Purification and Concentration of DNA from Aqueous Solutions

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ABSTRACT

This unit presents basic procedures for manipulating solutions of single- or double-stranded DNA through purification and concentration steps. These techniques are useful when proteins or solute molecules need to be removed from aqueous solutions, or when DNA solutions need to be concentrated. The Basic Protocol, using phenol extraction and ethanol (or isopropanol) precipitation, is appropriate for purification of DNA from small volumes (<0.4 ml) at concentrations lower than 1 mg/ml. Three support protocols outline methods to buffer the phenol used in extractions, concentrate DNA using butanol, and extract residual organic solvents with ether, respectively. An alternative to these methods is nucleic acid purification using glass beads, and this technique is also presented. These protocols may also be used for purifying RNA. The final two alternate protocols are used for concentrating RNA and extracting and precipitating DNA from larger volumes and from dilute solutions, and for removing low-molecular-weight oligonucleotides and triphosphates. *Curr. Protoc. Pharmacol.* 38:A.3C.1-A.3C.10. © 2007 by John Wiley & Sons, Inc.

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INTRODUCTION

This appendix presents basic procedures for manipulating solutions of single- or double-stranded DNA through purification and concentration steps. These techniques are useful when proteins or solute molecules need to be removed from aqueous solutions, or when DNA solutions need to be concentrated for reasons of convenience. The Basic Protocol, using phenol extraction and ethanol precipitation, is appropriate for the purification of DNA from small volumes (≤0.4 ml) at concentrations ≤1 mg/ml. Isopropanol may also be used to precipitate DNA, as described in Alternate Protocol 1. Three support protocols outline methods to buffer the phenol used in extractions (see Support Protocol 1), concentrate DNA using butanol (see Support Protocol 2), and extract residual organic solvents with ether (see Support Protocol 3). An alternative to these methods is nucleic acid purification using commercially available silica membrane spin columns, presented in Alternate Protocol 2. These protocols may also be used for purifying RNA.

The final two alternate protocols provide modifications to the basic protocol that are used for concentrating RNA and extracting and precipitating DNA from larger volumes and from dilute solutions (see Alternate Protocol 3), and for removing low-molecular-weight oligonucleotides and triphosphates (see Alternate Protocol 4).

PHENOL EXTRACTION AND ETHANOL PRECIPITATION OF DNA

BASIC PROTOCOL

This protocol describes the most commonly used method of purifying and concentrating DNA preparations. The DNA solution is first extracted with a phenol/chloroform/isoamyl alcohol mixture to remove protein contaminants, and then precipitated with 100% ethanol. The DNA is pelleted after the precipitation step, washed with 70% ethanol to remove

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salts and small organic molecules, and resuspended in buffer at a concentration suitable for further experimentation.

Materials

≤1 mg/ml DNA to be purified
25:24:1 (v/v/v) phenol/chloroform/isoamyl alcohol (made with buffered phenol; see Support Protocol 1)
3 M sodium acetate, pH 5.2
100% ethanol, ice cold
70% ethanol, room temperature
TE buffer, pH 8.0 (*APPENDIX 2A*)
Speedvac evaporator (Savant)

1. Add an equal volume of phenol/chloroform/isoamyl alcohol to the DNA solution to be purified in a 1.5-ml microcentrifuge tube.

   DNA solutions containing ≤0.5 M monovalent cations can be used. Extracting volumes ≤100 μl is difficult; small volumes should be diluted to obtain a volume that is easy to work with.

   High salt concentrations can cause the inversion of the aqueous and organic phases. If this happens, the organic phase can be identified by its yellow color.

2. Vortex vigorously for 10 sec and microcentrifuge 15 sec at maximum speed, room temperature.

   Phases should be well separated. If DNA solution is viscous or contains a large amount of protein, it should be microcentrifuged longer (1 to 2 min).

3. Carefully remove the top (aqueous) phase containing the DNA using a 200-μl pipettor and transfer to a new tube. If a white precipitate is present at the aqueous/organic interface, repeat steps 1 to 3.

   If starting with a small amount of DNA (<1 μg), recovery can be improved by reextracting the organic phase with 100 μl TE buffer, pH 8.0. This aqueous phase can be pooled with that from the first extraction.

4. Add 1/10 vol of 3 M sodium acetate, pH 5.2, to the solution of DNA. Mix by vortexing briefly or by flicking the tube several times with a finger.

   If the solution contained a high concentration of NaCl or sodium acetate (0.3 to 0.5 M) prior to the phenol extraction step, then no additional salt should be added. It is advisable to make appropriate dilutions to keep NaCl and sodium acetate concentrations below 0.5 M.

   For high concentrations of DNA (>50 to 100 μg/ml), precipitation is essentially instantaneous at room temperature. If ethanol precipitation is not desirable, residual organic solvents can be removed by ether extraction (see Support Protocol 3). In this case, no salt should be added.

   To prevent carryover of residual phenol, the aqueous phase can be reextracted with 24:1 (v/v) chloroform/isoamyl alcohol. However, this should not be necessary if the final pellet is washed well with 70% ethanol, or if an additional ethanol precipitation step is included.

5. Add 2 to 2.5 vol (calculated *after* salt addition) of ice-cold 100% ethanol. Mix by vortexing and place in crushed dry ice for 5 min or longer.

   This precipitation step can also be done in a −70°C freezer for 15 min or longer, or in a −20°C freezer for at least 30 min. A slurry of dry ice and ethanol may also be used, but tube labels are less often lost when crushed dry ice is used.

6. Microcentrifuge 5 min at maximum speed and remove the supernatant.

   For large pellets the supernatant can simply be poured off. For small pellets (<1 μg), aspirate off the ethanol supernatant with a pipetting device such as a Pasteur pipet or mechanical pipettor. This is best accomplished by drawing off liquid from the side of the tube opposite that against which the DNA precipitate was pelleted. Start at the top and move downward as the liquid level drops.

7. Add 1 ml room-temperature 70% ethanol. Invert the tube several times and microcentrifuge as in step 6.

   If the DNA molecules being precipitated are very small (< 200 bases), use 95% ethanol at this step.

8. Remove the supernatant as in step 6. Dry the pellet in a desiccator under vacuum or in a Speedvac evaporator.

   The DNA pellet will not stick well to the walls of the tube after the 70% ethanol wash and care must be taken to avoid aspirating the pellet out of the tube.

9. Dissolve the dry pellet in an appropriate volume of water if it is going to be used for further enzymatic manipulations requiring specific buffers. Dissolve in TE buffer, pH 8.0, if it is going to be stored indefinitely.

   DNA pellets will not dissolve well in high-salt buffers. To facilitate resuspension, the DNA concentration of the final solution should be kept at <1 mg/ml.

   If DNA is resuspended in a volume of TE buffer or water to yield a DNA concentration of <1 mg/ml, small quantities (<25 μg) of precipitated plasmids or restriction fragments should dissolve quickly upon gentle vortexing or flicking of the tube. However, larger quantities of DNA may require vortexing and brief heating (5 min at 65°C) to resuspend. High-molecular-weight genomic DNA may require one to several days to dissolve and should be shaken gently (not vortexed) to avoid shearing, particularly if it is to be used for cosmid cloning or other applications requiring high-molecular-weight DNA. Gentle shaking on a rotating platform or a rocking apparatus is recommended.

PRECIPITATION OF DNA USING ISOPROPANOL

ALTERNATE PROTOCOL 1

Equal volumes of isopropanol and DNA solution are used in precipitation. Note that the isopropanol volume is half that of the given volume of ethanol in precipitations. This allows precipitation from a large starting volume (e.g., 0.7 ml) in a single microcentrifuge tube. Isopropanol is less volatile than ethanol and takes longer to remove by evaporation. Some salts are less soluble in isopropanol (compared to ethanol) and will be precipitated along with nucleic acids. Extra washings may be necessary to eliminate these contaminating salts.

PREPARATION OF BUFFERED PHENOL AND PHENOL/CHLOROFORM/ISOAMYL ALCOHOL

SUPPORT PROTOCOL 1

For some purposes, fresh liquefied phenol (88% phenol) can be used without further purification. However, for purification of DNA prior to cloning and other sensitive applications, phenol must be redistilled before use, because oxidation products of phenol can damage and introduce breaks into nucleic acid chains. Redistilled phenol for use in nucleic acid purification is commercially available, but must be buffered before use. Appropriately buffered phenol is also commercially available, but is somewhat more expensive and should not be stored for long periods of time (e.g., >6 months).

CAUTION: Phenol can cause severe burns to skin and damage clothing. Gloves, safety glasses, and a lab coat should be worn whenever working with phenol, and all manipulations should be carried out in a fume hood. A glass receptacle should be available exclusively for disposing of used phenol and chloroform.

Materials

8-hydroxyquinoline
Liquefied phenol
50 mM Tris base (unadjusted pH ~10.5)
50 mM Tris·Cl, pH 8.0 (*APPENDIX 2A*)
Chloroform
Isoamyl alcohol

1. Add 0.5 g of 8-hydroxyquinoline to a 2-liter glass beaker containing a stir bar.
2. Gently pour in 500 ml liquefied phenol or melted crystals of redistilled phenol (melted in a water bath at 65°C).

   The phenol will turn yellow due to the 8-hydroxyquinoline, which is added as an antioxidant.

3. Add 500 ml of 50 mM Tris base.
4. Cover the beaker with aluminum foil. Stir 10 min at low speed with magnetic stirrer at room temperature.
5. Let phases separate at room temperature. Gently decant the top (aqueous) phase into a suitable waste receptacle. Remove what cannot be decanted with a 25-ml glass pipet and a suction bulb.
6. Add 500 ml of 50 mM Tris·Cl, pH 8.0. Repeat steps 4 to 6 so that two successive equilibrations with Tris·Cl are performed, ending with removal of the second Tris·Cl phase.

   The pH of the phenol phase can be checked with indicator paper and should be 8.0. If it is not, the Tris·Cl equilibration should be repeated until this pH is obtained.

7. Add 250 ml of 50 mM Tris·Cl, pH 8.0, or TE buffer, pH 8.0, and store at 4°C in brown glass bottles or clear glass bottles wrapped in aluminum foil.

   Phenol prepared with 8-hydroxyquinoline as an antioxidant can be stored ≤ 2 months at 4°C.

8. For use in DNA purification procedure (see Basic Protocol), mix 25 vol phenol (bottom yellow phase of stored solution) with 24 vol chloroform and 1 vol isoamyl alcohol. Store up to 2 months at 4°C wrapped in foil or in a dark glass bottle.

SUPPORT PROTOCOL 2

CONCENTRATION OF DNA USING BUTANOL

It is generally inconvenient to handle large volumes or dilute solutions of DNA. Water molecules (but not DNA or solute molecules) can be removed from aqueous solutions by extraction with *sec*-butanol (2-butanol). This procedure is useful for reducing volumes or concentrating dilute solutions before proceeding with the Basic Protocol.

Additional Materials *(also see Basic Protocol)*

sec-Butanol
25:24:1 (v/v/v) phenol/chloroform/isoamyl alcohol (made with buffered phenol; see Support Protocol 1)
Polypropylene tube

1. Add an equal volume of *sec*-butanol to the sample and mix well by vortexing or by gentle inversion (if the DNA is of high molecular weight). Perform extraction in a polypropylene tube, as butanol will damage polystyrene.

2. Centrifuge 5 min at 1200 × *g* (2500 rpm), room temperature, or in a microcentrifuge for 10 sec at maximum speed.

3. Remove and discard the upper (*sec*-butanol) phase.

4. Repeat steps 1 to 3 until the desired volume of aqueous solution is obtained.

5. Extract the lower, aqueous phase with 25:24:1 phenol/chloroform/isoamyl alcohol and ethanol precipitate (see Basic Protocol, steps 1 to 9) or remove *sec*-butanol by two ether extractions (see Support Protocol 3).

   Addition of too much sec-butanol can result in complete loss of the water phase into the sec-butanol layer. If this happens, add 1/2 vol water back to the sec-butanol, mix well, and spin. The DNA can be recovered in this new aqueous phase and can be further concentrated with smaller amounts of sec-butanol.

   The salt concentration will increase in direct proportion to the volume decrease. The DNA can be precipitated with ethanol to readjust the buffer conditions.

REMOVAL OF RESIDUAL PHENOL, CHLOROFORM, OR BUTANOL BY ETHER EXTRACTION

SUPPORT PROTOCOL 3

DNA solutions that have been purified by extraction with phenol and chloroform (see Basic Protocol) or concentrated with *sec*-butanol (see Support Protocol 2) can often be used without ethanol precipitation for enzymatic manipulations or in gel electrophoresis experiments if the organic solvents are removed by extraction with ether. Traces of ether are subsequently removed by evaporation. This procedure is useful only if the solute concentrations in the starting solution are compatible with what is needed in later steps. It is quite useful in purifying high-molecular-weight DNA, as mechanical shearing of large nucleic acid molecules can occur during precipitation with ethanol.

CAUTION: Ether is highly flammable and its vapors can cause drowsiness. All manipulations with ether should be carried out in a well-ventilated fume hood.

Materials

Diethyl ether
TE buffer, pH 8.0 (*APPENDIX 2A*)
Polypropylene tube

1. Mix diethyl ether with an equal volume of water or TE buffer, pH 8.0, in a polypropylene tube. Vortex vigorously for 10 sec and let the phases separate.

   Ether is the top phase.

2. Add an equal volume of hydrated ether to the DNA sample. Mix well by vortexing or by gentle inversion (if the DNA is of high molecular weight).

3. Microcentrifuge 5 sec at maximum speed or let the phases separate by setting the tube upright in a test tube rack.

4. Remove and discard the top (ether) layer. Repeat steps 2 and 3.

5. Remove ether by leaving the sample open under a fume hood for 15 min (small volumes, < 100 µl), or under vacuum for 15 min (larger volumes).

   The DNA solution will be free of organic solvents and will have salt concentrations that are roughly three-fourths of those that were in the aqueous solution before phenol extraction (solute concentrations are lowered in the two phenol/chloroform/isoamyl alcohol extractions steps).

ALTERNATE PROTOCOL 2

DNA PURIFICATION USING SILICA MEMBRANE SPIN COLUMNS

The use of glass beads or silica gel particles has become a popular method for isolating DNA. The evolution of this principle has resulted in the introduction of silica membrane spin columns. The basic principle of silica gel solid support spin columns is fairly simple. DNA is bound to the silica membrane spin column in the presence of a high concentration of chaotropic salt, contaminants are washed away, and the DNA is then eluted from the silica membrane in water or a low-salt buffer. The procedure below includes steps for when a bacterial lysate is used as the starting material.

The major advantage of silica membrane spin columns is the fact that the silica is bound to a solid support, which eliminates the problem of glass-bead contamination of the DNA sample. This method of DNA purification is quick and convenient, and can produce a high yield of pure DNA. Silica membrane spin columns are available from many companies, including Qiagen, Promega, Invitrogen, and Novagen, as kits including the columns and all appropriate buffers necessary for DNA purification.

Materials

1×10^9 cell/ml bacterial culture containing DNA of interest *or* 0.1 to 1 mg/ml DNA to be purified (5 to 10 μg DNA total)
6 M sodium iodide (NaI) solution (filter through filter paper, store up to 3 months in the dark at 4°C)
Resuspension buffer (see recipe)
Lysis solution: 0.2 M NaOH/1.0% (w/v) SDS (store indefinitely at room temperature)
Neutralization/binding solution (see recipe)
Wash buffer (see recipe)
TE buffer, pH 8.5 (*APPENDIX 2A*) *or* nuclease-free H_2O

Silica membrane spin columns (e.g., Qiagen, Promega, Invitrogen, Novagen)
1.5-ml microcentrifuge tubes

Prepare DNA solution

1. Harvest 1 to 5 ml of bacterial culture by centrifuging 1 min at 10,000 × *g*, room temperature, then discard the supernatant.

   To clean a DNA solution not generated from bacterial lysates, add 3 vol of 6 M NaI solution to DNA in a 1.5-ml microcentrifuge tube, mix, and proceed to step 6.

2. Add 250 μl cell resuspension buffer, resuspend the cell pellet by vortexing or pipetting, and transfer to 1.5-ml microcentrifuge tube.

3. Add 250 μl lysis solution and mix by inversion (do not vortex). Allow lysis to proceed 3 to 5 min; do not allow lysis reaction to proceed for more than 5 min.

4. Add 350 μl neutralization/binding solution and mix by inversion.

5. Microcentrifuge 10 min at maximum speed (a white pellet will form in the bottom of the tube).

Purify DNA on silica membrane spin column

6. Apply the supernatant to the silica membrane spin column.

7. Microcentrifuge spin column inside its collection tube for 1 min at maximum speed. Remove the spin column from the collection tube and discard the flowthrough. Reinsert the spin column into the collection tube.

8. Wash the spin column by adding 750 µl of wash buffer and microcentrifuging 1 min at maximum speed. Remove the spin column from the collection tube and discard the flowthrough.
9. Reinsert the spin column in the collection tube and microcentrifuge for an additional 1 min to remove any residual wash buffer (ethanol) from the column membrane.
10. Transfer the spin column to a 1.5-ml microcentrifuge tube and add 75 to 100 µl nuclease-free water or TE buffer, pH 8.5, to the center of the membrane. Let stand for 2 to 10 min, then microcentrifuge 1 min at maximum speed.
11. Collect DNA and store at 4°C until use.

PURIFICATION AND CONCENTRATION OF RNA AND DILUTE SOLUTIONS OF DNA

ALTERNATE PROTOCOL 3

The following adaptations to the purification procedure (see Basic Protocol) are used if RNA or dilute solutions of DNA are to be purified.

Purification and Concentration of RNA

The procedure outlined in the Basic Protocol is identical for purification of RNA, except that 2.5 vol ethanol should be used routinely for the precipitation (step 5). It is essential that all water used directly or in buffers be treated with diethylpyrocarbonate (DEPC) to inactivate RNase (see *UNIT 2.5*, Reagents and Solutions, for instructions).

Dilute Solutions of DNA

When DNA solutions are dilute (<10 µg/ml) or when <1 µg of DNA is present, the ratio of ethanol to aqueous volume should be increased to 3:1 and the time on dry ice (step 5) extended to 30 min. Microcentrifugation should be carried out for 15 min in a cold room to ensure the recovery of DNA from these solutions.

Nanogram quantities of labeled or unlabeled DNA can be efficiently precipitated by the use of carrier nucleic acid. A convenient method is to add 10 µg of commercially available tRNA from *E. coli*, yeast, or bovine liver to the desired DNA sample. The DNA will be co-precipitated with the tRNA. The carrier tRNA will not interfere with most enzymatic reactions, but will be phosphorylated efficiently by polynucleotide kinase and should not be added if this enzyme will be used in subsequent radiolabeling reactions.

Recovery of small quantities of short DNA fragments and oligonucleotides can be enhanced by adding magnesium chloride to a concentration of <10 mM before adding ethanol (step 4). However, DNA precipitated from solutions containing >10 mM magnesium or phosphate ions is often difficult to redissolve and such solutions should be diluted prior to ethanol precipitation.

DNA in Large Aqueous Volumes (>0.4 to 10 ml)

Larger volumes can be accommodated by simply scaling up the amounts used in the Basic Protocol or by using butanol concentration (see Support Protocol 2). For the phenol extraction (see Basic Protocol, steps 1 through 3), tightly capped 15- or 50-ml polypropylene tubes should be used as polystyrene tubes cannot withstand the phenol/chloroform mixture. Centrifugation steps should be performed for 5 min at speeds not exceeding 1200 × *g* (2500 rpm), room temperature. The ethanol precipitate (step 6) should be centrifuged in thick-walled Corning glass test tubes (15- or 30-ml capacity) for 15 min in fixed-angle rotors at 8000 × *g* (10,000 rpm), 4°C. Glass tubes should be silanized to facilitate recovery of small amounts of DNA (<10 µg).

ALTERNATE PROTOCOL 4

REMOVAL OF LOW-MOLECULAR-WEIGHT OLIGONUCLEOTIDES AND TRIPHOSPHATES BY ETHANOL PRECIPITATION

The use of ammonium acetate in place of sodium acetate allows the preferential precipitation of longer DNA molecules. Thus, small single- or double-stranded oligonucleotides (less than ~30 bp) and unincorporated nucleotides used in radiolabeling or other DNA modification reactions can be effectively removed from DNA solutions by two rounds of ethanol precipitation in the presence of ammonium acetate. This approach is not sufficient to completely remove large quantities of linkers as used in cloning procedures. If the nucleic acid is to be phosphorylated, this protocol should not be used because T4 polynucleotide kinase is inhibited by ammonium ions. Although the removal of unincorporated nucleoside triphosphates, reaction products, and small oligonucleotides is effective, it is not absolute and the procedure should not be used to purify DNA from these small molecules prior to detailed biochemical or analytical studies.

Additional Materials *(also see Basic Protocol)*

4 M ammonium acetate, pH 4.8

1. Add an equal volume of 4 M ammonium acetate, pH 4.8, to the DNA solution. Mix well.
2. Add 2 vol (calculated *after* salt addition) of ice-cold 100% ethanol. Vortex and set tube in crushed dry ice for 5 min.

   This precipitation step can also be done in a −70°C freezer for 15 min or longer, or in a −20°C freezer for at least 30 min. A slurry of dry ice and ethanol may also be used, but tube labels are less often lost when crushed dry ice is used.

3. Microcentrifuge 5 min at high speed, room temperature. Carefully remove supernatant and redissolve pellet in 100 µl TE buffer, pH 8.0.
4. Repeat steps 1 to 3.

   Reprecipitation is required, particularly if the DNA solution from step 1 contained Mg^{2+} or other divalent or polyvalent cations that will facilitate the precipitation of the oligonucleotides.

5. Proceed with steps 7 to 9 in Basic Protocol.

REAGENTS AND SOLUTIONS

Use deionized, distilled water in all recipes and protocol steps. For common stock solutions, see ***APPENDIX 2A****; for suppliers, see* ***SUPPLIERS APPENDIX****.*

Neutralization/binding solution

Add 477.65 g guanidine HCl and 49.09 g potassium acetate to 500 ml water and stir to dissolve. Adjust pH to ~4.2 with acetic acid, dilute solution to 1 liter with water, and filter sterilize. Store indefinitely at 4°C.

Resuspension buffer

50 mM Tris·Cl, pH 8.0 (*APPENDIX 2A*)
10 mM EDTA (*APPENDIX 2A*)
100 mg RNase A
Store indefinitely at 4°C.

Wash buffer

1 part 10 mM Tris·Cl, pH 7.5 (*APPENDIX 2A*)
1 part 100 mM NaCl (*APPENDIX 2A*)
4 parts 100% ethanol (final 80%)
Store indefinitely at room temperature

COMMENTARY

Background Information

It is often necessary to purify or concentrate a solution of DNA prior to further enzymatic manipulations or analytical studies. The most commonly used method for deproteinizing DNA is extraction with phenol, which efficiently denatures proteins and probably dissolves denatured protein (Kirby, 1957). Chloroform is also a useful protein denaturant with somewhat different properties—it stabilizes the rather unstable boundary between an aqueous phase and a pure phenol layer. The phenol/chloroform mixture reduces the amount of aqueous solution retained in the organic phase (compared to a pure phenol phase), maximizing the yield (Penman, 1966; Palmiter, 1974). Isoamyl alcohol prevents foaming of the mixture upon vortexing and aids in the separation of the organic and aqueous phases (Marmur, 1961). Denatured protein forms a layer at the interface between the aqueous and organic phases and is thus isolated from the bulk of the DNA in the aqueous layer. This procedure is rapid, inexpensive, and easy to perform.

Ethanol precipitation is useful for concentrating DNA solutions and for removing residual phenol and chloroform from the deproteinized aqueous solution. It is also useful for providing DNA that is relatively free of solute molecules when buffer conditions need to be changed. In the presence of relatively high (0.1 to 0.5 M) concentrations of monovalent cations, ethanol induces a structural transition in nucleic acid molecules which causes them to aggregate and precipitate from solution (Eickbush and Moudrianakis, 1978). However, because most salts and small organic molecules are soluble in 70% ethanol, ethanol precipitation and washing of the pellet will effectively desalt DNA. Although sodium chloride, sodium acetate, and ammonium acetate are each capable of inducing precipitation, it is more difficult to remove sodium chloride due to its lower solubility in 70% ethanol.

The silica membrane spin column protocol, modified from principles originally described in Vogelstein and Gillespie (1979), provides a simple, nontoxic method for removing DNA from contaminating impurities. In the presence of high chaotropic salt concentrations, DNA binds to a silica membrane inside a spin column. The resulting precipitate is washed to remove NaI (or other chaotropic salts like guanidine HCl or sodium perchlorate) along with impurities from the original sample, and subsequent suspension in water or TE buffer causes dissociation (elution) of the DNA from the silica membrane. Because fewer manipulations are required, this method is faster and easier to perform than organic-based extraction methods. However, the yields may somewhat lower, generally ranging from 50% to 75% of the starting material. The procedure seems to work best with DNA fragments larger than 500 bp, as some shorter fragments may bind tightly and irreversibly to the silica membrane. An advantage of silica membrane spin columns relative to glass beads (Vogelstein and Gillespie, 1979) is that they help to decrease shearing of DNA fragments that are larger than 3 to 10 kb.

One alternative for purifying DNA from residual protein is the use of StrataClean Resin (Stratagene), a nontoxic slurry of hydroxylated silica particles. Acidic hydroxy groups on the resin appear to bind proteins in a manner similar to phenolic hydroxyls, and at or near neutral pH display a high affinity for protein and low affinity for DNA. Protein bound to the resin is separated by centrifugation from nucleic acids remaining in solution; two or three extractions with the resin may be required to completely remove protein from a nucleic acid sample. Another product, Phase Lock Gel (an inert silica-based blend of intermediate density available from 5 Prime → 3 Prime; *SUPPLIERS APPENDIX*) improves recoveries in standard organic extractions by reducing loss of sample at the interface. During centrifugation, the normally fuzzy interface is compacted tightly below or within the gel. The gel/interface complex migrates discretely between the organic and aqueous phases, thus creating a tight partition which allows recovery of virtually all of the aqueous phase.

Critical Parameters

The oxidation products of phenol can damage nucleic acids and only redistilled phenol should be used. For complete deproteinization, extractions should be repeated until no protein precipitate remains at the aqueous/organic interface.

In general, alcohol precipitation of nucleic acids requires the presence of at least 0.1 M monovalent cation in the starting aqueous solution. Precipitation of nucleic acids at low concentrations requires cooling to low temperatures to give good recovery. Precipitation of nucleic acids at high concentrations (>0.25 mg/ml after addition of ethanol) is very rapid at

room temperature. Formation of a visible precipitate after adding alcohol and mixing well indicates complete precipitation, and no chilling or further incubation is needed.

In organic extraction, loss of nucleic acid at the interface and into the organic phase is minimized by back-extracting the organic phase. The most critical parameter in the purification of DNA using silica membrane spin columns is the pH of the chaotropic salt solution. For efficient binding to the silica membrane, the salt solution should be at pH 6.5. Many different companies sell silica membrane spin columns as kits supplied with all necessary reagents and buffers. One variable is the type of chaotropic salt solution supplied with the kit (NaI, guanidine HCl, or guanidine isothiocyanate). For the most part, these salt solutions are only a variation on the same theme and may be interchangeable between kits or columns from different vendors.

Anticipated Results

These procedures should result in virtually complete removal of proteins and quantitative recovery of nucleic acids. However, sequential extractions or precipitations require care and attention to detail to prevent accumulation of small losses at each step. It is particularly important to carefully recover the aqueous phase and reextract the organic phase to ensure full recovery of small amounts of DNA from phenol/chloroform extractions.

The yield of nucleic acids resulting from the silica membrane spin column procedure can be similarly improved (to $\leq$80% recovery) by subjecting supernatants to an additional binding step and increasing the amount of elution buffer to 100 μl or more.

Time Considerations

Approximately 90 min should be allowed for carrying out steps 1 through 9 of the Basic Protocol on twelve DNA samples in microcentrifuge tubes. Phenol buffering should be started $\geq$1 hr before equilibrated phenol is needed. Nucleic acids should not be left in the presence of phenol, but can be indefinitely precipitated in alcohol or dried after precipitation. The silica membrane spin column protocol can be performed on twelve samples in 15 to 20 min.

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