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A rapid and simple method for the isolation of high molecular weight cellular and chromosome-specific DNA in solution without the use of organic solvents

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A novel method has been developed for the isolation of high molecular weight DNA from eukaryotic cells and flow sorted chromosomes. This method includes removal of detergent and proteolytic digestion products by dialysing nucleic acid containing solutions against 20% (w/v) polyethylene glycol (PEG). Because lysates remain static during PEG dialysis, hydrodynamic shear degradation of DNA is reduced in comparison to conventional protocols utilizing chloroform and phenol extractions. Resulting preparations contain pure high molecular weight DNA (average fragment length approximately 500 kb) and non-degraded RNA with 260/280 values equal to, or greater than 1.8. PEG-isolated DNA can be used in all standard molecular biological applications, including restriction enzyme digestion, ligation, and cloning. Additionally, samples can be further processed using DNase and organic extraction to yield RNA suitable for northern blot analysis.

PEG isolation of cellular and chromosome-specific nucleic acids. This procedure works well when a maximum of $3x10^7$ diploid mammalian cells are processed to yield up to approximately 200 μg of genomic DNA. Cells (washed free of culture media), or flow sorted chromosomes, are resuspended within a sterile 15 ml polypropylene tube in 5.0 ml of autoclaved 10 mM Tris-HCl pH 8.0, 1 mM EDTA (TE buffer). Stocks of SDS and proteinase K are added to final concentrations of 0.1% and 100 μg per ml respectively. Tubes are gently inverted two times (to mix reagents) and incubated at 37°C overnight. Using an autoclaved Schleicher and Schuell model UH020/2A microdialysis apparatus, samples are dialysed/concentrated (without vacuum) at 22°C against four 60-minute changes of 20% w/v PEG 8000 (Sigma) in TE (autoclaved). The PEG phase is slowly stirred during these steps. Sample volumes are seen to decrease approximately 10-fold during PEG dialysis. Desalting is accomplished by microdialysis against two 15-30 min changes of TE, also at 22°C. Final processed samples are transferred to sterile polypropylene tubes for long-term storage at 4°C.

Further processing to obtain cellular RNA. Immediately following PEG dialysis and desalting, fresh PEG-isolated cellular nucleic acids are aliquoted into a sterile 1.5 ml polypropylene tube. Autoclaved 1 M $MgCl_2$ is added to 20 mM, and the sample is digested for 20 min at 37°C with a two-fold unit excess of RNase-free RQ1 DNase (Promega Biotech). Sample viscosity should decrease dramatically during DNase treatment. Immediately following incubation, SDS is added to a final concentration of 0.5% and the sample is incubated 65°C for 5 min. Following extraction with chloroform:phenol and ethanol precipitation, RNA is ready for subsequent manipulation (i.e., northern blot analysis). Full details of these procedures, results, and applications can be obtained from the authors upon request.

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