focus

Published as a service to the molecular biologist by Bethesda Research Laboratories

Vol. 7 No. 4 Fall 1985

Ethanol Precipitation of DNA

Jil A. Zeugin
James L. Hartley
Bethesda Research Laboratories

Ethanol precipitation of DNA is one of the most frequently used procedures in molecular biology. The most commonly used protocols require the addition of sodium ions and ethanol to the DNA solution, incubation in a dry ice bath (−70°C) or at −20°C for times ranging from a few minutes to overnight, then centrifugation to sediment the insoluble DNA.

We have investigated the effect of the amount of DNA, incubation temperatures and times, and centrifugation times on the recovery of DNA from ethanol precipitations. We find that precipitation of DNA is not significantly enhanced by long or low temperature incubation. However, longer centrifugations can give good recoveries of nanogram amounts of DNA.

Methods

Our approach was to mix various amounts of carrier DNA with radioactive DNA of known specific activity in a solution approximating a restriction enzyme buffer. Sodium acetate and ethanol were then added, and the mixtures were incubated and centrifuged under various conditions. The supernates were transferred to new tubes, and the radioactivity in the supernates and precipitates was determined by counting Cerenkov radiation. The percentage of recovered DNA was calculated from the counts in the precipitate and the total counts recovered.

Components of the standard reaction were:

1) ^{32}P-labeled DNA, 0.6 ng (approximately 60,000 cpm Cerenkov). This DNA was a 3.2 kb double-stranded, linear DNA labeled by the T4 DNA polymerase replacement method (1,2). It was purified from unincorporated nucleotides by gel filtration chromatography.
2) Nonradioactive carrier DNA, 0–10 μg. Herring sperm DNA was sonicated to an average length of approximately 300 bp.
3) DNA solutions. ^{32}P-labeled DNA was added to BRL Core Buffer™ [50 mM Tris-HCl (pH 7.5), 50 mM NaCl, 10 mM $MgCl_2$] containing 0.3 M sodium acetate (without pH adjustment) to give 0.6 ng DNA (approximately 60,000 cpm Cerenkov) per 250 μl of solution. Ten μl volumes of TC [10 mM Tris-HCl (pH 7.5), 1 mM cyclohexane-diaminetetraacetic acid (an EDTA analog)] containing 0, 10 ng, 100 ng, 1 μg or 10 μg of carrier DNA were added to 250 μl aliquots of this solution. Thus, the DNA concentrations in these solutions were 2.4 ng/ml, 40 ng/ml, 400 ng/ml, 4 μg/ml and 40 μg/ml.
4) Ethanol. All ethanol (absolute, denatured) was at room temperature when added to the DNA solutions.

For each experiment, 750 μl of ethanol were added to 260 μl of a DNA solution also at room temperature. Tubes (1.5 ml polypropylene) were inverted 10 times to mix, incubated at various times and temperatures, and centrifuged at 12,000xg at 6°C. Supernates were transferred to another 1.5 ml tube, and both the precipitated DNA and the supernates were counted in a liquid scintillation counter. The percentage of counts precipitated was calculated for each pair of tubes. All experiments were done in duplicate.

Results

Effect of Incubation Temperature. After addition of ethanol to the DNA solutions and mixing, tubes were incubated under the following conditions: 1) immersed in a bath of dry ice and ethanol (−70°C); 2) placed in a rack in a −20°C freezer; and 3) embedded in crushed ice in an ice bucket (0°C). After 10 minutes all tubes were centrifuged at 12,000xg at 6°C for 10 minutes.

Temperature of incubation had a small but consistent effect on recovery of the DNA (Figure 1). Recovery was better at 0°C, the warmest incubation temperature, than at −70°C. This effect has been seen in three other sets of experiments (data not shown). These data may be rationalized by observing that at −70°C the 75% ethanol mixtures are quite viscous, retarding migration of DNA complexes to the wall of the centrifuge tube. As expected, recoveries were better at higher DNA concentrations.

Effect of Incubation Times. Because the temperature of incubation was seen to have only a small effect on the percentage of labeled DNA precipitated at a given DNA concentration, the effect of incubation times at 0°C was examined. Ethanol (750 μl at room temperature) was added to DNA (260 μl at room temperature) and the tubes

continued on next page

Bethesda Research Laboratories / Life Technologies, Inc.
P.O. Box 6009 • Gaithersburg, Maryland 20877 U.S.A.
Telex: 64210 BRL GARG UW • To order: (800) 638-8992
Tech-Line™: (800) 638-4045 • In Maryland and outside the U.S.: (301) 840-8000

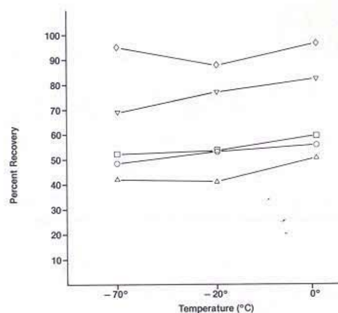

Figure 1. Effect of incubation temperature upon ethanol precipitation of DNA. All points are averages of two determinations. (○ = 0.6 ng, △ = 10 ng, □ = 100 ng, ▽ = 1 μg, and ◇ = 10 μg.)

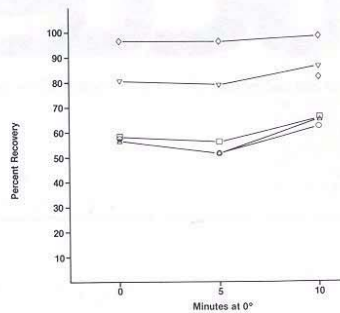

Figure 2. Effect of incubation time upon ethanol precipitation of DNA. Note that two values are plotted for 10 μg, 10 minutes incubation. The lower value is believed to be due to experimental error. All other points are averages of two determinations. (○ = 0.6 ng, △ = 10 ng, □ = 100 ng, ▽ = 1 μg, and ◇ = 10 μg.)

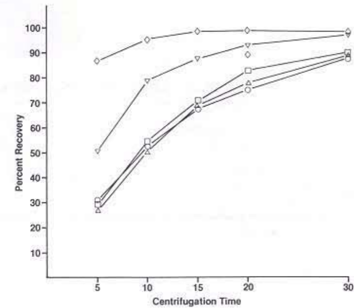

Figure 3. Effect of centrifugation time upon ethanol precipitation of DNA. Note that two values are plotted for 10 μg, 20 minutes centrifugation. The lower value is believed to be due to experimental error. All other points are averages of two determinations. (○ = 0.6 ng, △ = 10 ng, □ = 100 ng, ▽ = 1 μg, and ◇ = 10 μg.)

were left at room temperature (zero time) or placed in crushed ice for 5 or 10 minutes. All samples were centrifuged for 10 minutes.

Figure 2 shows that the time of incubation on ice had little effect on DNA recovery, although a small but consistent improvement was seen with 10 minute incubations on ice.

Effect of Centrifugation Times. The dependence of DNA precipitation upon extended centrifugation times was the third factor examined. Because of the minimal effects of incubation times and temperatures upon recovery, ethanol was added to the DNA solutions, and the tubes were mixed and immediately put into the centrifuge. Samples were centrifuged for 5, 10, 15, 20 or 30 minutes.

Dramatic improvements in the recovery of DNA were seen at longer centrifugation times. As expected, this effect was most pronounced at lower concentrations of DNA. Figure 3 shows that greater than 80% of 0.6 ng of DNA can be recovered from the standard precipitation reaction by simply centrifuging for 30 minutes. Almost 90% of 10 μg of DNA could be recovered with only five minutes of centrifugation.

Summary

The surprising conclusion from the data presented above is that incubation of an ethanol precipitation in a dry ice/ethanol bath has no beneficial effect on DNA precipitation, and, in fact, is somewhat counterproductive for small amounts of DNA. This effect has been seen in three other separate experiments, in addition to the experiment described here. Since the purpose of the centrifugation is to drive the DNA aggregate through the ethanol solution to the wall of the tube, it is perhaps not surprising that the increased viscosity of the ethanol at −70°C would retard the movement of the DNA aggregate, especially if the aggregate is small. In contrast, extended centrifugation times can result in good recovery of subnanogram amounts of DNA. Thus, on the basis of the data presented here, the majority of the time available for an ethanol precipitation procedure should be devoted to longer centrifugation times.

An observation that may also be of interest is that there was a marked improvement in recovery as the amount of DNA in our assay rose above a certain minimum amount. Recoveries of 0.6, 10 and 100 ng of DNA were quite similar under all conditions tested, while at 1 μg the recovery improved considerably, and at 10 μg recoveries were even better (Figures 1, 2 and 3). There may be an increase in DNA aggregate size between 100 ng and 1 μg of DNA per assay, resulting in improved migration of the aggregates through the ethanol.

References:

1. O'Farrell, P. (1981) *FOCUS 3:3*, 1.
2. Deen, K., Landers, T., and Berninger, M. (1983) *Anal. Biochem. 135*, 456.