DNA Isolation Procedures

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1 Introduction

Literally hundreds of protocols for DNA preparation from various sources of tissue have been published over the last few decades. To display all of these preparations would take volumes of manual space so instead we present in this chapter several of the preparations that have been used successfully in our laboratories. We also present a few "classical" procedures that are "tried and true" and nearly always work. In addition the www is an excellent source for protocols. Some forums exist for the dissemination of protocols for DNA and RNA isolation (DNA isolation protocols forums: http://www.nwfsc.noaa.gov/protocols.html, http://bric.postech.ac.kr/resources/rprotocol/; RNA isolation protocols forum: http://www.nwfsc.noaa.gov/protocols/methods/RNAMethodsMenu.html).

Myriad permutations of the traditional phenol/chloroform extraction methods (e. g., [1-5]) are still in use because they reliably produce high-quality DNA.

For DNA fragment analysis [6-7], we recommend using a cesium-chloride (CsC1) gradient (which takes 3-4 days) to minimize the possibility of amplifying nuclear mitochondrial sequences. Some investigators use salt-precipitation [8] before phenol/chloroform extraction and others follow phenol/chloroform extraction with further purification using a Centricon 30000 MW membrane (Amicon).

2 Materials

Organellar DNA

We include a CsCL preparation not merely out of tradition. Modern PCR techniques have all but eliminated the need for CsC1 gradient purification of target DNA. The reason we include this procedure is that the CsCl gradient method can also be used as a last resort when organellar DNA studies result in the discovery of organellar DNA inserted into the nuclear genome. The CsC1 gradient can be used to purify the organellar DNA away from the inserted organellar DNA that is contained within the nuclear genome, thus avoiding the problem of spurious results from nu-mtDNA. These methods use more time, are more susceptible to contamination because tubes are opened and closed more frequently, and are unpleasant due to the exposure to toxic chemicals.

When separating organellar DNA from nuclear DNA, fluorescent dyes (either Hoechst 33258 or Ethidium Bromide) are used to visualize the different types of DNA using a CsC1 gradient. Ethidium bromide (EtBr), similar to propidium

iodide, is an intercalating dye. Both dyes insert between the stacked purine and pyrimidine base pairs of double-stranded DNA. The intercalation of EtBr causes the DNA to become buoyant, resulting in DNA with a lower density (and therefore higher in the centrifuge tube) in a CsC1 gradient. As supercoiled DNA binds with EtBr, it relaxes the supercoil, such that it rewinds in the opposite direction. During this rewinding, more EtBr is bound to the DNA, so that the strands cannot bind to each other, unless a nick is introduced into the strand to cause relaxation. Therefore, linear DNA can bind more EtBr than plasmid DNA and its buoyant density is less than plasmid DNA. This results in the characteristic plasmid gradient where the supercoiled plasmid DNA is below that of the linearized genomic or nicked DNA.

Contrasted to EtBr, Hoechst 33258 does not intercalate into the DNA. It interacts with the large groove of the DNA molecule by hydrogen bonding. This blue fluorescing dye interacts more with A and T nucleotides rather than G and C nucleotides. Hoechst dye also decreases the buoyant density of the DNA. Since many plastid and mitochondrial genomes have a much higher ratio of AT:GC residues, these genomes will bind more Hoechst dye than the nuclear DNA which characteristically has lower AT:GC ratios (although this will vary depending on what organism is being sampled). The lower density mitochondrial DNA (mtDNA) and plastid DNA (pDNA) will migrate higher during centrifugation and can be separated from the nuclear DNA in this manner.

Plants and Algae

Plant and algal DNA isolation also present particular problems that oftentimes require the use of the traditional methods. The isolation of nucleic acids from plants and algae differs from most modern and generic techniques used for animal tissues due to the cellular structure of plant material *versus* animal tissues. Plants and macroalgae have cell walls mostly comprised of cellulose or some other complex polysaccharide, and the degree to which they must be separated from the nucleic acid material (particularly RNA) depends on the intended use of the nucleic acids. For example, RNA that will eventually be used to make cDNA library material must be completely free of any complex polysaccharides which decrease the amount of mRNA yields following the initial separation. The use of herbaria-preserved material has also proven to be valuable for obtaining DNA from rare or unique specimens. We include a protocol in this chapter that has been quite successful at extracting DNA from preserved plant specimens.

Interestingly enough, plant and macroalgal material can yield large quantities of nucleic acids, primarily due to their large genome sizes. In macroalgae, nuclear DNA per cell varies over four orders of magnitude (200-0.2 pg) and in algal species with smaller genomes, there can be a 1000-fold difference between the size of the nuclear genome and that of the plastid genome [9]. Nuclear DNA can exist in one to four copies per cell, depending on what stage it is in its life history. However, specific sub-regions within the nuclear genome are comprised of identical tandem repeats of the same sequence. One example of

this is the region of the nuclear rDNA that contains the genes for the large- and the small- subunit ribosomal RNAs plus spacer regions, both transcribed and non-transcribed regions. Certain angiosperm nuclear rDNA repeats can range from 9-12 kb in length and include large, non-transcribed regions that can vary even within a species (they are not conserved and they can vary in the number of tandem repeats [10].

Plants and macroalgae also contain two other genomes: mitochondrial and plastid. In both terrestrial plants and macroalgae, the plastid DNA genome is a double-stranded DNA circle, which contains the genes for plastid rRNA, tRNA and some other proteins. Plant genomes can range from 120-217 kilobases [11], in contrast to macroalgae, which range from 73 to over 400 kb. Plastid genomes of angiosperms have two copies of the region containing the ribosomal DNA (rDNA) genes, and are on opposite sides of the circular DNA molecule, but in reverse orientation (called an inverted repeat). Macroalgae may contain variations of this organization, with strings of tandem repeats of the rDNA, or have only a single copy of the ribosomal gene [10].

Eukaryotic mitochondrial genomes have a much greater variety than plastid genomes in size. While metazoan mitochondrial genomes are small circles (16-40kb) of conserved gene content [12, 13], angiosperm mitochondrial genomes are some of the largest known (200->2000 kb), with the greatest variation even within a genus [14]. Even certain species have several sizes of mitochondrial DNA molecules within an individual. Macroalgae, in contrast, have small mitochondrial genomes ranging from 100-500 kb pairs. They are found in both circular and linear forms, similar to fungi and protozoa. As with plastid DNA, there are identical copies of the mitochondrial DNA per cell, since each mitochondrion has one or more copies. Similar to metazoans, uni-parental inheritance is observed [15].

Microscopic Organisms

Because many of the traditional DNA isolation preparations for animals were originally developed for vertebrates and insects, microscopic organisms such as protozoa and extremely small animals pose difficult problems for DNA isolation. Because of the wide range of animals and microscopic organisms, we will focus on several protocols that have been developed for rapid and efficient isolation of DNA.

Isolation of DNA from museum-preserved specimens has always been difficult due to the nature of the liquids in which specimens are preserved. Previous fixatives like formaldehyde and other aldehyde mixtures work well in preserving macro- and ultra- structural components of the specimens. Unfortunately, because these types of chemicals bind tightly to the tissue matrix as well as to the nucleic acids retained inside them, processing the material for DNA can be difficult. Also, specimens may have initially been preserved in formalin or some other strong fixative, and then eventually placed in ethanol, which produces a sample from which it is difficult to obtain and therefore isolate DNA. The method described here for these types of “fixed” specimens may not always

be fruitful, but in many instances has proved to be successful for obtaining PCR templates of various loci used in phylogenetic analysis [16]. Due to the special nature of ancient specimens we dedicate an entire section to that subject.

Solutions

In this section we explain how to make the common stock solutions for Molecular Biology laboratories in the protocol boxes in this chapter. Other specific solutions are described in the relevant sections of this manual.

• 5 M ammonium acetate (NH_4Ac)
Dissolve 335 g of ammonium acetate (CH_3COONH_4; M.W. 77.08) in 800 mL of H_20 Adjust the volume to 1 liter with H_20.
Sterilize by filtration.

• 1 M ammonium sulfate
Dissolve 132.14 g of ammonium sulfate [$(NH_4)_2$ SO_4; M.W. 132.14) in 800 mL of H_2O Adjust the volume to 1 liter with H_2O.

• BCIP (5-bromo-4-chloro-3indolyl-phosphate, Boehringer-Mannheim) solution
Dissolve BCIP to a concentration of 50 mg/ml in dimethyl formamide.
Store aliquots at -20°C.

• Carlson lysis buffer
For 100 mls:
100 mM Tris, pH 9.5 (1.21g Tris base in 70 ml ddH2O, pH to 9.5 with HC1)
20 mM EDTA (0.76g)
1.4 M NaC1 (8.18g)
2% CTAB (2 g)
1% PEG 8000 or 6000 (1 g)
Stir overnight. Bring up to 100 ml volume. Add 2µl β-mercaptoethanol per 1 ml of buffer just before use.

• Chelex® 100 buffer
Chelex 100 buffer-0.001 M Tris, pH 8.0
0.05 mM EDTA
5% (w/v) Chelex® 100 resin

• 2x CTAB (cetyltrimethylammonium bromide) extraction buffer
100 mM Tris-HC1 (pH 8.0)
1.4 M NaC1
20 mM EDTA
2% (w/v) CTAB
0.1% (w/v) PVPP (polyvinyl polypyrrolidine)
0.2% (v/v) B-mercaptoethanol (add directly before use, but do not store above with this).

• CTAB/NaC1 solution (10% CTAB, 0.7 M NaC1)
Dissolve 10 g of CTAB in 80 mL of 0.7 M NaCl solution. Stir on a hot magnetic stirrer until the CTAB has dissolved. Adjust volume to 100 mL with 0.7 M NaC1 solution.

• DEPC (diethyl pyrocarbonate)-treated H
0.5% DEPC in H stir vigorously in a fume hood for an hour and let sit for 2 hours prior to autoclaving.

• Digestion buffer
100 mM NaC1
10 mM Tris pH 8.0
25 mM EDTA
Prepare aliquots of 100 mL

• DMSO (dimethylsulfoxide) buffer
From the new Sambrook & Russell [30] Purchase a high grade DMSO (HPLC grade or better). Divide the contents of a fresh bottle into 1 mL aliquots in sterile tubes. Close the tubes tightly and store at -20°C. Use each aliquot only once and then discard.

• DTAB (dodecyltrimethylammonium bromide) solution
8% DTAB
1.5 M NaCl
100 mM Tris (pH 8.8)
50 mM EDTA

• 0.5 M EDTA pH 8.0 (ethylenediaminetetraacetic acid)
Add 168.1 g of disodium ethy1enediaminetetraacetate to 800 mL of H Stir vigorously on a magnetic stirrer. Adjust the pH to 8.0 with NaOH (approximately 20 g of NaOH pellets). Adjust the volume to 1 liter with H
NOTE: The disodium salt of EDTA will not go into solution until the pH is adjusted to approximately 8.0.
Sterilize by autoclaving.

• Ethidium bromide solution (10 mg/mL)
Add 200 mg of ethidium bromide to 20 mL of H
Stir on a magnetic stirrer for several hours to ensure that the dye has dissolved. Store in a light-proof container (e.g., in a falcon tube wrapped in aluminum foil) at room-temperature.

• Glycine
2mg/ml of glycine in PBT.
Store at -20°C.

• 5 M guanidinium thiocyanate
Dissolve 59 g of guanidinium thiocyanate to 100 mL of H_2O. Heat at 65°C until dissolved in a water bath. Filter the solution through a Whatman No. 1 filter or equivalent (i.e., a Nalgene Filtration Unit).

• Guanidinium iso thiocyanate (GITC) homogenization buffer
4 M guanidinium iso thiocyanate (CH_5N_3•CHNS; M.W. 118.16)
0.1 M Tris-HC1 (pH 7.5)
1% β-mercaptoethanol
Dissolve 50 g of guanidinium iso thiocyanate in 10 mL of 0.1 M Tris-HC1 (pH 7.5) and RNAse-free, DNAse-free H_2O to 100 mL. Heat at 65°C until dissolved in a water bath. Filter the solution through a Whatman No. 1 filter or equivalent (i.e., a Nalgene Filtration Unit).
To avoid filtration, the solution may be prepared for half volume (25 g of guanidinium iso thiocyanate in 5 mL of Tris-HCl, and H_2O to 50 mL) in a sterile 50 mL Falcon tube using sterile spatulas for pouring the guanidinium iso thiocyanate, and sterile reagents.
This solution is stable and can be stored indefinitely at room temperature. Just before use, add β-mercaptoethanol to a final concentration of 1% (0.14 M).

•HM
1 mM $CaCl_2$
1.5 mM $NaHCO_3$
0.1 mM $MgCl_2$
0.08 mM $MgSO_4$
0.03 mM KNO_3
in Arrowhead spring H_2O

• Liftons buffer
100 mM EDTA
25 mM Tris-HC1 pH 7.5
1% SDS

• 4M lithium chloride (LiC1)
Dissolve 169.56 g of lithium chloride (LiCl; M.W. 42.39) in 800 mL of H_2O and adjust the volume to 1 liter with H_2O.

• 1 M Levamisole
Dissolve 60 mg of levamisole in 250 µl of RNase-free H_2O.
Make fresh stock for every use.

• Lysis buffer
100 mM EDTA
10 mM Tris-HC1 pH 7.5

• MAB
100 mM maleic acid
150 mM NaC1, pH 7.5

• 1 M magnesium chloride ($MgCl_2$)
Dissolve 203.31 g of magnesium chloride hexahydrate ($MgC1_3$ M.W. 203.31) in 800 mL of H_2O and adjust the volume to 1 liter with H_2O.
Dispense in aliquots and sterilize by autoclaving.
NOTE: $MgCl_2$ is extremely hygroscopic. Buy small bottles and do not store opened bottles for long periods of time.

• MOPS [3-(N-morpholino)propanesulfonic acid] buffer
0.1 M MOPS (pH 7)
0.5 M NaC1
0.1% Tween-20.

• NBT (4-nitroblue tetrazolium chloride, Boehringer Mannheim)
Dissolve NBT at 75mg/mi in 70% dimethyl formamide.
Store aliquots at -20°C.

• NTMT
100 mM NaC1
100 mM Tris HC1 (pH 9.5)
50 mM $MgCl_2$
0.1% Tween-20
2 mM levamisole (add on day of use).

• 4% Paraformaldehyde
Dissolve 10 g paraformaldehyde in 200 ml of DEPC-treated H at 65°C in a fume hood and cool on ice. Adjust pH to 7.5 with 5-10 µl NaOH. Add 25 ml 10x PBS and make volume up to 250 ml with DEPC treated H_2O.
Aliquots can be stored for several months at -20°C.

• PCI (phenol:chlorophora:isoamyl alcohol)
This is a solution of phenol, chloroform, and isoamyl alcohol, in a ratio of 25:24:1.
We recommend the use of commercially mixed PCI at a pH of 7.5-8.0, which avoids the hassle of handling phenol solutions.

• 5 M potassium acetate (KOAc) pH 7.5
Dissolve 49.1 g of potassium acetate (CH_3COOK M.W. 98.15) in 90 mL of RNAse-free, DNAse-free H_2O Adjust the pH to 7.5 with 2 M acetic acid. Adjust the volume to 100 mL with H_2O.
Aliquot and store at -20°C.

• 10x PBS (phosphate-buffered saline solution)
Dissolve 80 g of sodium chloride, 2 g of potassium chloride, 14.4 g of sodium phosphate, and 2.4 g of potassium phosphate in 800 mL of H_2O Adjust the pH to 7.4 with HC1. Adjust the volume to 1 L with H_2O.
Aliquot and sterilize by autoclaving on liquid cycle.
Store at room temperature.

• PBT (phosphate buffered saline, Triton X-100)
0.1% of Tween-20 in 1xPBS buffer.

• 10x PCR buffer
Dissolve 8.116 g of Tris Base (Molecular biology grade), 0.610 g of $MgC1_2$, and 2.227 g of ammonium sulfate in 90 mL of HC1. Stir until dissolved. Adjust the volume to 100 mL with H
Sterilize by autoclaving and aliquot in 1 mL eppendorf tubes.

• PCR buffer w/non-ionic detergents
50 mM KC1
10 mM Tris-HC1, pH 8.3
2.5 mM $MgC1_2$
0.1 mg/mL gelatin
0.45% NP-40
0.45% Tween-20
Prepare aliquots of 10 mL in sterile 10 mL falcon tubes using sterile reagents since this buffer cannot be filtered or autoclaved. Use 0.5 mL of 1 M potassium chloride, 0.1 mL of 1 M Tris-HC1(pH 8.3), 25 µL of 1 M magnesium chloride, 1 mg of gelatin, 450 µL of NP-40, and 450 µL of Tween-20 and bring the volume up to 10 mL in RNAse-free, DNAse-free d H_2O.

• PK buffer
Dilute 10 ml 1M Tris-HC1 and 2 ml 0.5 M EDTA in RNase-free H_2O, and adjust volume to 200 ml with RNase-free H_2O.

• 1 M potassium chloride (KC1)
Dissolve 7.45 g of potassium chloride (KC1; M.W. 74.55) in 80 mL of H and adjust volume to 100 ml with H_2O.

• Proteinase K (20mg/mL)
Use at a concentration of 50-60 µg/mL with a reaction buffer containing 0.01 M Tris (pH 7.8), 0.005 M EDTA, and 0.5% SDS.
Incubate at 37-56°C
Store Proteinase K at -20°C.

• RNA preparation binding buffer
0.5M NaC1

10mM TrisCl pH 7.4
1mM EDTA pH 8.0,
0.1% SDS
Mix appropriate amounts of RNAse-free stock solutions of Tris-HC1, NaC1 and EDTA. Autoclave the mixture and allow to cool to 65°C. Then add appropriate amount of SDS from a 10% stock solution.

• RNA preparation elution buffer
10 mM TrisCl pH 7.4
0.1 mM EDTA pH 8.0
Mix appropriate amounts of RNAse free stock solutions of Tris-HC1 and EDTA. Autoclave the mixture and allow to cool to 65°C.

• RNA preparation washing buffer
0.1M NaC1
10mM TrisCl pH 7.4
1mM EDTA pH 8.0
0.1% SDS
Mix appropriate amounts of RNAse free stock solutions of Tris-HC1, NaC1 and EDTA. Autoclave the mixture and allow to cool to 65°C. Then add appropriate amount of sodium lauryl sulfate sarcosinate from a 10% stock solution.

• 5% sarkosyl buffer
Dissolve 25 g of sarkosyl in 15 mL of 5 M sodium chloride, 25 mL 1 M Tris-HC1 (pH
8.0), 15 mL 0.5 M EDTA, and 400 mL of H Adjust the volume to 500 mL with H_2O.
Do not refrigerate or autoclave.

• 4% SDS buffer
Dissolve 20 g of SDS (Sodium Dodecyl Sulfate; also called Sodium Lauryl Sulfate) in 30 mL of 5 M sodium chloride, 25 mL 1 M Tris-HCI (pH 8.0), 100 mL 0.5 M EDTA, and 300 mL of H_2O. Adjust the volume to 500 mL with H_2O.
Do not refrigerate or autoclave.

• 10% SDS
Dissolve 10 g of SDS (Sodium Dodecyl Sulfate) in 80 mL of H_2O, and adjust the volume to 100 mL with H_2O.

• SDS/Sarkosyl lysis buffer
3 volumes of 4% SDS buffer
1 volume of 5% Sarkosyl Buffer
5 μg/mL proteinase K
0.12% of β-mercaptoethanol
Add 200 μL of Proteinase K (2Omg/mL) and 100 μL of β-mercaptoethanol to 60 mL of 4% SDS buffer, and 20 mL of 5% sarkosyl buffer.
Do not refrigerate or autoclave.

• 20x SSC (pH 4.5)
3 M NaC1
0.3 M sodium citrate

• Silica solution
Weigh out 4.8 grams silica dioxide into a 50 ml polypropylene tube
Add 40 ml distilled water and agitate
Let stand 24 hrs
Pipette 35 ml off
Bring up to 40 ml with distilled water and agitate
Let stand 5 hrs
Pipette 36 ml off
Add 48 ml HC1
Aliquot into 1.5 ml tubes and store in dark

• 3 M sodium acetate (NaOAc) (pH 5.2 and 7.0)
Dissolve 40.82 g of sodium acetate trihydrate ($CH_3COONa•3H_2O$; M.W. 136.08) in
80 mL of H_2O. Adjust the pH to 5.2 with glacial acetic acid or adjust the pH to 7.0 with dilute acetic acid. Adjust the volume to 100 mL with H_2O.
Sterilize by autoclaving.

• 2 M sodium acetate (pH 4.0)
Dissolve 27.22 g of sodium acetate trihydrate ($CH_3COONa•3H_2O$; M.W. 136.08) in 80 mL of H_2O. Adjust the pH to 4.0 with glacial acetic acid. Adjust the volume to
100 mL with H_2O.
Sterilize by autoclaving.

• 5 M sodium chloride
Dissolve 292.2 g of sodium chloride (NaC1; M.W. 58.44) in 800 ml of H_2O.
Adjust the volume to 1 liter with H_2O.
Sterilize by autoclaving.

• Solution I
50 mM glucose
25 mM Tris-HC1 (pH 8.0)
10 mM EDTA (pH 8.0)
Solution I can be prepared in batches of 100 mL. Autoclave for 15 minutes on liquid cycle. Store at 4°C.

• STE (Sodium Chloride-Tris-EDTA) buffer
0.1 M NaC1
10 mM Tris-HCI (pH 8.0)
1 mM EDTA (pH 8.0)

• 50x TAE (Tris-Acetate Buffer)
Dissolve 242 g of Tris base, 57.1 mL of glacial acetic acid, and 100 mL of 0.5 M EDTA (pH 8.0) in H_2O up to 1 liter.
The 50x TAE is the concentrated stock solution. Use lx TAE as working solution (0.04 M Tris-acetate, 0.001 M EDTA).

• 5x TBE (Tris-Borate/EDTA buffer)
Dissolve 54 g of Tris base, 27.5 g of boric acid, and 20 mL of 0.5 M EDTA (pH 8.0) in H_2O up to 1 liter. Stir until dissolved.
The 5x TBE is the concentrated stock solution. Use 0.5x TBE (0.045 M Tris borate, 0.001 M EDTA) as electrophoresis buffer.
NOTE: A precipitation forms when concentrated solutions of TBE are stored for long periods of time. Discard any batches that develop a precipitate.
NOTE: 10x TBE buffer is commercially available, and it constitutes a good solution for laboratories not using much TBE buffer, or for laboratories with high budgets.

• TBST solution
135 mM NaCl
2.7 mM KCl
25 mM Tris HC1 (pH 7.5)
0.1% Tween-20
2 mM levamisole (add on day of use).

• TE Buffer Solution (pH 7.4, 7.6, 8.0)
10 mM Tris-HCl, pH 7.4
1 mM EDTA, pH 8.0
10 mM Tris-HC1, pH 7.6
1 mM EDTA, pH 8.0
10 mM Tris-HCl, pH 8.0
1 mM EDTA, pH 8.0

• 10 mM Tris-HC1 (pH 7.4, 7.6 and 8.0)
Dissolve 121.1 g of Tris base in 800 ml of H_2O. Adjust pH to the desired value by adding concentrated HCl.
pH 7.4 add 70 ml
pH 7.6 add 60 ml
pH 8.0 add 42 ml.
Other pHs desired can be obtained by titrating the HCl. Adjust the final volume of the solution to 1000 ml with H_2O.

3 Methods

3.1 Kits

Various commercially available DNA extraction kits and systems are becoming increasingly popular because of their ease of use, limited labor, and ability to consistently produce high-quality DNA. Because of proprietary considerations of the manufacturer, the composition of some components in these kits is not revealed to the user. We describe a few below that we have used with success in our laboratories. Not all kits are economically favorable to use. Qiagen (http://www.qiagen.com) produces several kits (e.g., QIAamp DNA Mini Kit #51304, #51306; DNeasy Tissue Kit #69504, #69506) which seem to be economically feasible for extracting large numbers of samples. These kits contain a stable proteinase K solution, unidentified non-phenol/chloroform buffers, a silica-gel membrane spin-column to isolate and purify high-quality DNA for PCR, and easy-to-follow instructions. These kits cost approximately $1.50 to $1.80 per sample. Other kits, which are expensive but adequate for large numbers of samples include: Nucleospin (http://www.clonetech.com) and Isoquick (Microprobe Corp.). Bio 101 (http://www.bio101.com) sells a Kit which employs special tubes containing a ceramic lysing matrix, a machine that violently shakes samples (twelve at a time), and a subsequent purification process. This system is ideal for small samples or organisms with sturdy cell walls like plants.

3.2 The Generic DNA Preparation

Most DNA preparation protocols outline the steps involved in three major phases of DNA isolation. The first phase (in *italic*) contains preparatory procedures and the solutions used during these steps. We also include in this phase of the preparation any steps that involve maceration or dispersion of tissue. The second phase of the protocols (in **bold**) is the actual isolation steps required to separate the nucleic acids from the rest of the cellular proteins and debris. The third phase (in normal font) in these preparations includes steps required to purify nucleic acids from impurities that will interfere with sub sequent enzymatic manipulations. It should be noted that when protocols fail, the phenol/chloroform prep will usually work on any remainder of the sample. Resuspension of the isolated DNA should be accomplished so that a DNA collection is standardized for stock concentration. We routinely attempt to resuspend our samples to a concentration of 1µg DNA/µl of solution. The DNA is resuspended in dH_2O or in TE Buffer Solution. Dilutions of the stock solutions can then be made to accommodate the concentrations used for PCR or cloning purposes.

Since some of the preparations we present in this chapter are organism-dependent, we list them in a phylogenetic context. Next to the major groups of organisms in Figure 1 we list the protocols that work best for that group. This does not mean that a preparation listed for bacteria will not work on other organisms; some mixing and matching of protocols can be accomplished to maximize efficiency and yield of DNA preparation.

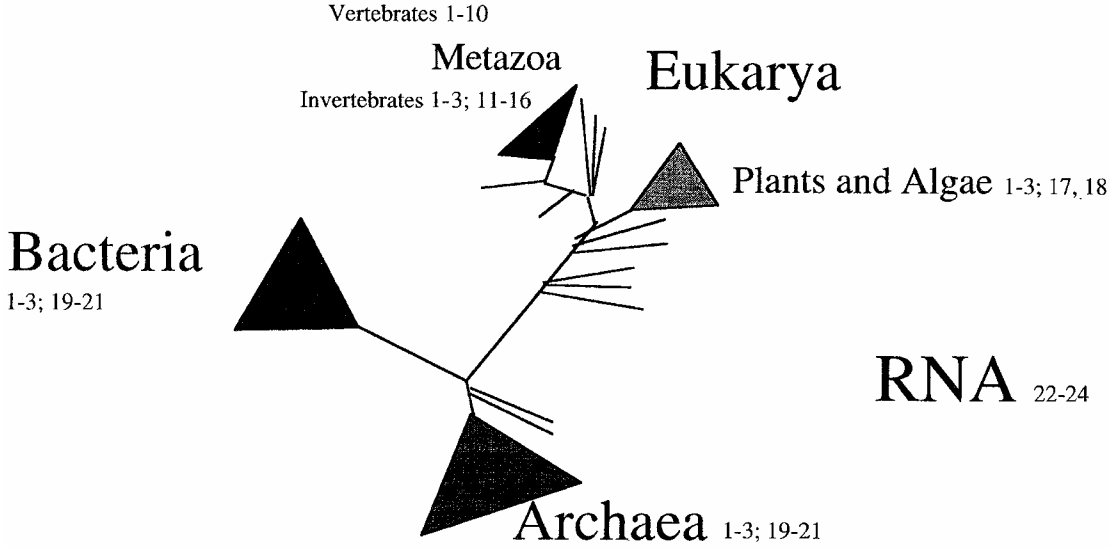

Figure 1 Highly condensed "tree of life" showing the various DNA isolation techniques listed in this chapter next to the group of organisms most appropriate for the protocol.

4 Protocols

Protocol 1 Traditional phenol/chloroform extraction for vertebrates or invertebrates.

(Time: 2 days)

1. Different starting materials will require different maceration and dispersion methods.
2. Liquid nitrogen powdering is a common method for dispersing tissues where large starting amounts are available.
3. Smaller amounts of starting material can be dounced with a commercial homogenizer or with a homemade douncer.
4. The macerated dispersed material is placed in an extraction buffer in a ratio of 3 extraction buffer volumes to 1 sample volume. A common extraction buffer is the Lifton buffer, among many others.
5. Add 1/50 volume of Proteinase K (10 mg/mL) and incubate the sample at 65°C for 2 hrs to overnight.
6. Add 1/10 volume of 5 M potassium acetate and mix well by inverting. Incubate on ice for 30 min.
7. Spin tubes at 10,000 rpm for 10 min and transfer supernatant to new tube.

8. Add an equal volume of PCI.
9. Mix the solution by inverting the tube several times.
10 Spin tubes at 3000 to 5000 rpm for 5 min. The upper aqueous phase containing nucleic acids and the lower organic phases should separate. Transfer upper phase to a new tube.
11. If a sample is especially rare, a back extraction can be performed on the phenol phase by adding an equal volume of TE buffer solution and mixing, followed by spinning tubes at 3000 to 5000 rpm. The aqueous phase from this separation and the aqueous phase from the previous spin can then be combined.
12. Add an equal volume of chloroform to the tube and mix by inverting the tube several times.
13. Spin the tubes again at 3000 to 5000 rpm for 5 min.
14. Remove upper aqueous layer to transfer a new tube.

15. Add two volumes of ice-cold 95-100% ethanol, invert the tubes several times and allow the DNA to precipitate at -20°C for 10 min.
16. The DNA can be collected by spinning the solution at top speed in a microcentrifuge.
17. The ethanol is gently poured off and the pellet is resuspended in 0.5 mL of TE buffer solution.
18. 0.3 ml of 5M ammonium acetate and 1 ml of 100% ethanol are added to the resuspended DNA for a second precipitation. The solution can be placed at -20°C for 10 min.
19. The tubes are spun at top speed in a microcentrifuge and the ethanol supernatant is poured off gently.
20. The resulting pellet is resuspended in TE buffer solution or dH_2O.

Protocol 2 Crude total cellular miniprep

(Time: 3 hours)

This procedure is highly recommended when large numbers of individuals are to he processed for systematic or population genetic studies.

1. The equivalent of no more than 100 µL of tissue is placed in a microcentrifuge tube.
2. Add 200 µL of grinding buffer and dounce the tissue using a commercial or homemade dounce.
3. Add 200 µL of mini lysis solution.
4. Place tubes at 65°C for 30 min.

5. Add 60 µL of 5M potassium acetate and mix the solution by inverting.
6. Place tubes on ice for 30 min.
7. Spin tubes at max speed in a microcentrifuge and transfer supernatant to a new tube.

8. Add 1 mL of ice-cold 95-100% ethanol and mix solution by inverting. Let tubes sit for 30 min at room temperature
9. Pellet the DNA by spinning at maximum speed in a microcentrifuge and remove ethanol supernatant
10. Resuspend pellet in 100 µL of 0.5M ammonium acetate ($N H_4OAc$).
11. Add 400 µ1 of 70% ethanol and mix the tubes by inverting. Let tubes sit at room temperature for 10 min.
12. Pellet the DNA by spinning at maximum speed in a microcentrifuge for 10 min.
13. Pour off ethanol and allow pellet at bottom of tube to air dry for 30 min to one hour.
14. Resuspend the pellet in 50 to 100 µL of dH_2O or TE buffer solution.

Protocol 3 Separation of nuclear and organellar DNAs using cesium chloride gradients

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(Time: 2 to 3 days)

This protocol describes steps after obtaining high molecular weight nucleic acids most typically through phenol chloroform preps.

1. Measure the volume of solution of total DNA that will be used for the cesium chloride gradients. For every mL of solution, add 1 g of finely ground cesium chloride (using a mortar and pestle) to the solution slowly, keeping it warm by placing the tube in one's hands. Agitate the tube gently.
2. While the cesium chloride is dissolving, add 15 mL of ethidium bromide (10 mg/mL stock) to a Beckman 13 mL quickseal tube.
3. Add the DNA/cesium chloride solution to the quickseal tube till the volume reaches the lower part of the neck of the seal.
4. Balance tubes, and seal.
5. Centrifuge at 40,000 rpm for 40-48 hrs. Once centrifugation is complete, carefully remove tubes (do not disturb the gradient!) and place in a rack.
6. Use a small, hand-held UV lamp to visualize the bands. There should be at least 4 bands in the tubes. The lowest density band (and therefore the highest one) is the mitochondrial DNA, the next one is the plastid DNA. The third lower band is sheared rDNA and the bottom band (and this should contain the most DNA) is the nuclear DNA.
7. Using a large gauge needle (18 is okay), puncture a hole next to the fraction that is to be isolated. If all 3 fractions are to be isolated, then start with the topmost band (mitochondrial) and work down the layers.
8. Slowly draw up the band until everything is collected, and place in separate collection tubes.

9. Extract DNA with 100% iso-butanol two to three times to remove the ethidium bromide by mixing an equal volume of iso-butanol and inverting the tubes several times. The aqueous phase (top) containing the DNA should lose the pink color of ethidium bromide with each wash. Extract one extra time after all ethidium bromide appears to be removed from aqueous phase.
10. After collecting all 3 types of DNAs, the solutions must be dialyzed in TE buffer solution (usually several changes of 1-2 L overnight) at 4°C. This will remove all the cesium chloride
11. After dialysis, the DNA can be used for several techniques (PCR, restriction, digest, Southern and Northern blot analysis, genomic libraries). This solution must be kept at 4°C (freezing the DNA will shear the longer strands).

Protocol 4 Standard vertebrate isolation protocol using CHELEX®

(Time: approximately 15 min)

Chelating resin (available from Sigma as Chelex, [17]) works by denaturing proteins and removing inhibitory ions [18]. There are several reasons to prefer this method over others. In as little as 12 min, one can have DNA suitable for PCR. The risk of contamination resulting from opening and closing of tubes is greatly reduced over other methods, because the tube is opened just one time to add the tissue sample. It is inexpensive ($1/gm), which is important when processing large numbers of samples. It requires little tissue; all that is needed for a successful reaction (300 µL), is a piece of tissue approximately the size of the period at the end of this sentence. Chelex samples work with a variety of tissue preparations including blood, muscle and liver. Finally, Chelex is a very successful method; only a small percentage of reactions do not work with this preparation. Following is a minimum-step method:

1. Add a small piece of tissue (< I mg) to 300 µL of 5% Chelex® (5% weight to volume chelating resin in ultrapure water).
2. Incubate at 100°C for 12 min.
3. Vortex once or twice during incubation.

4. Use 0.8-1.0 µL in a 10-20 µL PCR reaction.

5. Store at -20 or -80°C.
6. Some of the modifications of the Chelex® protocol include: a longer or shorter incubation time (e.g., 10-15 min); more complicated incubation profiles: a) 100°C for 10 min, then 70°C for 20 min; b) 5 hr at 56°C; vortex; then 15 min at 95°C; 20% Chelex; boil for 8 min; vortex; spin.
7. Brief homogenization of tissue in DMSO buffer before adding it to the Chelex improves extraction and PCR amplification.
8. Spin the samples briefly (e. g., 5 min at 13,000 rpm) before drawing off supernatant for PCR.

9. In each case, reactions can be left at room temperature overnight after incubation. Vortexing one or more times after the sample has had time to extract seems to be essential for better PCR.
10. A common problem with reactions that do not produce a PCR product on the first attempt is too much DNA. This is often remedied by using some portion of the supernatant in a subsequent "re-chelexing" reaction. A disadvantage of this method is that the DNA product is not readily quantifiable using ethidium-bromide-infused agarose gels because of the small amount of DNA recovered.

Protocol 5 Isolation of DNA from museum-preserved specimens-formalin

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(Time: 3 hours to 1 day)

1. Initially the specimen or tissue sample is placed in a TE buffer to rid the tissue of any remaining fixative.
2. This pre-incubation can be from one hour to overnight, depending on the specimen size and the type of fixation that it has undergone (if it comes directly from formalin or if it has been preserved in ethanol following formalin fixation).
3. Place each sample in an eppendorf tube, add 500 µL of STE buffer containing 0.2% SDS and 250 µL of 10 M ammonium acetate.
4. Place tube in a heating block at 55°C to preheat.

5. Place a small amount of tissue (the size of a match head) into each tube and grind the tissue with a sterile teflon eppendorf grinder (Kontes).
6. After tissue has been ground, return to the heating block and incubate for 1 hour.
7 Centrifuge tubes in a microcentrifuge at 14,000 rpm for 5 min to pellet the cell debris and precipitated proteins.

8. Transfer supernatant to a new tube and add 2 volumes of ice-cold 100% ethanol.
9. Mix gently by inverting tubes.
10. Place tubes at -20°C overnight, or at -80°C for 1 hour until DNA precipitates.
11. Centrifuge tubes at 4°C at 14,000 rpm for 15 min.
12. Remove supernatant and add the same volume of cold 70% ethanol.
13. Spin tubes at 4°C at 14,000 rpm for 10 min.
14. Pour off ethanol and dry tubes completely.
15. Resuspend the pellet in 50 µL of TE buffer overnight at 4°C or for 30 min at 40°C.
16. Use between 1-5 µL of solution for PCR reactions (depending on PCR reaction volume).

Protocol 6 Enriched cytoplasmic nucleic acid preparation from animals

Minced, ground, powdered or dounced tissue is brought up to 5 mL using any homogenization buffer.

1. Add 100 µL of 10% stock of Nonidet P-40 or Triton X-100 and incubate on ice for 5 min.
2. Spin solution at 2,500 rpm in SS34 rotor. Recover supernatant, as nuclei will pellet.
3. Several low speed (2,500 rpm) spins may be necessary to remove majority of nuclei.

4. Add an equal volume of PCI.
5. Mix the solution by inverting the tube several times.
6. Spin tubes at 3,000 to 5,000 rpm for 5 min. The upper aqueous phase containing nucleic acids and the lower organic phases should separate.
7. If a sample is especially rare, a back extraction can be performed on the phenol phase by adding an equal volume of TE buffer and mixing, followed by spinning tubes at 3,000 to 5,000 rpm. The aqueous phase from this separation and the aqueous phase from the previous spin can then be combined.
8. Add an equal volume of chloroform to the tube and mix by inverting the tube.
9. Spin the tubes again at 3,000 to 5,000 rpm for 5 min.
10. Remove upper aqueous layer.

11. Add two volumes of ice-cold 95-100% ethanol and allow the DNA to precipitate at -20°C for 10 min.
12. The DNA can be collected by spinning the solution at top speed in a microcentrifuge.
13. The ethanol is gently poured off and the pellet is resuspended in 0.5 mL of TE buffer.
14. 0.3 mL of 5 M ammonium acetate and 1 mL of 95-100% ethanol are added to the resuspended DNA for a second precipitation. The solution can be placed at -20°C for 10 min again.
15. The tubes are spun at top speed in a microcentrifuge and the ethanol supernatant is poured off gently.
16. The resulting pellet is resuspended in TE buffer or dH_2O.

Protocol 7 Plucked feathers using CHELEX®

1. Using a sterile razor blade, cut off approximately 5 mm from the base of a plucked feather (calamus)

2. Place in a 0.8 mL tube containing 250 µL sterile 5% Chelex® (Bio-Rad).

3. Incubate at 100°C for 15 min; vortex 2x's for 15 sec during incubation period.
4. Allow solution to cool to room temperature and spin for 30 sec at maximum speed.

5. Transfer supernatant to fresh, sterile 0.8 mL tube.
6. Store at 4°C, or at -20°C for long-term storage.

Protocol 8 Preparation for caviar and other fish tissues.

This method works well for fish tissue samples, fin snips, and eggs, even when the latter are processed (salted and pasteurized).

1. Place a single egg or small piece of tissue in about 200 µL of Lifton buffer and add 20 µL of a 20 mg/ml proteinase K solution. If an egg sample is being processed, use the pipette tip to crush the egg. Incubate at 55-65°C for a minimum of one hour with gentle rocking. The yield of the extraction increases with the amount of incubation time. For caviar that is degraded or spoiled, or suspicious tissue samples, incubate overnight. A general rule is that once the tissue is completely dissolved, you can proceed to the next step.

2. Add 300 µL of phenol and 25 µL 5M potassium acetate, pH 4.8 to the above solution (after one hour to overnight incubation) and shake vigorously. Allow about 5-10 min incubation time with gentle rocking. Spin 14,000 rpm for 5 min. At this point, the aqueous phase is on top. The whitish material at the interface contains denatured proteins and carbohydrates and should be avoided. Remove top layer (aqueous phase) and transfer to new tube. Save the remaining solution.
3. Add 300 µL of chloroform and gently mix, incubate, and spin as above in step 2. If separate layers are not evident after spinning, the wrong phase was removed during step 2 (occasionally the phases flip because the DNA is heavier than the phenol). If so, use the saved solution from step 2 and repeat step 3 (i.e., add the chloroform to this layer, mix, incubate, and spin). Remove top layer (aqueous phase) and transfer to a new tube. Again, the interface should be avoided.
4. Add 1 mL of 100% ethanol to the aqueous phase removed in step 3 and mix. Place at -20°C for a minimum of 30 min. The extraction can also be left at -20°C overnight at this point if necessary.

5. Spin at 14,000 rpm for 5 min. Pour off supernatant, being careful not to lose the pellet (sometimes the pellet will not be visible). Resuspend the pellet in 200 µL of RNAse free, sterile water and then add 500 µL 100% EtOH and 25 µL 7.5M NH_4OAc. Place at -20°C for 20 min.

6. Spin at 14,000 rpm for 5 min. Pour off supernatant and allow inverted tube to dry completely or centrifuge under speed vacuum without heat for 1-2 min. Resuspend pellet in 50 µL sterile H_2O.
7. If a high-quality extraction is obtained, use approximately 1 µL of a 1:100 dilution of the extraction for a 25 µL or 50 µL total PCR reaction. A dilution series for the appropriate amount for PCR can be performed.
8. If PCR reactions do not work using several dilutions, they may contain impurities or inhibitors in the extraction. This often happens with caviar egg extractions. In this case, try using the GeneClean (BIO 101) kit following manufacturer's specifications (extractions should be resuspended in 30 µL RNAse-free sterile H_2O. Be sure to keep solutions sterile at this step (by UV treating everything) as this can introduce contaminants.

Protocol 9 Avian tissue and feathers from museum skins using the QIAgen DNAeasy® Tissue Kit

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(Time: 3 hours; also used for molted feathers for maximum yield)

1. Working in a UV hood or clean area restricted to ancient DNA extractions and using a sterile razor blade, shave off a thin tissue fragment (~1.5 x 1.5 x 3 mm) from ventral side of the appropriate digit of the foot (varies among avian groups) or approximately 5 mm from the base of a plucked feather (calamus) from each specimen [19].
2. Place approximately one-half of tissue sample or entire feather sample in a sterile 1.5 mL tube. Negative extraction controls should be carried out throughout the entire procedure.
3. Add 180 µL of Buffer ATL and 20 µL Proteinase K (20 mg/mL) to 1.5 mL tubes containing tissue.
4. Incubate at 55°C rotating until tissue is completely dissolved (may take up to 48 hours or more).
5. In order to guarantee full digestion, it may be necessary to add additional 20 µL aliquots of Proteinase K to the sample.
6. If additional Proteinase K is added, the volumes of subsequent solutions of Buffer AL and ethanol must be scaled up accordingly.

7. After tissue is completely digested, add 200 µL Buffer AL (or scaled up volume).
8. Incubate at 65°C for 15 min.
9. Add 200 µL of 95-100% ethanol (or scaled-up volume) and mix thoroughly by vortexing.
10. Incubate at 4°C for 1 hour.
11. Place the provided spin column inside 2 mL collection tube and add entire volume of sample to spin column and centrifuge for 1 min at 8,000 rpm; discard filtrate and collection tube.

12. Place spin column in fresh 2 mL collection tube and add 500 µL of Buffer AWl and centrifuge for 1 min at 8,000 rpm; discard filtrate and collection tube.
13. Place spin column in fresh 2 mL collection tube and add 500 µL of Buffer AW2 and centrifuge for 3 min at maximum speed to dry DNeasy membrane; discard filtrate and tube.
14. Place spin column in fresh 1.5 mL tube and add 50 µL Buffer AE preheated to 70°C [19].
15. Incubate at room temperature for 45 min.

16. Centrifuge for 3 min at 8,000 rpm.
17. A second elution may be performed by transferring spin column to a fresh 1.5 mL tube and repeating step 12; incubate sample for 10 min at room temperature and repeat step 14.
18. Store at 4°C, or at -20°C for long-term storage.

Buffers ATL, AL, AWl, AW2, and AE are supplied with the DNAeasy® Tissue Kit.

Protocol 10 Focal samples using the QIAamp® DNA Stool Mini Kit

(Time: 4 to 6 hours)

1. Using a sterile razor blade, cut 180-220 mg from surface of stool (thin slices) and place in a sterile 2 ml tube.
2. Add I mL of Digestion buffer (100 mM NaCl, 10 mM Tris-HC1 pH 8.0, 25mM EDTA), 2% SDS, and 20 µL Proteinase K (20 mg/mL) to each 2 mL tube containing stool; above Digestion buffer not provided by QIAgen; substituted for Buffer ASL.
3. Incubate at 65°C overnight or until well digested (solids may still be present); mix by vortexing.
4. Centrifuge sample for I min at full speed to pellet stool particles.
5. Transfer supernatant to a fresh 2 mL tube and discard pellet.
6. Add 1 InhibitEX tablet to each sample and vortex immediately and continuously for 1 min; incubate suspension at room temperature for 5 min.
7. Centrifuge sample for 3 min at full speed to pellet stool particles and InhibitEX.
8. Immediately pipet supernatant into a fresh 1.5 mL tube (this step may be repeated as needed in order to ensure maximum recovery of sample; discard pellet when all spins completed).
9. Pipet 600 µl supernatant from step 7 into a fresh 2 mL tube containing 25 µL Proteinase K (20 mg/mL); (if greater than 600 µL recovered from step 7, carry the remaining solution through the following procedure in separate tubes using scaled volumes of all solutions; samples will be consolidated at step 15).

10. Add 600 µL Buffer AL and vortex for 15 sec (QIAgen notes that it is essential that the sample and Buffer AL are thoroughly mixed to form a homogenous solution).
11. Incubate at 70°C for 10 min.
12. Add 600 µL of ethanol (95-100%) to the lysate and vortex.
13. Place the provided spin column inside 2 mL collection tube and add 600 µL lysate from step 11 directly on to the QIAamp spin column.
14. Centrifuge for 1 min at full speed; place the spin column in a fresh 2 mL collection tube and discard filtrate.
15. Add a second aliquot of 600 µL lysate and centrifuge for 1 min at full speed; place the spin column in a fresh 2 mL collection tube and discard filtrate.
16. Repeat step 14 with a third aliquot of 600 µL lysate; if greater than 600 µL recovered from step 7, then add parallel sample to the spin column and repeat step 14.
17. Place spin column in fresh 2 mL collection tube and add 500 µL Buffer AW1 and centrifuge for 1 min at full speed; discard filtrate and collection tube.
18. Place spin column in fresh 2 mL collection tube and add 500 µL Buffer AW2 and centrifuge for 2 min at full speed; discard filtrate and collection tube.
19. Place spin column in fresh 2 mL collection tube and centrifuge for 2 min at full speed to dry column.
20. Place spin column in fresh 1.5 mL tube and add 50 µL Buffer AE preheated to 70°C.
21. Incubate at room temperature for 45 min.

22 Centrifuge for 3 min at 8,000 rpm a second elution may be performed by transferring spin column to a fresh 1.5 mL tube and repeating step 19 incubate sample for 10 min at room temperature and repeat step 21.
23. Store at 4°C or -20°C for long-term storage.

InhibitEX and buffers AL, AW1, AW2 and AE are supplied with the QIAamp® DNA Stool Mini Kit.

Protocol 11 Quick DNA extraction for invertebrates and arthropods

(Time: 2-3 hours)

This method has been used with fresh, frozen or ethanol-preserved tissues of many different metazoan phyla (Porifera, Cnidaria, Ctenophora, Echinodermata, Hemichordata, Priapula, Arthropoda, Platyhelminthes, Phoronida, Brachiopoda, Bryozoa, Nemertea, Mollusca, Annelida, Sipuncula, Echiura and Pogonophora). It is especially recommended for organisms that have mucous-type secretions such as molluscs and platyhelminths. This method does not yield good PCR products for specific organisms that present certain types of body pigmentation, especially Onychophora and some Diplopoda (Arthropoda, Myriapoda) Tissues are homogenized in a solution of guanidinium thiocyanate following a modified protocol for RNA extraction from Chirgwin et al. [20].

1. Homogenize the tissue sample in 1 volume (e. g., 400 µl of 4M guanidinium thiocyanate homogenization buffer and 0.1M beta-mercaptoethanol.
2. If the tissue sample cannot be disrupted easily, grind the sample to a fine powder in liquid nitrogen with a mortar and pestle and pour the powder into the guanidinium thiocyanate buffer.

3. Mix well, shaking for 10 min to 1 hour at room temperature. Add 1 volume of PCI and vortex.
4. Centrifuge for 4 min at 12,000 rpm in a microcentrifuge.
5. Carefully remove the upper (aqueous layer) with a micropipette and transfer to a clean tube. Be careful not to disturb the debris on the interface.
6. Add 1 volume of chloroform:isoamyl-alcohol (24:1) and vortex. Centrifuge for 4 min at 12,000 rpm in a microcentrifuge.
7. Carefully remove the upper layer with a micropipette and transfer to a clean tube. Repeat steps 6-8 if the sample is dirty.
8. Add 0.1 volumes (ca. 40 µL) of 3 M sodium acetate pH 5.2 and 2 volumes (ca. 800 µL) of ice-cold 95% ethanol (or 100% ethanol).
9. Precipitate the DNA at -80°C for 20 min (or from 2 hours to overnight at -20°C).
10. Centrifuge the precipitate for 20 min at 12,000 rpm in a microcentrifuge.
11. Discard the ethanol solution by decantation and wash the pellet with 1 mL of 70% ethanol.
12. Centrifuge the precipitate for 5 min at 12,000 rpm in a microcentrifuge.
13. Discard the ethanol solution and dry in a vacuum centrifuge (or at 55°C).
14. Resuspend the pellet in 50-100 µL of TE buffer (pH 7.6). Incubating the sample at 45-60°C can facilitate dissolution of the pellet.
15. Use 1-2 µL for PCR.

It is recommended to use small amounts of tissue (i.e., a leg of a small spider is enough). The dirtier the sample, the worse the PCR works. But do not expect to see a band in an agarose gel after the extraction if small amounts of tissue are used. For long-term storage of the samples, proteinase-K and RNAse treatments are necessary.

Protocol 12 DNA isolation from small insects and crustaceans

(Time 4-24 hours)

This protocol has been used to obtain DNA samples from frozen live or ethanol-preserved crustaceans and insects (1-3 mm in length). It can be used to extract DNA from small portions of larger crustaceans or insects. All steps are performed at room temperature unless specified.

1. For grinding specimens, use a razor blade to cut off the cap and rim of a 0.67 mL microcentrifuge tube. Make a pestle by inserting a 1.5 mL blue pipette tip into the smaller 0.67 mL microcentrifuge tube (this is a cheaper and time saving alternative to using and resterilizing ground glass homogenizers).
2. Rinse alcohol-preserved specimens in sterile distilled water briefly to remove excess alcohol. Frozen or live animals can be ground directly.
3. Grind animal on inside wall of larger tube (with pestle) in 20-30 µL of Lysis buffer. Once sufficiently ground, add Lysis buffer to a total volume of 1 mL. Add 100 µL of 10% SDS and 4 µL of Proteinase K (20 mg/mL) [keep enzyme mixture on ice] and mix.
4. Incubate at 55°C with rotation/shaking for a minimum of 4 hours to overnight.

5. Add 500 µL of equilibrated phenol (pH 8.0) and shake tube well for 5-10 min.
6. Centrifuge for 10 min at 12,000 rpm in a microcentrifuge.
7. Remove aqueous phase (usually the top layer) and place into fresh tube. Try not to collect any portion of the bottom layer when sample is transferred. Repeat steps 3-5 on the aqueous sample once (or until interface between lower organic phase and upper aqueous phase is clean).
8. Add 500 µL of chloroform, place on rotator for 5-10 min, and centrifuge for 10 min. Remove aqueous phase (top layer) and place in a new tube.
9. At this point you can stop and continue with the DNA precipitation steps at a later time, or amplify directly from the sample (if concerned about losing DNA at precipitation steps, storage is not recommended for long term).

10. Add cold 100% ethanol to sample tube from step 6 above (fill to top).
11. Place at -80°C for 15 min.
12. Centrifuge for 25 min at 12,000 rpm in a microcentrifuge.
13. Discard alcohol carefully by pipetting, without disturbing pellet (if pellet is visible). Check pipette tip before ejecting to ensure that pellet was not drawn into pipette tip during alcohol removal. If pellet is inside pipette tip, return pellet to tube and spin again.
14. Add 1000 µL of 70% ethanol and vortex for a few sec.
15. Centrifuge at 12,000 rpm in a microcentrifuge for 10 min. Check for pellet as above.
16. Air dry at room temperature or place in a vacuum centrifuge without heat.
17. Resuspend DNA pellet in 100 µL sterile water.
18. Place DNA sample in a 50-55°C water bath for 5-10 min to dissolve DNA pellet before amplification. Thoroughly mix DNA sample before any further procedures. Use 1-2 µL of sample for PCR.
19. Keep a working DNA sample in the refrigerator (i.e., 30 µL aliquot) to avoid freeze/thaw cycles and place remainder of DNA sample at -20°C for future use.

Protocol 13 DTAB — CTAB preparation

(Time: 1 hour)

This preparation works well on a wide variety of tissues Phillips and Simon [21] have used this preparation to isolate DNA from pinned insects The preliminary step in this approach is to poke holes in the pinned insect exoskeleton prior to the first step below After the DTAB soak the pinned insect can be washed in chloroform and returned to its place in the collection We have used the preparation on pinned insect body parts such as leg and wing tissues We find that the insect parts should be broken into small fragments prior to placing them in the DTAI3 solution The full preparation is given below

1. The tissue is soaked in 600 µL of DTAB solution (8% DTAB, l.5 M NaC1, 100 mM Tris-HC1 (pH 8.8), and 50 mM EDTA) at 68°C for at least one hour or preferably overnight.

2. An equal volume of chloroform:isoamyl alcohol (24:1) is added to the specimen in DTAB and mixed by inverting.
3. This mixed solution is spun at 10,000 g for 2 min.
4. The aqueous layer is removed and placed into another tube. An equal volume of chloroform:isoamyl alcohol (24:1) is added to the aqueous phase for a second extraction.
5. The mixed solution is centrifuged at 10,000 g for 2 min and the aqueous layer is removed and placed into a new tube.
6. Add 900 µL of water and 100 µL of CTAB (5% CTAB, 0.4 M NaC1) to the aqueous layer from above.
7. Gently mix by inverting tubes and leave at room temperature for 2 min.
8. Spin tubes at 10,000 g for 10 min.
9. Pour off supernatant and resuspend pellet in 300 µL of 1.2 M NaC1. This step exchanges CTAB.

10. Precipitate the DNA in 750 µL of 100% ethanol. Mix and leave at room temperature for 10 min.
11. Spin at 10,000 g for 10 min. Pour off ethanol supernatant.
12. Wash pellet in 70% ethanol.
13. Centrifuge at 10,000 g for 10 min and air dry.
14. Resuspend pellet in 20-40 µL of dH_2O or TE buffer.

Protocol 14 DNA isolation from microscopic animals

(Time 1 hour)

This protocol has been used to obtain DNA samples of organisms smaller than 1 mm in length such as *Macrobiotus* (Tardigrada) and Pauropoda (Arthropoda, Hexapoda) The method is based on a direct lysis of the animal tissues a modification of the protocol by Higuchi [22]. Method described in Giribet et al. [23].

1. Pour 400 µL of PCR buffer with non-ionic detergents (50 mM KC1, 10 mM Tris-HCl pH 8.3, 2.5 mM $MgCl_2$ 0.1 mg/mL gelatin, 0.45% NP-40, 0.45% Tween-20) in an eppendorf tube.
2. Add 0.6 µL of Proteinase K (10 mg/mL) per 100 µL buffer (a total of 2.4 µL). Add one to three live individuals.
3. Incubate at 60°C for 1 hour with occasional vortexing.

Use 10 to 25 µL for PCR. This method is not suitable for long-term DNA storage, and will yield DNA for just a few PCR reactions.

Protocol 15 Insect preparation — quick and dirty

This preparation can be used on small insects. Storage of DNA made from this preparation is not recommended.

1. Whole small insect (or part of a larger insect, such as a leg) is ground with a pipette tip in 100 µL of "quick and dirty" extraction buffer (1X PCR Buffer) in a 1.5 mL tube.

2. The ground insect in solution is placed in a boiling water bath for 15 min.
3. The tube is centrifuged at 14,000 rpm to get rid of debris.
4. The supernatant containing crude DNA preparation is ready for PCR and can be transferred to a new tube.

5. The crude DNA solution is stored at -20°C.
Different concentrations of the crude preparation need to be tested for PCR amplification.

Protocol 16 Centricon 30 concentration and purification (Millipore)

(Time: 2 hours)

Useful for tiny or very rare specimens, hut is more expensive than ethanol precipitation. Follow general directions as provided with concentrators. In general, the Centricon step is used as a substitute for ethanol precipitation.

1. The samples that are purified using this approach can be prepped to this stage by most of the protocols mentioned in this chapter.

2 Add samples to filter tubes and cap
3. Spin for 10 min at 5,000 rpm in standard fixed-angle centrifuge.
4. Add sterile water to the fill line on the Centricon tube.
5. Spin for 30 min (Repeat steps 3 & 4, twice).

6. Invert the tubes and spin again for 3 min. The sample will be collected at the bottom of the lid.
7. Transfer the samples into labeled tubes and add 100 µL of water.
8. Use 1-2 µL of your sample for PCR. Keep a working DNA sample in the refrigerator (i.e., 30 µL aliquot) to avoid freeze/thaw cycles and place the remainder of DNA sample at -20°C for future use.
9. For both precipitation protocols, an assay gel for DNA extractions is not performed since the amount of DNA is small and usually not detectable (however, the amount isolated is usually sufficient for PCR).

Protocol 17 DNA isolation from plants and algae

(Time: 6-8 hours)

Obtaining chemically pure DNA from plant and macroalgal material is usually a difficult and tedious process. If pure cultures can be obtained, they are the most ideal material used for nucleic acid purification. If field-collected specimens are the only sources available, then they must be cleaned and all epiphytic material (animal, plant or fungi) must be removed. Optimal materials are the tissues of actively growing parts, or those producing gametes or spores. A good example is the reproductive structure in red algae, the cystocarp, which produces large amounts of nuclei and organelles relative to other parts of the organism, and is usually devoid of epiphytic material. Most material obtained in this manner yields small amounts of DNA, but the nucleic acid material is pure.

1. After cleaning, the plant/macroalgae is cut into smaller pieces, so that the tissue can be easily frozen with liquid N_2 and then ground to a fine powder with a mortar and pestle.
2. The material must be kept cold at all times during this process, particularly if the separated nucleic acids will be used for genomic analysis (library construction, restriction mapping, etc.).
3. Combine the ground tissue in an insulated container and keep covered with liquid nitrogen until all the material has been pulverized. This can be done by placing the powder in a plastic beaker, covering to prevent contamination, and placing it in a -80°C freezer until all material is crushed.
4. After all the material has been ground to a fine powder, it can be added to a number of various standard lysis solutions.

5. Add the powder slowly to a prewarmed (50°C) SDS/Sarkosyl lysis buffer, so that it does not freeze the buffer, keeping it in an incubated water bath. It is best to start with approximately 50-100 mL of buffer, and gradually add tissue and more buffer when necessary. If the solution is still thick once it reaches 50°C, add more buffer, but try to keep the total volume at a minimum to reduce the amount of reagents (and therefore cost).

6. Continue to incubate this solution at 50°C for 1.5-2 hours. If the extraction solution is thick, or there are large amounts of tissue in the slurry, you can filter the solution through cheesecloth. It is necessary to remove all the liquid from the cheesecloth (which contains DNA) so that the yield is high. Alternatively, the solution can be centrifuged (5,000-10,000 g) for 10 min at 4°C. Both of these steps can be skipped if there is not much undigested material in the solution.
7. Once the digestion is complete, the filtered (or unfiltered) solution can be put into 35 mL Oakridge or Sepcor centrifuge tubes (these must be phenol- and chloroform-resistant, and are tolerant of high-speed centrifugation).
8. Fill the tubes half full, and to this add an equal amount of room temperature PCI.
9. Mix the solution well by inverting the tubes several times (no vortexing) and centrifuge at 10,000 g for 20 min at room-temperature.
10. Transfer the aqueous phase with a wide-bore pipet (a 10 mL plastic pipet with the end cut off is perfect), making sure that the interface is not transferred to the new tube.
11. Fill the new tube only half full, and add an equal amount of chloroform:isoamyl alcohol (24:1) to the sample.
12. Repeat mixing and centrifugation. Transfer the aqueous layer to a large glass beaker.

13. Slowly add 0.2-0.3 volumes of 95-100% ice-cold ethanol while constantly swirling. A large quantity of polysaccharide may precipitate. The level of precipitate may vary among species, but generally marine species produce large amounts of polysaccharides. After this step, the solution should be slightly more viscous.
14. Transfer the supernatant to new Oakridge/Sepcor tubes and add an equal volume of PCI at room temperature.
15. Invert the mixture several times for 10-15 min.
16. Centrifuge for 15 min at 10,000 g.
17. Remove the aqueous layer with a wide-bore pipet (reduces shearing of the DNA). Pool the fractions from all the tubes and place in a beaker on ice. Keep the solution cold from this step till the end. Add this solution to pre-chilled Oakridge/Sepcor tubes, only filling half full.
18. Add an equal volume of chloroform:isoamyl alcohol (24:1).
19. Invert tubes to mix for 10 min.
20. Centrifuge at 10,000 g for 10 min.
21. Remove the aqueous layer, place in a chilled beaker on ice, and measure the total volume.
22. Add exactly 1/10th the volume of 5 M NaC1.
23. Mix gently and add exactly two volumes of ice-cold 95% ethanol and mix by pouring between two beakers. The DNA will precipitate out into long, stringy threads.

24. Spool the DNA using a bent glass rod and transfer the DNA to a small beaker containing ice-cold 70% ethanol. The DNA will still contain a considerable amount of carbohydrates which have co-precipitated with the DNA.
25. Cover and place at -20°C overnight.
26. Following the 70% ethanol wash, take the DNA out of the alcohol and place in a 50 mL falcon tube.
27. Drain off most of the ethanol completely. Add TE buffer (pH 8.0), until the DNA is completely dissolved.
28. Depending on how large the pellet is, this may take 1-2 days. Make sure that the solution is kept cold (in the refrigerator or on ice).

Protocol 18 Plant DNA isolation from herbarium and fresh collected specimens.

Modified from Struwe et al. [24].

1. Place 0.5 to 1.0 cm square piece of plant tissue in a tube and rapidly macerate tissue. The most efficient way to do this is to use a FastPrep machine.
2. Mix 500 µL of Carlson lysis buffer and 75 µL of beta-mercaptoethanol into each tube. Samples can be left at 4°Cfor extended periods of time (1 week) in this solution.
3. Incubate extraction at 74°C for 20 min to 1 hour.

4. Remove samples to room temperature and let cool.
5. Add 575 µL of PCI to each tube and mix gently.
6. Spin tubes at 14,000 for 2 min to separate organic and aqueous phases.

7. While tubes are spinning prepare GENECLEAN® (Bio 101) glass milk by vortexing to get glass beads into solution.
8. Remove 300 µL of upper aqueous layer from step 6 to a new eppendorf tube.
9. Add 900 µL of sodium iodide (NaI; concentration not specified in the Kit) from the GENECLEAN® kit and 20 µL of glass milk per tube.
10. Mix gently for 10 min at room temperature.
11. Spin tubes at 14,000 rpm for 30 sec to pellet glass milk.
12. Pour off supernatant and add 900 µL of New™ Wash concentrate.
13. Resuspend glass milk pellet by gently breaking the pellet with a pipette tip and shaking the tube.
14. Spin tubes at 14,000 rpm for 30 sec to pellet glass milk.
15. Repeat steps 12-14 twice more.
16. Use a pipette to remove the last 10 or 20 µL of New™ Wash concentrate without disrupting glass milk pellet.
17. Resuspend glass milk pellet in water or TE buffer and place at 50°C for 10 min to elute DNA away from glass milk beads.
18. Spin tubes at 14,000 rpm for 2 min and transfer supernatant with DNA to a new tube.
19. Store DNA in freezer.

Protocol 19 Bacterial genomic DNA preparation

(Time 2 hours)

This protocol is probably generally applicable to a wide variety of bacterial species.

1. Spin down 5 ml of saturated cell culture in eppendorf tube.
2. Resuspend pellet in 500 µL of ice cold Solution 1 ([5]; used for small-scale preparations of plasmid DNA by lysis by alkali) containing lysozyme (final concentration 2mg/mL) Incubate on ice for 10 min.

3. Add 50 µL of 10% SDS buffer Incubate at 37°C for 5-10 min until clear and viscous.
4. Put into a new tube (or a phase lock gel; phase lock gel is not necessary but makes life a lot easier).
5. Add 550 µL of phenol (freshly equilibrated with an equal volume of 0.3 M NaOAc).
6. Mix gently by inversion and centrifuge at 4°C for 15 min.
7. Transfer top layer to a new tube (or phase lock gel) and repeat step 5.
8. Transfer top layer to eppendorf and add one-tenth volume of 3 M NaOAc.
9. Spin for 3 min and transfer supernatant.

10. Add 2 volumes of 100% ethanol and mix by inverting.
11. Cool sample at -80°C for 5 min.
12. Centrifuge for 15 min at 4°C.
13. Remove and discard the supernatant.
14. Vacuum dry the pellet and resuspend in 50-100 µL of water.

This is a modified version of a protocol from Current Protocols in Molecular Biology [25].

Protocol 20 Isolation of DNA from prokaryotes: CTAB

(Time 2-3 hours)

The isolation of prokaryotic nucleic acid is much less work-intensive than those described for plants and macroalgae. Most Bacteria and Archaea have cell walls that can be easily broken through and lysed for the isolation of DNA and RNA. Ideally the material used should be grown from pure culture. Depending on the type of organism used for genetic material, either agar or liquid cultures can yield similar results, as long as there is no contamination in the process. Extra care must be taken when using nutrient rich media, since most airborne bacteria can grow in this as well.

1. For liquid cultures, spin down 1-3 mL of culture and remove the media.
2. Add 567 µL of TE buffer to the pelleted cells. If agar media are used, choose one colony from the petri dish (using a sterile toothpick) and place the toothpick with the colony into a sterile eppendorf tube containing the 567 L of TE buffer.
3. Resuspend the pellet by repeated pipetting, or by gently vortexing the toothpick with the solution so that the cells become resuspended.
4. Add 30 µL of 10% SDS and 3 µL of a 20 mg/mL solution of proteinase K. Mix and incubate for 1 hour at 37°C.

5. After incubation, add 100 µL of 5 M NaC1 and mix.
6. Afterwards add 80 µL of a CTAB/NaC1 solution (0.7 M NaC1, 10% CTAB).
7 Incubate this solution at 65°C for 10 min
8. After incubation, add an equal volume of chloroform: isoamyl alcohol (24:1) and mix.
9. Centrifuge 5 min, and transfer the aqueous solution to a new tube. Be careful not to transfer the interface. Add another equal volume of PCI and mix well.
10. Centrifuge at 14,000 rpm for 5 min and transfer supernatant to a new tube.
11. Repeat first extraction again (chloroform: isoamyl alcohol alone).

12. Add 0.6 volumes of isopropanol and mix gently until the DNA precipitates.
13. Centrifuge and remove isopropanol. Add 1 mL of 70% ethanol to wash the salt away from the DNA.
14. Centrifuge, and discard the ethanol, drying on the benchtop at room temperature.
15. Resuspend the pellet in 50-100 µL of TE buffer and keep at 4°C.

This DNA can be used for restriction digests, Southern and Northern blot analysis, genomic library construction, and PCR.

Protocol 21 Isolation of DNA from prokaryotes: Chelex®

Isolation Time: 1 hour

Another quick method to isolate DNA from prokaryotes for PCR amplification via the Chelex® method.

1. Bacterial cultures are pelleted and placed in 200 µL of Chelex® 100 buffer (Chelex 100 buffer-0.001 M Tris-HC1, pH 8.0; 0.05 mM EDTA; 5% (w/v) Chelex® 100 resin).
2. Grind the cells with the Chelex(r) 100 buffer (the resin helps this process) with a sterile teflon eppendorf grinder (Kontes). Resuspending the cells helps break open the cells during this process.

3. Heat extract the DNA by incubating the tubes at 80°C for 25 min or longer, and then boiling for 10 min.
4. Another heat extraction method is to autoclave the cells for 10 min on slow exhaust, making sure a small hole is punctured through the top of the eppendorf tube to allow it to equilibrate in the autoclave.

5. Once the heat extraction is complete, centrifuge the tubes and avoid cell debris when pipetting.
6. Use from 1-5 µL of extract for PCR. Extracts can be stored at -20°C for several months.

This method can also be used for isolating template DNA from dried botanical museum specimens, particularly the spores from any reproductive organ that is still intact [26].

Protocol 22 Isolation of total RNA from tissues and cultured cells

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The following protocol was modified from the guanidinium thiocyanate/phenol DNA purification method described by Chomzynski & Sacchi [27] and Chomzynski [28]. Guanidinium iso thiocyanate is a very powerful protein denaturant, which inactivates RNases during the extraction procedure. This protocol allows the isolation of total RNA within 4 hours and provides both high yield and purity of undegraded RNA preparations. It is especially recommended for full-length cDNA synthesis and RT-PCR reactions. This method also permits recovery of total RNA from small quantities of tissues or cultured cells.

From Tissues

1. Freeze the tissue in liquid nitrogen or on dry ice immediately after dissection. It is possible to store tissue at -80°C for several months.
2. Cut the frozen tissue into small pieces (less than 0.5 cm cubes) and transfer in an appropriate tube according to the amount of tissue (do not use polycarbonate tubes with guanidine iso thiocyanate). Add 1 mL of GITC solution (4 M guanidinium iso thiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% sarkosyl, 0.1 M 2-mercaptoethanol, stored in light-proof vessel) per 100 mg tissue.
3. Homogenize small amounts of tissue by passing pieces through a syringe fitted with a 18-21 gauge needle. Break up the tissue by grinding it against the side of the tube with the needle. If the tissue is difficult to homogenize in this manner, or if larger amounts will be processed, a Dounce or power homogenizer may be used. It is also possible to grind up the frozen tissue with a mortar and pestle with liquid nitrogen.

From Cultured Cells

1. Wash the cells in ice cold phosphate buffered saline (PBS) solution and transfer to a sterile microcentrifuge tube.

2. Pellet the cells by centrifugation (10 min at 1,000 rpm) and add the appropriate amount of GITC solution (1 mL per 10^7 cells). Pass the suspension through a plastic syringe fitted with an 18-21 gauge needle at least 3 times.
3. Proceed with the RNA isolation.

4. Add the following solutions to the homogenate and mix thoroughly by inverting the tube after the addition of each reagent (all quantities and volumes in this protocol are calculated for 100 mg tissue or 10^7 cells): 0.1 mL of 2 M sodium acetate (pH 4.0), 1 mL of acid phenol (pH 5.0) and 0.2 mL of chloroform.
5. Shake the suspension vigorously for 30 sec.
6. Incubate on ice for 15 min.
7. Centrifuge the samples at 10,000 rpm for 20 min at 4°C.
8. Transfer the upper aqueous phase (containing the RNA) in a fresh tube. Be careful not to disturb the interphase containing proteins and DNA.

9. Add 1 mL 100% isopropanol to the aqueous phase, mix and precipitate the RNA by incubating the solution at -20°C for 1 hour.
10. Centrifuge at 14,000 rpm for 20 min at 4°C.
11. Resuspend the RNA pellet in 0.5 mL GITC solution and transfer to a fresh 1.5 mL microcentrifuge tube.
12. Precipitate RNA by adding 0.5 mL 100% isopropanol and incubating at - 20°C for 1 hour.
13. Centrifuge for 10 min at 4°C (14,000 rpm), wash the RNA pellet twice with 70% ethanol and air dry the precipitant.
14. Resuspend RNA in 50 μL RNase-free water or TE buffer. If mRNA will be isolated, resuspend in 5 mL STE/0.5 M NaCl/Proteinase K (200 μg/mL) and proceed with the poly(A)+ selection procedure (see below). The RNA can be stored at -80°C for several months. For long-term storage, RNA is best kept in formamide [29].

Note: Since guanidinium iso thiocyanate does not irreversibly inactivate RNases, it is important to carefully separate the RNA-containing aqueous phase from the interphase and lower organic phase. If residual RNase contaminates the sample after deproteinization, these enzymes can become active again following the removal of the denaturant.

Protocol 23 Isolation of poly(A)+ RNA: selection of polyadenylated RNA from total RNA by Oligo-deoxythymidine (DT) Cellulose Chromatography

Poly(A)+ RNA can be isolated from total RNA preparations using oligo(dT) cellulose selection or directly from tissue and cell culture samples. The purification of mRNA from total RNA is recommended for tissue sources and cell lines rich in RNases in order to minimize possible RNA degradation during the extraction process. Total RNA is first isolated from tissue or cultured cells and mRNA is isolated by poly(A)+ selection using oligo(dT) cellulose (Boehringer or equivalent)

1. Prepare oligo(dT) cellulose (Boehringer or equivalent) in the following way:
2. Wash 1 g of oligo(dT) cellulose in 50 mL of 0.2 M sodium hydroxide in sterile water for 30 min at room temperature on a rotating wheel. Neutralize by washing twice in 50 mL of 500 mM Tris-HCI (pH 7.4), followed by six changes of sterile water. Wash in 50 mL of RNA preparation binding buffer to equilibrate the salt concentration, recover by centrifugation and then resuspend in 50 mL of RNA preparation binding buffer.
3. Resuspend total RNA from Protocol 22 in 5 mL STE/0.5 M NaCl/Proteinase K 200 μg/mL and add 0.25 mL of oligo(dT) cellulose. Allow the RNA to bind, incubating for at least 1 hour on a rotating wheel at room temperature.
4. Spin down oligo(dT) cellulose in lysate at 2,000 rpm for 1 min. Remove the supernatant and resuspend in 15 mL binding buffer. Repeat this step twice in order to remove unbound (mainly ribosomal) RNA.
5. Transfer oligo(dT) cellulose to alkali-treated econo-column (BioRad®). Alkali treatment of columns will be done by washing the columns with 0.2 M NaOH, then several volumes of binding buffer.
6. Wash oligo(dT) cellulose with 10 mL RNA preparation binding buffer followed by 2 mL RNA preparation washing buffer.
7. Elute RNA with 1 mL RNA preparation elution buffer. Ethanol precipitate RNA by adding 100 μL 5 M NaC1 and 2.5 mL 100% ethanol. Freeze at -20°C.
8. Spin down RNA, wash twice with 70% ethanol, air dry the pellet and resuspend in an appropriate volume of RNase-free water or TE buffer. Use an aliquot for OD 260/280 measurement. Store at -80°C.
9. Regenerate the oligo(dT) cellulose in the following way:
10. Wash in several volumes of RNA preparation elution buffer, re-treat with 0.2 M NaOH, spin down and resuspend in 500 mM Tris-HC1 (pH 7.4). Wash for 15 min, spin, check the pH of the supernatant and resuspend again in 500 mM Tris-HC1 (pH 7.4) if it is still alkaline. Resuspend the neutralized oligo(dT) cellulose in sterile water, wash to remove the salt, spin, and resuspend in absolute ethanol. Store at -20°C protected from light.

Protocol 24 Kits and commercial reagents for the isolation of RNA

Several Kits are available to purify mRNA from total RNA and/or directly from cell and tissue samples. Some examples are listed in the following.

- The PolyATtract® mRNA Isolation Systems from Promega can be used to isolate mRNA from total RNA samples as well as directly from tissue or cultured cell samples (PolyATtract® System 1000). These systems utilize streptavidin paramagnetic particles which eliminates the need for oligo(dT) cellulose columns. Biotinylated oligo(dT) probe is annealed in solution to the poly(A)+ RNA in a total RNA sample. The hybrids are captured with the MagneSphere® Streptavidin Paramagnetic Particles in a Magnetic Separation Stand and the non-hybridized HNA is washed away. The mRNA can be isolated in about 45 min. The PolyATtract® Series 9600TM mRNA Isolation System provides reagents for the processing of mRNA from numerous small samples for RT-PCR or other applications in a 96 well plate format. It is available with or without reagents for performing first-strand cDNA synthesis.
- The MagNA Pure LC mRNA Isolation Kit from Boehringer is also based on magnetic bead technology. It is especially designed to isolate mRNA from blood, blood cells and cultured cells. Purified mRNA is suitable for RT-PCR reactions and other typical mRNA downstream applications such as Northern blotting, Northern ELISA, ribonuclease protection assay, and preparation of cDNA libraries.
- Stratagene's Poly(A) Quik® mRNA Isolation Kit is based on oligo(dT) cellulose chromatography and can be used to isolate mRNA from total RNA samples. Each column is designed to accommodate up to 500 μg of total RNA in volumes ranging from 200 to 1000 μL. It allows the isolation of poly(A)+ RNA from total RNA in about 15 min.
- The FastTrack™ Kit (Invitrogen) can be used to isolate mRNA directly from $lx10^7$ to $3x10^8$ cells, 0.4 gram of tissue, or 0.2-1 mg of total RNA in about 90 min. For smaller amounts of cells or tissues the Micro Kit is available (samples ranging in size from $1x10^2$ to $5x10^6$ cells, 10-200 mg of tissue, or 100 μg of total RNA). The procedure requires no ultracentrifugation or guanidinium lysis and is based on oligo(dT) cellulose binding. Obtained mRNA can be used for cDNA library construction, substracted probe generation, primer extension studies, Northern blot analysis, RNase protection assays oocyte microinjection and *in vitro* translation.

Commercial reagents for the isolation of total RNA similar to the protocol described in this section are available from a number of different vendors. Two examples are listed in the following:

- TRIzol (Gibco/BRL) is an acid phenol extraction reagent containing detergents and guanidinium iso thiocyanate. The preparation procedure to isolate total RNA using TRIzol is pretty much similar to the GITC protocol described in this section (Protocol 22). The RNACIean™ systems from Hybaid are also based on the guanidinium thiocyanate and phenol extraction method and can be used for the isolation of total RNA from tissues and cells (RNAC1ean™) as well as from liquid samples (RNAC1ean™ LS). For the removal of polysaccharides from total RNA, which might be necessary in some cases for an exact quantification of the RNA (polysaccharides may absorb UV light at 260 nm), the "RNAC1ean™ Extension" system can be used.

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