Precipitation of DNA by polyethylene glycol and ethanol

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The use of polyethylene glycol (PEG) for precipitation of DNA has been described in the literature (1–6). We present here conditions for precipitation of DNA by PEG that give improved recoveries of DNA so that precipitation by PEG is comparable to that by ethanol, in terms of recovery, over a wide range of DNA concentrations. The problem of precipitation of low-molecular-weight DNA fragments by PEG was also investigated.

The recovery of DNA obtained on precipitation with PEG or ethanol was determined as follows: HindIII-digested pUC13 DNA was labelled with [α-^{32}P]dATP by end-filling using Klenow fragment of DNA polymerase I and the labelled DNA precipitated by adding 0.5 volumes of 40% (W/V) PEG 8000 (Sigma) or 2 volumes of 95% (v/v) ethanol and keeping for 10 min at the desired temperature. The precipitated DNA was then collected by centrifugation at the desired temperature for 30 min and the radioactivity in the pellet estimated by measuring Cerenkov radiation.

In our study, the conditions used in the literature for precipitation of DNA by PEG [0.5–1 M NaCl, 5–10% (w/v) PEG, 4°C] did not give good recoveries of DNA when present at low (less than 1 μg/ml) concentrations. Maximum recoveries of DNA were observed when precipitation of DNA was carried out at a PEG concentration of 13% in the presence of 10 mM $MgCl_2$ at room temperature (20–22°C). Under these conditions, the recoveries obtained with PEG were comparable to those obtained with ethanol (Fig. 1). We have found that precipitation with ethanol is best done at room temperature if recovery alone is the criterion. Precipitation of DNA at room temperature has the added advantage of reducing salt contamination; in fact, precipitation with PEG or ethanol at room temperature enabled us to recover DNA from CsCl gradient without any dialysis.

Precipitation of ^{32}P-labelled HpaII digest of pBR322 DNA (5 μg/ml) with PEG or ethanol (in the presence of 10 mM Mg^{2+}, at room temperature) followed by electrophoresis and autoradiography (Fig. 2) showed that only fragments above 150 bp were efficiently precipitated by PEG while ethanol precipitated even 26 and 34 bp fragments efficiently. Thus, while PEG cannot be used for precipitation of DNA fragments less than 150 bp in size, the reagent is ideally suited for removal of low-molecular-weight DNA fragments such as those obtained on double digestion with restriction enzymes.

Washing twice with 70% ethanol by centrifugation for 5 min at room temperature was found to effectively remove the left-over PEG. DNA thus obtained could be digested well with restriction enzymes and gave good transformation efficiency.

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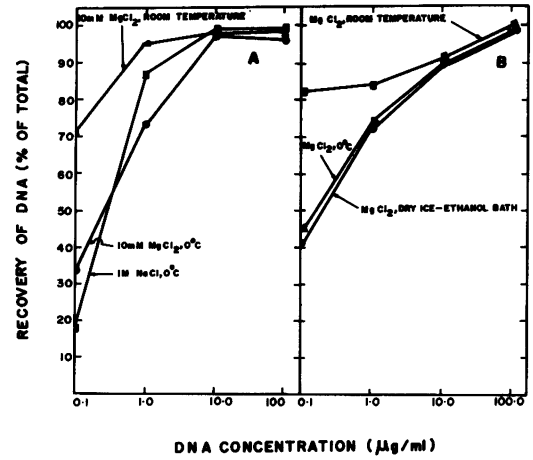

Figure 1. Precipitation of DNA with PEG (**A**) or ethanol (**B**). The precipitation temperatures were as indicated in the figure. The samples precipitated at room temperature were spun at the same temperature. Samples precipitated at 4°C or at the temperature of dry ice–ethanol bath were spun at 4°C.

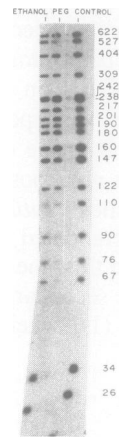

Figure 2. Precipitation of DNA fragments of low molecular weight by PEG or ethanol. HpaII digest of pBR322 was labelled with [α-^{32}P]dATP by end-filling and precipitated with PEG or ethanol in the presence of 10 mM Mg^{2+} at room temperature. The precipitates and the unprecipitated HpaII digest (control) were analysed on 8% polyacrylamide gel containing 7 M urea and the gel subjected to autoradiography. The numbers on the right side indicate the sizes of fragments in base pairs.