ISOLATION AND PURIFICATION OF DNA

SECTION I

This section begins with a protocol describing basic techniques for purifying and concentrating DNA samples (*UNIT 10.1*), followed by similar protocols for purifying DNA from mammalian tissue (*UNIT 10.2*) and isolating and purifying bacterial plasmids (*UNIT 10.3*).

IMPORTANT NOTE: The smallest amount of contamination of DNA preparations by recombinant phages or plasmids can be disastrous. Many person-years have been wasted reisolating previously cloned sequences that contaminated preparations of DNA used to create recombinant DNA libraries and many researchers have been embarrassed to find that the "extra" genes they found on their Southern blots were actually contaminating plasmid DNA. All materials used for preparation of plasmid or phage DNA should be kept separate from those used for preparation of genomic DNA, and disposable items should be used wherever possible. Particular care should be taken to avoid contamination of commonly used rotors.

Purification and Concentration of DNA from Aqueous Solutions

UNIT 10.1

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 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 the first alternate protocol. Three support protocols outline methods to buffer the phenol used in extractions, concentrate DNA using butanol, and extract residual organic solvents with ether. An alternative to these methods is nucleic acid purification using glass beads, presented in the second alternate protocol. These protocol 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, and for removing low-molecular-weight oligonucleotides and triphosphates.

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 contaminents, then precipitated with 100% ethanol. The DNA is pelleted after the precipitation step, washed with 70% ethanol to remove salts and small organic molecules, and resuspended in buffer at a concentration suitable for further experimentation.

Materials

DNA to be purified (≤1 mg/ml) in 0.1 to 0.4 ml volume
25:24:1 phenol/chloroform/isoamyl alcohol (made with *buffered* phenol; first support protocol)
3 M sodium acetate, pH 5.2 (*APPENDIX 2*)
100% ethanol, ice cold
70% ethanol, room temperature
TE buffer, pH 8.0 (*APPENDIX 2*)
Speedvac evaporator (Savant)

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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 monovalent cations ≤0.5 M 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 room temperature.

   Phases should be well separated. If DNA solution is viscous or contains a large amount of protein, spin 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 (second support protocol). In this case, do not add salt.

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. Spin 5 min in a fixed-angle microcentrifuge 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 pipettor. This is best accomplished by drawing off liquid from the side of the tube opposite that which the DNA precipitate was pelleted against. Start at the top and move downward as the liquid level drops.

7. Add 1 ml of 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 keep the DNA concentration of the final solution 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

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.

BUFFERING PHENOL

SUPPORT PROTOCOL

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. Regardless of the source, the phenol must be buffered before use.

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
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 of 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 (i.e., two successive equilibrations with 500 ml of 50 mM Tris·Cl, pH 8.0).

   The pH of the phenol phase can be checked with indicator paper and should be 8.0. If it is not, repeat steps 3 to 7 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.
8. For use in DNA purification procedure (basic protocol), mix 25 vol phenol (bottom yellow phase of stored solution) with 24 vol chloroform and 1 vol isoamyl alcohol.

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

SUPPORT PROTOCOL

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

sec-butanol
25:24:1 phenol/chloroform/isoamyl alcohol (made with *buffered* phenol; first protocol)
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.
3. Remove and discard the upper (*sec*-butanol) phase.
4. Repeat steps 1 to 3 above 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 as in steps 1 to 9 of the basic protocol, or remove *sec*-butanol by two ether extractions as described in the second support protocol.

   Addition of too much sec-butanol can result in complete loss of the water phase into the sec-butanol layer. If this happens, add ½ 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

DNA solutions that have been purified by extraction with phenol and chloroform (first basic protocol) or concentrated with *sec*-butanol (second support protocol) 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 2*)
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 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 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 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).

PURIFICATION OF DNA USING GLASS BEADS

ALTERNATE PROTOCOL

The use of a glass beads suspension allows the rapid and efficient purification of DNA from contaminating proteins, RNA, or organic solvents. DNA in solution is adsorbed onto glass beads in the presence of sodium iodide. The DNA–glass beads suspension is washed to remove solution contaminants, and DNA is subsequently eluted into water or a low-salt buffer. Although faster than traditional extraction protocols, this method may result in somewhat reduced yields.

Additional Materials

6 M sodium iodide (NaI) solution
DNA in a 50- to 200-µl volume
Wash solution
TE buffer, pH 8.0 (*APPENDIX 2*)
Glass beads suspension

NOTE: The above materials are also available as commercial kits (e.g., Glas-Pac, National Scientific Supply; GeneClean, Bio101; and Qiaex Gel Extraction Kit, Qiagen).

1. Add 3 vol NaI solution to DNA in a 1.5-ml microcentrifuge tube. Add glass beads suspension as follows: for amounts of DNA <5 µg, use 5 µl glass beads suspension; for amounts of DNA >5 µg, use 5 µl plus an additional 1 µl for each 0.5-µg increment above 5 µg. Incubate 5 min at room temperature.

   For example, if 4 µg of DNA were being purified, 5 µl of the glass beads suspension would be used; if 7 µg of DNA were being purified, 5 µl + 4 µl = 9 µl glass beads suspension would be required.

   Longer incubation times with occasional mixing will improve the binding efficiency, especially with larger volumes.

2. Microcentrifuge DNA/glass beads complex 5 sec. Remove and discard supernatant.

   To enhance yield, save the supernatant and reincubate with another sample of glass beads suspension as in step 1.

3. Wash the DNA/glass beads pellet three times with 500 µl wash solution. Lightly vortex the mixture to resuspend, then microcentrifuge briefly to pellet the beads.

4. Resuspend pellet in TE buffer, pH 8.0, at 0.5 µg/µl. Incubate 2 to 3 min at 45°C to elute DNA from the glass beads.

5. Microcentrifuge 1 min and transfer the DNA-containing supernatant to a fresh tube. Store at 4°C until use.

ALTERNATE PROTOCOLS

PURIFICATION AND CONCENTRATION OF RNA AND DILUTE SOLUTIONS OF DNA

The following adaptations to the 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 4.1*, 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, increase the ratio of ethanol to aqueous volume to 3:1 and extend the time on dry ice to 30 min (step 5). Carry out microcentrifugation in a cold room for 15 min 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 as described in the second support protocol. For the phenol extraction (steps 1 through 3), use tightly capped 15- or 50-ml polypropylene tubes, as polystyrene tubes cannot withstand the phenol/chloroform mixture. Perform centrifugation steps 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 (see *APPENDIX 3*) to facilitate recovery of small amounts of DNA (<10 µg).

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

ALTERNATE PROTOCOL

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.

Additional Materials

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 (67% final). Vortex and set tube in crushed dry ice for 5 min.
3. Microcentrifuge 5 min at high speed, room temperature. Carefully remove supernatant and redissolve pellet in 100 µl TE buffer.
4. Repeat steps 1 to 3, then proceed to step 5.

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

5. Add 1 ml of room-temperature 70% ethanol to the tube and invert several times. Microcentrifuge 5 min at high speed, room temperature.
6. Discard ethanol and dry pellet as in basic protocol (step 9).

   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.

REAGENTS AND SOLUTIONS

Glass beads suspension

Transfer a volume of ~200 to 300 µl of 200-µm glass beads (National Scientific Supply) into a 1.5-ml microcentrifuge tube; add an equal volume of water. Vortex briefly to suspend just before using.

If glass beads do not come acid-washed, prepare as follows: Wash by soaking 1 hr in concentrated nitric acid. Rinse thoroughly with water. Dry in a baking oven, cool to room temperature, and store at 4°C until needed.

6 M sodium iodide (NaI) solution

Dissolve 0.75 g Na_2SO_3 in 40 ml H_2O. Add 45 g NaI (Sigma #S8379) and stir until dissolved (~30 min). Filter through Whatman paper or nitrocellulose and store 3 to 4 months in the dark (in aluminum foil). Discard if precipitate is observed.

Wash solution

20 mM Tris·Cl, pH 7.4
1 mM EDTA
100 mM NaCl

Add an equal volume of 100% ethanol and store 3 to 4 months at 0°C.

COMMENTARY

Background Information

It is often necessary to purify or concentrate a solution of DNA prior to further enzymatic manipulations or analytical studies. This is important, for example, when DNA-modifying enzymes need to be removed prior to digestion with a second enzyme or when DNA preparations contain contaminants that inhibit additional enzyme reactions. The most commonly used method for deproteinizing a solution of 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 use of the mixture also reduces the amount of aqueous solution retained in the organic phase (compared to a pure phenol phase) in order to maximize the yield (Penman, 1966; Palmiter, 1974). Isoamyl alcohol is added to prevent foaming of the mixture upon vortexing and to aid in the separation of the organic and aqueous phases (Marmur, 1961). The 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 on multiple samples.

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 going from one modification reaction to another. 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, and its use is discouraged.

The glass beads protocol, modified from Vogelstein and Gillespie (1979), provides a simple, nontoxic method for removing DNA from contaminating impurities in various enzymatic reactions and isolation procedures (the protocol can also be adapted for eluting DNA from agarose gels; see *UNIT 10.5*). In the presence of high salt, DNA binds to the small glass particles. The resulting precipitate is washed to remove NaI and impurities from the original sample, and subsequent suspension in water or TE buffer causes dissociation (elution) of the DNA from the glass. Because fewer manipulations are required, this method is faster and easier to perform than the other organic-based extraction methods described here. However, the yields are also 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; smaller-length fragments apparently bind tightly and irreversibly to the glass. DNA fragments larger than 3 to 5 kb may become sheared by the glass, although the suspension provided with the Qiaex gel extraction kit consists of activated silica-gel particles that allow efficient binding and subsequent elution of DNA fragments ranging from 50 bp to 50 kb in length.

Many commercial products now available either facilitate or serve as alternatives to the purification methods presented here. One such alternative for purifying DNA from residual protein in enzymatic reactions is StrataClean Resin (Stratagene), a nontoxic slurry of hy-

droxylated 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 (available from 5 Prime → 3 Prime; *APPENDIX 5*), improves recoveries in standard organic extractions by reducing loss of sample at the interface. The gel consists of an inert silica-based blend of intermediate density. During centrifugation, the normally fuzzy interface is compacted tightly below or within the gel. The gel/interface complex migrates discreetly between the organic and aqueous phases, thus creating a tight partition which allows recovery of virtually all of the aqueous phase.

Critical Parameters

Oxidation products of phenol can damage nucleic acids and only redistilled phenol should be used. To ensure complete deproteinization, extractions should be repeated until no protein precipitate is present 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 protocols, loss of nucleic acid at the interface and into the organic phase can be minimized by back-extracting the organic phase.

To ensure a reasonable yield from the glass beads method, the resulting NaI and wash supernatants should be extracted with the glass suspension a second time. The procedure works much more efficiently with DNA fragments ≥500 bp.

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 glass beads procedure can be similarly improved (to ≤80% recovery) by increasing incubation times by 5 to 10 min (i.e., doubling or tripling the incubation time) and by subjecting supernatants to an additional binding step.

Time Considerations

Approximately 90 min should be allowed for carrying out steps 1 through 12 of the basic protocol on twelve DNA samples in microcentrifuge tubes. Buffering of phenol should be started ≥1 hr before the 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 glass beads protocol can be performed on twelve samples in 15 to 20 min.

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