DNA isolation from small tissue samples using salt and spermine

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Common DNA isolation methods rely upon protein denaturation by organic solvents such as phenol and chloroform. These solvents pose some risk to the user and require special disposal procedures. We previously reported a method for isolating DNA from peripheral blood lymphocytes by precipitation with spermine (1). This procedure proved less effective with other tissues, especially lung, and the spermine precipitates often took several days to dissolve in buffer. We have modified our procedure to include a pre-extraction of proteins with concentrated NaCl, as described by Miller *et al.* (2). This current, improved procedure provides good recovery of purified DNA even with small samples (<200 mg) and a variety of tissue and cell types.

Weigh, mince, and homogenize tissues in 3.0 ml of 10 mM Tris-HCl, 400 mM NaCl, 2 mM EDTA, pH 8.2, using the highest speed setting on a Tissumizer homogenizer (Tekmar, Cincinnati, OH). For tissue samples larger than approximately 300 mg, increase the volume of buffer by 1 ml per 100 mg tissue weight. Add 0.2 ml of 10% SDS and 0.5 ml of proteinase K solution (2 mg/ml proteinase K in 1% SDS and 2 mM Na_2EDTA) to the tissue homogenates, and digest the samples for 4 hours at 37°C in a shaking water bath. After digestion, add 1.5 ml of saturated NaCl solution to each sample, vortex for 20 seconds, and centrifuge the samples at 17,000 *g* for 15 min at 4°C. Decant the DNA-containing supernatant into a clean tube, discarding the precipitated protein pellet. Add two volumes of room temperature absolute ethanol and mix by inverting several times. Collect the buoyant DNA precipitate with a pipette tip, transfer to a microfuge tube containing 0.5 ml of TE Buffer (10 mM Tris-HCl, 0.2 mM EDTA, pH 7.5), redissolve the DNA at 37°C. To each 0.5 ml of DNA solution, add 36.25 µl of an RNAse mixture (10 µl 10 mg/ml RNAse A, 1.25 µl 5000 U/ml RNAse T_1, in 25 µl 1M Tris-HCl, pH 7.4), and incubate at 37°C for 30 min. Add 11 µl of proteinase K (50 mg/ml) and incubate again at 37°C for 30 min.

To precipitate the DNA, add 50 µl of 100 mM spermine tetrahydrochloride in TE buffer and cool on ice. Add another 20 µl of the spermine solution after 5 minutes to ensure complete precipitation of the DNA. Keep the samples on ice another 5 minutes, then centrifuge at 17,000 *g* for 15 minutes at 4°C. Discard the supernatant and extract the spermine from the DNA pellets by suspending the remaining pellet in 1 ml of 0.3 M LiCl and 15 mM sodium citrate in 75% ethanol. Chill the sample at −20°C for 2 hours, then centrifuge for 15 minutes at 4°C at 17,000 *g*. This treatment, which displaces the spermine from the DNA, is repeated twice, with 30 minutes at −20°C. After washing the remaining DNA pellet with 90% ethanol, dissolve in 200 µl TE buffer, and add 0.1 ml of 5 M ammonium acetate and 0.4 ml of cold absolute ethanol. The DNA is allowed to precipitate either at −20°C overnight or at −80°C for 30 min. Centrifuge the samples for 15 minutes at 4°C at 17,000 *g* and wash the pellet twice in 70% ethanol by suspension and recentrifugation. The DNA pellet is dissolved in 1 ml of TE buffer.

This technique has been applied to several tissue types, including several from which it is difficult to isolate DNA using phenol-chloroform extraction techniques, and has resulted in good yields of DNA, even when starting with small amounts of tissues. For example, the average DNA yield from newborn strain A mouse lungs (lung weight between 120 and 197 mg) was 2.8 µg DNA/mg tissue. In comparison, phenol/chloroform extraction protocols yielded only 0.2 µg DNA/mg for the same tissue. C57 mouse liver and lung yielded 2.4 and 3.3 µg DNA/mg tissue, respectively, using this technique. Rat peripheral blood lymphocytes yielded 4.0 µg DNA/10^6 cells using this technique, and rat lung yielded 3.2 µg DNA/mg tissue, while phenol/chloroform extraction yielded only 0.7 µg DNA/mg lung tissue.

The purity of the DNA isolated by this method was assessed by an analytical anion exchange HPLC method (3) that measures both DNA and RNA. In samples prepared by this salt and spermine protocol, we have not observed any material eluting in the region where RNA standards appear. In contrast, DNA isolated by phenol-chloroform protocols often have detectable peaks corresponding to the RNA region, accounting for as much as 15% of the total UV absorbing material eluted (data not shown). In our experience, the ratio of A_{260}/A_{280} is not an adequate index for DNA purity, since samples with significant RNA contamination, determined by HPLC, may have A_{260}/A_{280} ratios between 1.8 and 2.0.

This manuscript has been subjected to Agency review, but does not necessarily reflect the views of the Agency, and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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