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Ethanol precipitation of DNA with linear polyacrylamide as carrier

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Linear polyacrylamide is a very efficient neutral carrier for precipitating picogram amounts of nucleic acids with ethanol. Polyacrylamide has several advantages over other available carriers for working with DNA or studying DNA-protein interactions.

Preparation of linear polyacrylamide: prepare a 5% acrylamide solution (without bis-acrylamide) in 40 mM Tris-HCl, 20 mM Na acetate, 1 mM EDTA, pH 7.8. Add 1/100 vol. of 10% ammonium persulfate and 1/1000 vol. TEMED, and let polymerize for 30 min. When the solution has become viscous, precipitate the polymer with 2.5 vol. ethanol, centrifuge, and redissolve the pellet in 20 vol. of water by shaking overnight. The 0.25% linear polyacrylamide solution obtained can be stored in the refrigerator for several years.

Precipitation of DNA: to DNA in >0.1 M salt, add 10–20 μg of linear polyacrylamide and 2.5 vol. ethanol. Leave at −70°C in a dry ice–ethanol bath for 10 min. and centrifuge for 10 min. at top speed in a microcentrifuge. Carefully remove the supernatant, wash the pellet with ethanol and dry. Polyacrylamide can also be used to precipitate RNA with ethanol or proteins with acetone.

Figure 1 shows that precipitating picogram amounts of DNA by the above method in the presence of polyacrylamide results in complete recovery of fragments larger than 20 base pairs, whereas most of the DNA is lost if no carrier is used. Also note that very short DNA fragments do not coprecipitate with polyacrylamide, which permits separation of labeled DNA from unreacted nucleotides by precipitation after labeling reactions. Polyacrylamide has been used in several laboratories for most of the common manipulations of DNA, including enzyme reactions, gel electrophoresis, cloning (3), DNA-protein interactions (2), and appears inert in all experiments. For example, unlike tRNA, it is not phosphorylated with polynucleotide kinase, and Figure 2 shows an example where glycogen interferes with DNA-protein interactions whereas polyacrylamide does not.

REFERENCES

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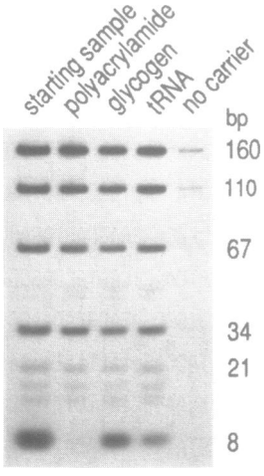

Figure 1. Precipitation of DNA fragments in the presence of different carriers. A mixture of 5′ end-labeled DNA fragments of different lengths (~1000 cpm each, total DNA ~20 pg) was ethanol-precipitated as described in the text in the presence of the indicated carriers. The precipitates were redissolved and electrophoresed on a 10% polyacrylamide gel; an autoradiogram is shown.

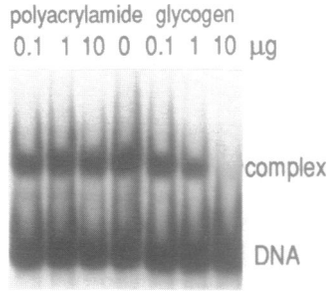

Figure 2. DNA-protein interactions in the presence of polyacrylamide or glycogen. The labeled late-strand DNA of the SV40 control region was mixed with a sequence-specific single-strand-binding protein (1) in the presence of the indicated amounts of polyacrylamide or glycogen. Formation of the DNA-protein complex was assayed by gel-retardation (2).