetate, pH 7.8). 3) Fill minicolumn reservoir with same buffer, introduce excised agarose sample. 4) Mount minicolumn into a standard tube gel electrophoresis apparatus (all air bubbles to be avoided in reservoir as well as in the outlet). 5) Electrophoresis 20 min, 5-8 mA/column (100 V). 6) Elute detached and cleared columns with elution buffer (e.g. 2M NaCl, 1mM EDTA, 10 mM Tris-HCl, pH 7.4); nucleic acids appear within 10 to 15 drops of it. Recovery : 65 to 90 % for RNA (50 to 240 nucleotides long) and DNA (60 bp to 8 kbp). Supercoiled plasmid DNA 20 to 50 %). Columns may be reused at least 5 times.

This technique is of even greater value to us in collecting DNA or RNA from large numbers (from 10^8 to 10^9 cells) of agarose encapsulated nuclei, chromatin, or nucleoskeletons (Jackson, D.A. and Cook, P.R. (1985) EMBO J. 4, 919-925). The minicolumns are cut to syringe format, and on top supplemented with a 50 ml syringe (or an equivalent Eppendorf Repettor Tip™) to admit about 30 ml of agarose beads containing the encapsulated material. Electrophoresis (conditions same as above), immersing the negative electrode directly into the syringe, the positive electrode in a buffer-filled beaker into which the minicolumn outlet is submersed. With this method we obtain the nucleolytic products, according to the performed analysis, from a highly diluted form in the beads, at sufficiently high concentration, and free of interfering substances for further experimentation.