APPENDIX 2

Basic Procedures

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Appendix 2 details commonly used molecular biology methods that are frequently referred to in this volume. These methods are intended to aid investigators unfamiliar with these basic procedures. Many variations and modifications of these procedures exist (e.g., see Sambrook et al. [1989], *Molecular cloning: A laboratory manual*), but the generic procedures provided here are applicable to most situations.

QUANTITATION OF CELL CONCENTRATION

Measuring the OD_{600} of a Culture

OD_{600} readings can be used to estimate the number of bacteria or yeast in a culture and to monitor growth. The relationship of light scattering of a culture to the actual cell number is dependent on the growth conditions (e.g., medium, temperature, strain) of the culture. However, approximations are generally adequate.

BACTERIAL CULTURES

For bacteria grown in LB (or equivalent rich) medium:

OD_{600} of $1 \cong 5 \times 10^8$ (to 1×10^9) cells/ml

Precise correlations can be determined for each strain and growth condition by plating dilutions of a culture on solid medium and counting colonies.

To determine the bacterial cell concentration, set the spectrophotometer to a wavelength of 600 nm and adjust the spectrophotometer to zero with a sample of fresh medium (the same medium that was used for growing the culture) in a cuvette with a 1-cm path length. The OD_{600} of an undiluted actively growing *E. coli* culture ranges from 0.1 to approximately 1 during log growth phase. Typically, a saturated overnight bacterial culture grown in rich medium (e.g., LB medium) has an OD_{600} of approximately 5. Cells grown in TB medium or Super Broth reach different densities.

YEAST CULTURES

For yeast grown in rich medium:

OD_{600} of $1 \cong 1 \times 10^7$ cells/ml

This conversion factor for yeast cell concentration varies with haploid versus diploid cells and among different genetic backgrounds. The precise conversion factor should be determined empirically by comparing the actual cell count obtained with a hemocytometer and the corresponding OD_{600} measurement for any previously uncharacterized strain.

To determine the yeast cell concentration, follow the procedure above for bacterial cells but use the conversion factor for yeast cell concentration.

A saturated diploid yeast culture grown in YPD medium contains approximately 10^8 cells/ml and has an OD_{600} of approximately 10–15. Saturated cultures grown in minimal medium reach an OD_{600} of approximately 3–5. A medium-sized yeast colony contains approximately 10^6 cells. A "matchhead full" of yeast contains 1×10^7 to 5×10^7 cells.

Counting the Cells in a Hemocytometer

A hemocytometer consists of two chambers, each of which is divided into nine 1-mm squares. A glass coverslip is supported 0.1 mm above the squares, providing a 1 mm x 1 mm x 0.1 mm (10^{-4} cm^3 or 10^{-4} ml) volume per square. The cell concentration per milliliter is the average count per square x 10^4. This method is used to determine accurately cell concentrations for yeast and mammalian cells. Cell counts for viable mammalian cells can be determined by exclusion of the dye trypan blue.

1. Clean the hemocytometer under running H_2O. Rinse with 70% ethanol. Wipe dry with a Kimwipe.

2. Dilute the cells in medium at a concentration of approximately 20–50 cells per 1-mm square on the hemocytometer and suspend them uniformly (i.e., there should be no clumps of cells).

   Note: Viable mammalian tissue-culture cells can be quantitated by using trypan blue, a dye excluded from viable cells. If viable cells must be counted, add 20 µl of 0.4% trypan blue solution to 20 µl of cell suspension and mix thoroughly.

   0.4% Trypan blue solution

   Dissolve 40 mg in sufficient PBS (pH 7.0) to make a final volume of 10 ml. (This stock solution can also be purchased.) This solution can be stored at 4°C for at least 6 months.

3. Place a clean hemocytometer coverslip on the hemocytometer. Load each chamber of the hemocytometer with cell suspension, allowing capillary action to draw the solution into the chamber.

4. Using 100x magnification on a microscope, count the cells in the four large corner squares and the central large square for each chamber (see Figure 1). Count the cells touching the top and left central lines but not those touching the bottom and right central lines.

   Notes: If more than 200 cells are counted per square, the cell suspension is too concentrated and must be diluted for more reliable cell counting. Reliable cell counts are typically 20–50 cells per square.

   If desired, keep a separate count of nonviable and viable mammalian cells stained with trypan blue. Nonviable cells stain blue, but viable cells appear clear with a distinct, sharp outline.

5. Calculate the cell concentration as follows:

   number of cells/ml = average cell count per square x 10^4 x dilution factor

   Note: The dilution factor in this calculation should include the factor of two if cells were stained with trypan blue.

6. Calculate the total number of cells in the original suspension as follows:

   total number of cells = number of cells/ml x total ml of cell suspension

7. If trypan blue exclusion was used to determine cell viability, calculate the cell viability as follows:

$$\text{cell viability} = \frac{\text{total number of viable cells}}{\text{total number of viable + nonviable cells}}$$

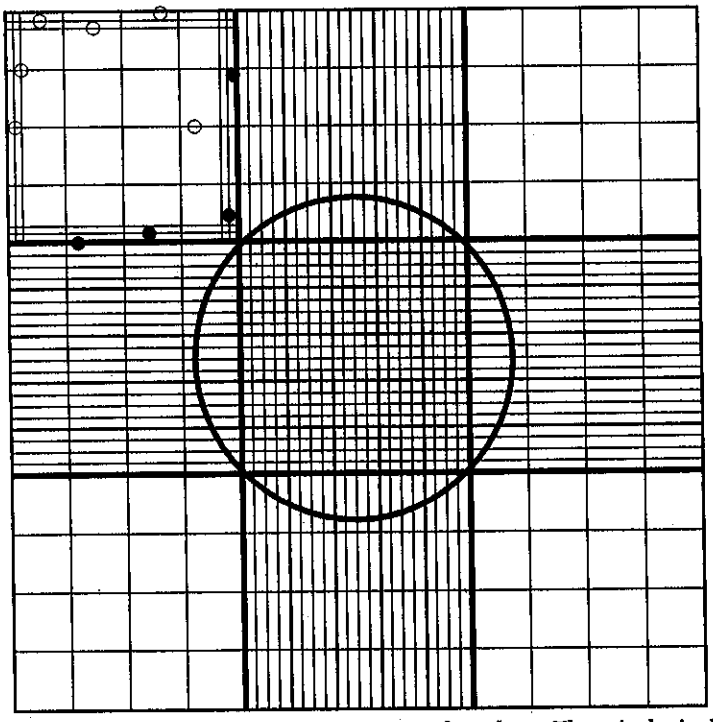

Figure 1 Standard hemocytometer chamber. The circle indicates the approximate area covered at 100x magnification (10x ocular and 10x objective). Count the cells touching the top and left central lines (*open circles*) but not those touching the bottom and right central lines (*closed circles*). Count the cells in the four large corner squares and the central large square for each chamber (one chamber is represented here). The corner square equals 1 mm.

STANDARD METHODS USED FOR ISOLATING DNA

This section describes several standard methods used during the purification of DNA. These protocols represent individual steps in the overall process of isolating high-quality DNA.

Extraction of DNA Samples with Organic Chemicals

Extractions with organic chemicals (phenol, chloroform, ether) are frequently used during the purification of DNA. Extraction of aqueous DNA solutions with phenol removes contaminating materials, particularly proteins (including degradative enzymes). Phenol must be equilibrated to a pH above 7.6; otherwise, the DNA tends to partition into the organic phase instead of the aqueous phase. DNA samples are generally extracted once or twice with phenol alone, phenol:chloroform (1:1), or phenol:chloroform:isoamyl alcohol (25:24:1). Typically, samples are then extracted once or twice with chloroform (or in some cases ether) to remove residual phenol, which may interfere with future manipulations of the DNA. Chloroform also denatures proteins. Isoamyl alcohol is often added to the chloroform to reduce foaming. The following precautions should be observed during the use of hazardous organic chemicals:

- Perform all extractions with organic chemicals in tubes constructed of material that is resistant to phenol and chloroform (e.g., glass and polypropylene). Polycarbonate tubes are not resistant to these organic reagents and should never be used.
- Perform all extractions with organic reagents in a chemical fume hood to avoid inhalation. Wear gloves, protective clothing, and safety glasses to avoid contact with the skin.
- Cap all tubes before mixing or centrifuging.
- Immediately rinse any areas of skin that come in contact with phenol with a large volume of H_2O and wash with soap and H_2O. Phenol causes severe burns to the skin and has an anesthetic effect, reducing the perceived severity of the injury. Seek immediate medical attention.
- Use extreme caution in handling flammable organic chemicals, particularly ether.
- Consult the local institutional safety officer regarding the proper disposal of organic chemical waste.

PREPARING EQUILIBRATED PHENOL

Phenol readily undergoes oxidation and must therefore be redistilled before use. Since redistilled phenol is acidic, a neutralization step with Tris base and then an equilibration step with TE (pH 8.0) or a similar buffer must be performed. Phenol that has been redistilled and preequilibrated is commercially available from many suppliers.

In the protocol below for equilibrating redistilled phenol, the antioxidant 8-hydroxyquinoline and *m*-cresol (optional) are added to extend the storage life of the phenol stock solution. The addition of 8-hydroxyquinoline also provides a yellow color, which aids in detecting the phenol phase during extractions.

1. In a chemical fume hood, thaw a 500-g bottle of **phenol** at room temperature or 37°C. Place the bottle of phenol in a clean beaker filled with clean distilled H_2O so that the phenol will leak into the H_2O if the phenol bottle breaks.

   phenol (see Appendix for Caution)

2. (*Optional*) Use a glass pipette or a glass graduated cylinder to add 70 ml of ***m*-cresol** to the thawed phenol. The total volume should now be approximately 0.8 liter. Stir gently with a magnetic stirring bar.

   ***m*-cresol** (see Appendix for Caution)

3. Add 0.8 g of **8-hydroxyquinoline** to make a final concentration of 0.1% (w/v).

   Note: This powder turns the phenol yellow.

   8-hydroxyquinoline (see Appendix for Caution)

4. Add approximately 50 ml of H_2O and stir to mix.

5. Stop stirring and allow the phases to separate.

6. Check the pH of the aqueous (upper) phase with a pH stick.

   Note: The aqueous phase should be very acidic.

7. Remove the aqueous phase by aspiration with a glass pipette.

8. Neutralize the phenol with Tris base as follows:

   a. Add approximately 0.8 liter of 1 M Tris base and stir to mix.

   b. Stop stirring and allow the phases to separate.

   c. Check the pH of the aqueous (upper) phase with a pH stick. If the pH is less than 5.0, remove the aqueous phase by aspiration and repeat steps a–c.

   d. Once the pH is above 5.0, completely remove the aqueous phase by aspiration.

9. Equilibrate the phenol with TE (pH 8.0) or a similar buffer by repeating step 8 at least three times using TE (pH 8.0) instead of Tris base.

10. Divide the phenol into aliquots and overlay the phenol with TE (pH 8.0). Store in dark bottles at 4°C for up to 1 month or in polypropylene tubes at –20°C for up to 6 months.

EXTRACTING WITH PHENOL, PHENOL:CHLOROFORM, OR PHENOL:CHLOROFORM:ISOAMYL ALCOHOL

Phenol alone can be used for extractions. A mixture of phenol and chloroform, with or without isoamyl alcohol can also be used in the protocol below. In all extractions, the phenol must first be equilibrated to a pH above 7.6 so that the DNA partitions into the aqueous phase.

1. Add 1 volume of **phenol** saturated with TE (pH 8.0) or a similar buffer (for preparation, see pp. 617–618) to the DNA sample. Mix gently by vortexing for 2–3 minutes or by inverting the capped tube for up to 5 minutes to form an emulsion.

   Notes: Either phenol:**chloroform** (1:1) or phenol:chloroform:isoamyl alcohol (25:24:1) can replace the phenol.

   Large volumes (2–20 ml) can be conveniently extracted in 15- or 50-ml polypropylene tubes. Small volumes (<0.5 ml) can be extracted in 1.5-ml microcentrifuge tubes. Volumes of 0.5–2 ml should be divided into aliquots in microcentrifuge tubes.

   For maximal recovery, the DNA should be dissolved in at least 100 µl of TE (pH 8.0).

   To recover RNA, use **DEPC**-treated H_2O to dissolve the RNA and to equilibrate the phenol.

   phenol, chloroform, DEPC (see Appendix for Caution)

2. For large volumes, centrifuge at 5000*g* at room temperature for 5 minutes to separate the organic and aqueous DNA-containing phases. For small volumes, centrifuge in a microcentrifuge at 12,000*g* for 5 minutes.

   Notes: Complete separation of the phases should be apparent. If not, repeat the centrifugation for a longer period of time and/or at a higher speed.

   Shorter periods of centrifugation may be sufficient for small volumes.

   The lower layer should be the organic phase and the upper layer should be the aqueous phase. The phases are often separated by a white or yellowish pellicle of precipitated protein and cellular debris.

3. Carefully remove the aqueous layer with a pipette and transfer it into a new tube for further extractions or precipitation of the DNA. Avoid touching the precipitated protein layer at the interface. Discard the organic phase.

   Notes: If the interface is noticeably cloudy (an indication of the presence of excess protein), a second extraction is recommended.

   Many investigators perform a series of extractions before precipitating the DNA with alcohol. Extraction with phenol:chloroform (1:1), phenol:chloroform:isoamyl alcohol (25:24:1), and then chloroform:isoamyl alcohol (24:1) is common for large amounts of DNA. Other investigators extract with phenol, phenol:chloroform (1:1), or phenol:chloroform: isoamyl alcohol (25:24:1) and then remove the residual phenol by extraction with chloroform (see p. 620).

   In some specialized protocols elsewhere in this manual, diethyl ether is recommended as an alternative to chloroform for removing phenol. Ether should be saturated with H_2O to prevent loss of volume from the aqueous phase. When extractions are performed with diethyl ether, the aqueous DNA-containing phase is the lower phase because of the lower density of ether. Since the DNA is in the lower phase, multiple extractions can be performed in the same tube. Ether in the upper phase causes an inverted meniscus at the interface, readily revealing the continued presence of ether. After removal of as much ether as possible with pipetting aids, residual traces of ether can be evaporated. The DNA can then be recovered by precipitation with alcohol.

EXTRACTING WITH CHLOROFORM TO REMOVE PHENOL

After extraction with phenol, chloroform is often used to remove residual phenol from the DNA. The DNA will remain in the aqueous (upper) phase. In general, one to two extractions with chloroform are performed before the DNA is recovered by precipitation with alcohol.

1. Add 1 volume of **chloroform** to the DNA sample and mix gently by vortexing for 1–2 minutes or by inverting the capped tube to form an emulsion.

   Notes: Chloroform:isoamyl alcohol (24:1) can replace the chloroform.

   Large volumes (2–20 ml) can be conveniently extracted in 15- or 50-ml polypropylene tubes. Small volumes (<0.5 ml) can be extracted in 1.5-ml microcentrifuge tubes. Volumes of 0.5–2 ml should be divided into aliquots in microcentrifuge tubes.

   chloroform (See Appendix for Caution)

2. For large volumes, centrifuge at 5000*g* at room temperature for 5 minutes to separate the organic and aqueous DNA-containing phases. For small volumes, centrifuge in a microcentrifuge at 12,000*g* for 5 minutes.

   Notes: The lower layer should be the organic phase and the upper layer should be the aqueous phase.

   Complete separation of the phases should be apparent. If not, repeat the centrifugation for a longer period of time and/or at a higher speed.

3. Carefully remove the aqueous layer with a pipette and transfer it into a new tube. Discard the organic phase.

Concentration of DNA Samples by Precipitation with Alcohols

CHOOSING THE ALCOHOL

DNA is readily recovered from aqueous solution by precipitation with alcohol in the presence of monovalent cations (e.g., sodium or ammonium). Both ethanol and isopropanol are widely used for this purpose, with the choice between them depending mainly on the volume of aqueous phase (2–2.5 volumes of ethanol is required, whereas only 1 volume of isopropanol is required). Precipitations with ethanol are usually effective at removing unwanted salt in DNA preparations.

CHOOSING THE SALT

If the existing monovalent cation concentration is low, either sodium acetate or ammonium acetate is added to the DNA before precipitation. Occasionally, NaCl is substituted. The choice between sodium or ammonium is determined by the subsequent use of the DNA. Although most of the salts should be removed by the end of the procedure, small amounts of a specific residual salt can cause problems. For example, DNA ligase can be inhibited by sodium ions, whereas CIP and bacteriophage T4 polynucleotide kinase can be inhibited by ammonium ions. Neither ion inhibits most other enzymes.

Washing precipitated DNA with 70% ethanol removes most of the salt. In situations where it is critical to remove all of the salt, the use of ammonium acetate as the source of salt in the precipitation should be considered. Residual ammonium acetate can be removed by drying the DNA pellet under vacuum. This procedure is often used with low-molecular-weight DNA but is not practical or desirable for large genomic DNA (see pp. 628–629) since the drying step would desiccate the genomic DNA, requiring a lengthy resuspension/dissolution period.

High concentrations of EDTA (10 mM) or phosphate (1 mM) in the DNA solution should be avoided since they may coprecipitate with the DNA. Dilution of the DNA solution or removal of these salts by use of spin columns must be performed before precipitation.

MAXIMIZING THE DNA YIELD

Temperature was once thought to be a key factor in efficiently precipitating DNA. Incubations at 4°C or in dry-ice/ethanol baths are not necessary but are still commonly used. The critical factor in the recovery of small amounts of DNA is the length of the centrifugation step. Longer periods of centrifugation will aid in the recovery of particularly small amounts of DNA.

If the DNA concentration is low, addition of *E. coli* tRNA can help in recovering the DNA. The tRNA should be extracted with phenol and boiled to remove contaminating DNases. tRNA specifically prepared for precipitating DNA is also commercially available.

COLLECTING THE DNA

For large-volume precipitations (>2 ml) in which the DNA concentration is high, DNA strands will form a visible precipitate, which collects into a compact

mass of material that can easily be removed by "spooling." Spooling large genomic DNA separates the DNA from the bulk of the RNA, which has been copurified but remains in solution (this eliminates the need to add exogeneous RNase, which may be contaminated with nucleases), and washing with 70% ethanol helps remove the majority of the salts. If the DNA is very dilute, the precipitated material may not be visible. In this case, the DNA must be recovered by centrifugation.

1. Add either 0.1 volume of 3 M sodium acetate (pH 5.2) or 0.3 volume of 10 M ammonium acetate to the DNA sample. Mix thoroughly by vortexing or by inverting the tube.

   Notes: The final concentration of sodium acetate should be approximately 300 mM.

   In most cases, the desired final concentration of ammonium acetate is 2–2.5 M. The stock solutions of ammonium acetate are usually at a pH of 7.0 or 7.4.

   For aqueous volumes of up to 0.5 ml, 1 μg of tRNA can be added before precipitation with alcohol. A carrier should only be added when its presence will not interfere with future uses of the DNA. For example, DNA ligation using bacteriophage T4 DNA ligase can be performed in the presence of tRNA carrier.

2. Add 2–2.5 volumes of absolute ethanol or 1 volume of isopropanol. Mix thoroughly by vortexing or by inverting the tube. Incubate for at least 15 minutes.

   Notes: Incubations can be performed at room temperature. Low-temperature incubations (on ice or at –20°C or colder) are not necessary but are still commonly used.

   The critical factor in the recovery of small amounts of DNA is the centrifugation step below, but use of prolonged precipitation periods may help maximize the DNA yield.

   If the concentration is high, a stringy white precipitate will appear almost immediately.

3. Collect the DNA precipitate by spooling if the DNA concentration is high and the size of the DNA is reasonably large; if the DNA is small or very dilute, collect the DNA by centrifuging.

   To collect visible DNA by spooling:

   a. Spool the stringy precipitated DNA on a pasteur pipette (i.e., slowly wind the DNA around the tip of a pasteur pipette).

   b. Wash the spooled DNA by repeatedly dipping it into a separate tube containing 70% ethanol. Do not dry the DNA.

   c. Place the DNA spooled on the pipette tip in a new tube containing a suitable volume of TE (pH 8.0) or another appropriate solution. Allow it to sit until the DNA is released from the pipette tip. Once the DNA has been released, mix gently by low-speed vortexing or by flicking the tube with a finger to help the DNA dissolve.

      Note: The DNA should dissolve quickly, but allowing it to sit at 4°C overnight is sometimes necessary.

   To collect DNA by centrifuging:

   a. For large volumes, centrifuge in a 15- or 30-ml Corex tube in a Sorvall SS34 or HB6 rotor (or equivalent) at 12,000*g* at 4°C for 30 minutes. For small volumes, centrifuge in a 1.5-ml microcentrifuge

tube in a microcentrifuge at 12,000*g* for 15 minutes. Decant the supernatant and invert the tube on a paper towel to drain.

Note: In general, centrifugation for 15 minutes is adequate to collect most DNA samples. Longer periods of centrifugation (e.g., 30 minutes) will aid in the collection of particularly small amounts of DNA.

b. To wash the DNA pellet, add 70% ethanol (5 ml for 15- or 30-ml tubes, 1 ml for microcentrifuge tubes) and gently rotate the tube, recentrifuge for 5 minutes, and decant the supernatant.

Note: This step should rinse the walls of the tube but keep the DNA pellet intact.

c. Dry the DNA pellet under vacuum for 5–10 minutes in a SpeedVac Concentrator or allow the DNA pellet to air dry.

Note: Small volumes of low-molecular-weight DNA are often dried under vacuum. Drying under vacuum is not practical or desirable for large genomic DNA since it would desiccate the genomic DNA and necessitate a lengthy resuspension/dissolution period (see p. 628).

d. Dissolve the DNA in TE (pH 8.0) or another appropriate solution.

4. Determine the DNA concentration (see pp. 624–627) and adjust to the appropriate concentration for storage or immediate use.

Quantitation of DNA

The accurate measurement of DNA concentration is essential for many applications. Several methods are in common usage for measuring DNA concentration, three of which are provided here. These methods are based largely on spectrophotometric measurement of UV absorbance or binding of fluorescent dyes.

UV ABSORBANCE

An advantage of the spectrophotometric method for DNA quantitation is that the amount of protein contamination in the sample can also be determined by measuring the OD_{280}. The disadvantage of this method is that it is sensitive to contaminating RNA, which can lead to an overestimation of the DNA concentration.

1. Set the spectrophotometer to a wavelength of 260 nm (in the UV spectrum). For a DNA sample dissolved in TE (pH 8.0), adjust the spectrophotometer to zero with TE (pH 8.0) in a quartz cuvette with a 1-cm path length.

2. Dilute the sample and measure the OD_{260}.

3. If the DNA solution is too dilute (i.e., the OD_{260} is <0.05), repeat the measurement with a more concentrated DNA sample.

   Note: Most spectrophotometers are accurate at an OD_{260} ranging from 0.05 to approximately 0.8.

4. Set the spectrophotometer to a wavelength of 280 nm and readjust to zero. Measure the OD_{280} of the sample.

   Note: Pure DNA will have a ratio of OD_{260}/OD_{280} of approximately 1.8. A ratio that is very different from 1.8 (i.e., <1.5 or >2) may be indicative of either residual protein or organic solvents in the DNA sample. In this case, extract the DNA sample with **phenol:chloroform** again and then precipitate with alcohol again.

   phenol, chloroform (see Appendix for Caution)

5. Calculate the DNA concentration as follows:

$$\text{double-stranded DNA concentration in } \mu g/ml = \text{measured } OD_{260} \times \frac{50\ \mu g/ml}{1\ OD_{260}} \times \text{dilution factor}$$

$$\text{single-stranded DNA concentration in } \mu g/ml = \text{measured } OD_{260} \times \frac{36\ \mu g/ml}{1\ OD_{260}} \times \text{dilution factor}$$

   Note: An OD_{260} of 1 corresponds to 50 µg/ml of double-stranded DNA or 36 µg/ml of single-stranded DNA.

BINDING OF ETHIDIUM BROMIDE

Ethidium bromide binds to double-stranded DNA by intercalation. It absorbs UV light at 260 nm and emits fluorescence at 590 nm. The amount of fluorescence is proportional to the amount of DNA.

DNA concentrations of dilute solutions or very small sample volumes that cannot be subjected to spectrophotometric quantitation can be estimated by binding of ethidium bromide. Samples are analyzed by agarose gel electrophoresis and compared with DNA samples of known concentration. This method can detect as little as 1–5 ng of DNA.

This method has the advantage that it is insensitive to contamination with RNA, which runs ahead of the DNA on the gel. To avoid misinterpretation of the DNA concentration due to binding of RNA to the dye, be sure to stain with ethidium bromide after running the gel instead of including the dye in the gel and electrophoresis running buffer.

1. Dilute a DNA standard with TE (pH 8.0) to make DNA concentrations of 2, 1, 0.5, 0.25, and 0.125 µg/ml.

   Notes: Be sure to prepare dilutions of the DNA standard covering a broad range of DNA concentrations so that they encompass the DNA samples of unknown concentration.

   For genomic DNA, uncut bacteriophage λ DNA is a good standard since it migrates in a conventional agarose gel at a size similar to that of genomic DNA.

2. Mix 10 µl of each diluted standard and the DNA sample of unknown concentration with 2 µl of a 6x gel-loading solution. Analyze on an agarose gel.

   Note: Choose gel conditions such that DNA samples migrate at limiting mobility; 0.7–1% agarose gels generally suffice. The gel can be run at high voltage (≥100 mA) for 30 minutes; the samples just need to enter the gel.

3. Place the gel in 1x electrophoresis buffer containing **ethidium bromide** at a final concentration of 0.2–0.5 µg/ml and stain for 1 hour to detect the bands.

   Note: Staining for 10–15 minutes is frequently sufficient to detect bands.

   ethidium bromide (see Appendix for Caution)

4. Place the gel in 1x electrophoresis buffer and destain for 1 hour.

5. Photograph the gel using a **UV** transilluminator.

   UV irradiation (see Appendix for Caution)

6. Estimate the concentration of the DNA sample by locating the diluted standard with the fluorescence intensity that most closely matches that of the sample.

7. Repeat the analysis with a broader range of DNA standard concentrations if the dilutions of the DNA standard do not encompass the sample of unknown concentration.

FLUORIMETRY

The fluorochrome Hoechst 33258 binds to DNA. DNA quantitation by fluorimetry takes advantage of the specific excitation of the DNA-bound fluorochrome with UV light at 365 nm and the subsequent emission at 458 nm. This protocol was developed for use with the Hoefer minifluorimeter model TKO 100 but other fluorimeters can be adapted for this purpose. DNA at concentrations greater than 10 ng/ml can be quantitated even in the presence of contaminating RNA and protein.

1. Turn on the fluorimeter 15 minutes before use and set the sensitivity dial to maximum.

2. Add 2 ml of 1x Hoechst 33258 solution to the cuvette supplied with the fluorimeter. Place the cuvette in the fluorimeter and adjust to zero.

1x Hoechst 33258 solution

Component and final concentration	Amount to add per 100 ml
1x TEN buffer	10 ml of 10x
H_2O	90 ml
0.1 µg/ml Hoechst 33258	10 µl of 1 mg/ml (in H_2O)

Prepare just before use. (The 1 mg/ml stock solution of Hoechst 33258 dye can be stored in a foil-wrapped container at 4°C for up to 6 months.)

10x TEN buffer

Component and final concentration	Amount to add per 100 ml
100 mM Tris-Cl	10 ml of 1 M (pH 7.5 at 25°C)
10 mM EDTA	2 ml of 0.5 M (pH 8.0)
1 M NaCl	20 ml of 5 M
H_2O	68 ml

Store at room temperature for up to 6 months.

3. Replace the contents of the cuvette with 2 ml of either salmon sperm DNA or bacteriophage λ DNA at a concentration of 0.5 mg/ml in 1x Hoechst 33258 solution. Shake the cuvette well.

   Note: The size of the standard DNA does not affect the amount of Hoechst dye that binds.

4. Place the cuvette in the fluorimeter and set the fluorimeter sensitivity to 500.

5. Replace the contents of the cuvette with 2 ml of 1x Hoechst 33258 solution.

6. Place the cuvette in the fluorimeter and set the fluorimeter to zero.

7. Replace the contents of the cuvette with 2 ml of the DNA sample diluted in 1x Hoechst 33258 solution. Shake the cuvette well.

8. Place the cuvette in the fluorimeter and take a reading within 2 seconds.

   Note: The reading must be taken immediately because the signal decays with time.

9. Calculate the DNA concentration by adjusting the reading (which corresponds to the DNA concentration in μg/ml) for the dilution in step 7.

Dialysis of DNA

Dialysis is a common procedure performed to remove unwanted salts in DNA samples. Standard dialysis in tubing can be used for large volumes, whereas spot dialysis (drop dialysis) can be used for very small volumes. Precipitations with ethanol can also be used to remove unwanted salt effectively (see pp. 621–623), but resuspension/dissolution of HMW genomic DNA (>50 kb) precipitated with ethanol can take a long time (hours to days).

STANDARD DIALYSIS OF DNA

Removal of salt from genomic DNA can best be achieved by standard dialysis. This gentle procedure minimizes shearing, thus maintaining the large size of HMW DNA.

1. Prepare sterile dialysis tubing as follows:
   a. Cut dry dialysis tubing into convenient lengths (10–20 cm).

      Note: Always wear gloves when handling dialysis tubing.

   b. Place the tubing in a 2-liter glass beaker containing 1 liter of 100 mM sodium bicarbonate/1 mM EDTA and boil on a hot plate for 15 minutes. Using a sterile blunt rod or a 10–25-ml glass pipette, occasionally submerge the tubing as it bubbles up in the boiling solution.

      Note: Do not overload the beaker.

      100 mM Sodium bicarbonate/1 mM EDTA

Component and final concentration	Amount to add per 1 liter
100 mM sodium bicarbonate	8.4 g
1 mM EDTA	2 ml of 0.5 M (pH 8.0)
H_2O	to make 1 liter

      Prepare just before use.

   c. Allow the solution to cool completely, and then thoroughly rinse the inside of each piece of tubing with sterile H_2O.
   d. Place the tubing in 1 liter of 1 mM EDTA (pH 8.0) and boil for 15 minutes.
   e. Store the dialysis tubing in 1 mM EDTA (pH 8.0) at 4°C for up to 1 year.

2. Just before use, wash the inside and the outside of the dialysis tubing with sterile H_2O.

3. Pour (or use a wide-bore pipette tip [at least ~2-mm internal diameter] to gently pipette) each genomic DNA sample into a separate piece of sterile dialysis tubing.

   Note: It is best to pour the DNA into a dialysis bag held above a clean, sterile glass beaker so that the DNA sample is not lost if it does not go into the bag.

4. Dialyze against 1 liter of TE (pH 8.0) at 4°C for 12 hours with three changes of buffer.

   Note: If the DNA sample contains residual proteinase K from the purification procedure, **PMSF** can be added to inhibit any trace amounts of proteinase K remaining from the initial lysis of cells. Dialyze against 1 liter of TE (pH 8.0) containing 100 μM PMSF (prepared just before use) at 4°C for 12 hours with two changes of buffer. Residual proteinase K would cause degradation of enzymes used in subsequent analysis of the DNA.

   PMSF (see Appendix for Caution)

SPOT DIALYSIS

Salts or other small molecules (e.g., from ligations of vector to insert DNA) can be removed by spot dialyzing (drop dialyzing) the samples.

1. Place 20–30 ml of TE (pH 8.0) in a 10-cm petri dish. Place the petri dish where it will be undisturbed by contact or vibration.

2. Using blunt forceps, gently place a filter (Millipore VSWP, 0.025-mm pore size, 47-mm diameter) on top of the TE with the shiny side facing up. Allow the filter to wet for approximately 1 minute.

   Note: If more than one sample will be dialyzed simultaneously, mark the filters with pencil or waterproof pen before applying the samples. Either a single sample of 20–400 μl or as many as four 50-μl samples can be dialyzed on a single filter.

3. Slowly pipette the sample onto the surface of the filter. Cover the dish and dialyze at room temperature for 20 minutes to 2 hours.

   Note: Brief dialysis (<1 hour) may increase the sample volume if the applied sample contains a high concentration of salt or sucrose. Prolonged dialysis (>4 hours) results in loss of sample volume.

4. Transfer the dialyzed sample into a 0.5-ml microcentrifuge tube and place on ice.

   Notes: Do not try to transfer the entire sample, since this is likely to submerge the filter and result in the loss of all of the remaining samples.

   The expected recovery is approximately 80–90% of the sample volume.

ASSESSING THE EXTENT OF RADIOLABELING IN DNA PROBES BY PRECIPITATION WITH TCA

Random priming (Feinberg and Vogelstein [1983] *Anal. Biochem. 132:* 6–13) is the most commonly used procedure to prepare radiolabeled DNA probes. Random hexameric oligonucleotides are used to prime the incorporation of radiolabeled dNTPs into the probe DNA. The high efficiency of incorporation of [α-^{32}P]dNTPs by the Klenow fragment of *E. coli* DNA polymerase I results in the synthesis of probes with specific activities of more than 10^9 cpm per microgram of DNA. Typically, 90% of the isotopically labeled dNTPs will be incorporated. The efficiency of incorporation of [^{32}P]dNTPs can be determined by the separation of the labeled DNA from the unincorporated dNTPs.

Unless extremely clean backgrounds are required, no special procedures to remove unincorporated dNTPs are needed. The probe sample can be added directly to hybridization mixtures. In cases where high sensitivity and low backgrounds are important, the unincorporated [^{32}P]dNTPs can be removed by the use of column chromatography or precipitations with spermine.

The protocol below for precipitation with TCA is a simple method for quantitating the efficiency of radiolabeling. To determine the percentage of [^{32}P]dNTPs incorporated into newly synthesized probe DNA, an aliquot of the reaction mixture is treated with TCA and the resulting precipitated DNA is captured on Whatman GF/C glass-fiber filters. The unincorporated dNTPs are washed away. The amount of radioactivity incorporated into the precipitated DNA can be determined by Cerenkov counting. The ratio of the cpm captured by precipitation with TCA to the total cpm in an equivalent aliquot can be used to calculate the efficiency of radiolabeling.

1. For each radiolabeling reaction, label two Whatman GF/C glass-fiber filters (2.4-cm diameter) with a blunt pencil so that they can be identified later. Pin the filters onto a Styrofoam block covered with aluminum foil.

2. Spot 1 µl of **radiolabeling reaction mixture** on each of the two filters. Allow the filters to dry completely at room temperature or under a heat lamp.

   radioactive substances (see Appendix for Caution)

3. Transfer one filter into a beaker containing 100 ml of ice-cold 10% TCA/100 mM sodium pyrophosphate. Wash on a platform shaker (or with swirling) for 3 minutes. Decant the washing solution. Repeat the wash two more times using fresh washing solution each time. Do not wash the second filter.

   Notes: The unincorporated dNTPs are eluted from the filter; the ^{32}P-labeled DNA is retained.
   If available, use a vacuum manifold to wash the filter.

10% TCA/100 mM sodium pyrophosphate

Component and final concentration	Amount to add per 1 liter
10% **TCA**	100 ml of 100% (w/v)
100 mM sodium pyrophosphate decahydrate	44.6 g
H_2O	to make 1 liter

Dissolve the sodium pyrophosphate in sufficient H_2O to make a final volume of 0.9 liter. Add the TCA and mix. Store at 4°C for up to 6 months.

TCA (see Appendix for Caution)

4. Place the filters in a beaker containing 90% ethanol and swirl a few times. Remove the filters and dry completely.

5. Place the washed filter and the unwashed filter in separate scintillation vials.

6. Measure the radioactivity in each vial for 1 minute using the ^3H channel on the scintillation counter.

Notes: The efficiency of detection of Cerenkov radiation is a function of the instrument and the geometry of the vials. Completely dry filters should be detected at approximately 25% efficiency.

The addition of a **toluene**-based scintillation fluid to the vials will give 100% efficiency of detection for ^{32}P. However, as long as both filters are being measured in the same manner, the calculation of the percentage incorporated will be identical, regardless of the counting method used.

toluene (See Appendix for Caution)

7. Calculate the percentage of [^{32}P]dNTPs incorporated as follows:

$$\text{percentage incorporated} = \frac{\text{cpm on washed filter}}{\text{cpm on unwashed filter}} \times 100$$

8. Calculate the specific activity of the synthetic DNA probe as follows:

$$\text{cpm/}\mu\text{g of probe} = \frac{\text{cpm on washed filter} \times \mu\text{l of total labeling reaction}}{\mu\text{g of input DNA}}$$

Note: The specific activity of the probe is calculated by using the amount of input DNA. The high specific activity of the radiolabeled dNTPs results in an insignificant amount of total DNA synthesized relative to the input DNA.

DILUTION AND STORAGE OF OLIGONUCLEOTIDES

Newly synthesized oligonucleotides are best stored as lyophilized pellets in microcentrifuge tubes at −20°C. Simple procedures for suspending, quantitating, and storing oligonucleotides are provided here.

1. Suspend the oligonucleotide in H_2O to make a final concentration of 20 μM. The table below indicates the volume of H_2O to add to 1 OD unit of dried oligonucleotide of various lengths to obtain a 20 μM solution. (When measured in a quartz cuvette with a 1-cm path length, an OD_{260} of 1 corresponds to 33 μg/ml [33 ng/μl] of single-stranded oligonucleotide [unmodified].)

Length (nucleotides)	Concentration in ng/μl for a 20 μM solution	Volume of H_2O to add (μl)[a]
15	100	330
17	112	295
20	132	250
25	165	200
32	211	156
40	264	125

[a]The volumes required can be calculated as in the following example: For a 20-mer, a 20 μM solution contains 132 μg/ml. Since an OD_{260} of 1 corresponds to 33 μg in 1 ml,

$$\frac{33\ \mu g\ \text{in}\ 1\ OD_{260}}{132\ \mu g/ml} = 250\ \mu l$$

To prepare a 20 μM stock solution, suspend 1 OD unit of a 20-mer in 250 μl of H_2O.

2. To confirm the concentration of the resulting oligonucleotide stock solution, measure the OD_{260} of a 30-fold dilution in H_2O in a cuvette with a 1-cm path length and then perform the following calculation:

$$\text{concentration in } \mu g/ml = \text{measured } OD_{260} \times \frac{33\ \mu g/ml}{1\ OD_{260}} \times \text{dilution factor}$$

Note: For example, if the OD_{260} measurement is 0.133 for a 30-fold dilution of a 20-mer, the calculation is:

$$0.133\ OD_{260} \times \frac{33\ \mu g/ml}{1\ OD_{260}} \times 30 = 131.7\ \mu g/ml$$

This confirms that the concentration of the 20-mer oligonucleotide stock solution is 131.7 μg/ml or approximately 20 μM.

3. Store oligonucleotide stock solutions and dilutions prepared as working solutions at −20°C. These solutions are stable for at least 1–2 years.

STORAGE AND SHIPMENT OF BIOLOGICAL SAMPLES

This section provides commonly used methods for storing and shipping various types of biological samples: bacterial stocks, yeast stocks, mammalian tissue-culture cells, blood or tissue samples, and DNA. Compliance with local, state, and federal regulations for the shipment of biohazardous materials is the responsibility of the investigator. Consult the local institution for further guidelines.

Bacterial Stocks

Most bacterial stocks are frozen in 7% DMSO or 15% glycerol at –80°C for long-term storage. Viability of frozen cells depends on the specific strain and the health of the cells at the time of freezing. Cultures to be stored are typically started from a single colony and grown in a suitable medium with agitation overnight (~10–15 hours).

DMSO STOCKS

Transfer 1 ml of an overnight culture into a labeled 1.5-ml screwcap cryotube and add 80 µl of DMSO. (Use DMSO from a bottle specifically dedicated for bacterial stock preparation. Never pipette directly from the stock bottle; aseptically remove an aliquot from the bottle and use the aliquot of DMSO to prepare the cultures.) Cap the tube and mix gently. Store at –80°C. Long-term viability of stocks depends on the particular strain, but some bacterial stocks have been known to maintain good viability for up to 10 years after initial storage in DMSO.

GLYCEROL STOCKS

Transfer 0.5 ml of an overnight culture into a labeled 1.5-ml screwcap cryotube and add 0.5 ml of sterile 30% glycerol. Cap the tube and mix gently. Store at –80°C. Long-term viability of stocks depends on the particular strain.

Alternatively, grow bacteria in medium containing 8–10% glycerol in plastic multiwell plates and store at –80°C. This method is typically used for storing cosmid, bacteriophage P1, BAC, and cDNA libraries.

RETRIEVAL OF FROZEN BACTERIAL STOCKS

Never thaw frozen bacterial stocks in DMSO or glycerol. Use a sterile loop, sterile wooden stick, or sterile disposable pipette to scratch the surface of the stock. Streak appropriate agar plates (e.g., LB agar plates) for single colonies. Recap the frozen stock and return it to storage at –80°C. Incubate the plate overnight at 37°C. The colonies on a plate can be used for up to 1 week to inoculate cultures. Plates should be stored upside down at 4°C during this time.

SHIPMENT OF BACTERIAL STRAINS BY MAIL

In general, most bacterial strains can be shipped by several different methods that maintain good viability. Agar stab cultures have traditionally been used to store and ship bacterial strains. Parafilm-sealed petri dishes streaked for single colonies or sterile filter disks impregnated with bacterial culture can also be shipped. The latter are aseptically transferred to appropriate medium upon receipt and a fresh overnight culture is grown. Overnight cultures can be shipped in cryotubes at room temperature. Upon receipt, the culture is streaked on plates of appropriate selective medium and single colonies are isolated. Frozen DMSO and glycerol stocks can be shipped on dry ice.

In general, agar stab cultures of nonplasmid-containing strains can be stored at room temperature for many years. They are not appropriate for long-term storage of plasmid-bearing strains because of loss of the plasmid under nonselective conditions. However, plasmid-bearing strains can be conveniently shipped in agar stab cultures. Immediately upon receipt, the cells must be streaked on plates of selective medium and either DMSO or glycerol stocks should be made.

Agar stab cultures are prepared in 3-ml glass vials with rubber gaskets in the screwcaps (e.g., Wheaton) as follows: Place 2 ml of liquified LB top agar (0.7% agar; some investigators use 1–1.2% agar) in each vial. Autoclave the vials with the caps loosened. Allow to cool to room temperature, tighten the caps, and store at room temperature until needed. Use a sterile loop or sterile wooden stick to pick an isolated single colony and stab it through the center of the agar to the bottom of the vial. Tighten the cap.

Bacterial strains should only be shipped at room temperature in moderate weather conditions. Hot summer weather may prove to be lethal during shipment.

Yeast Stocks

Yeast stocks are typically frozen in 20% glycerol at –80°C for long-term storage. Viability of frozen yeast cells depends on the specific strain and the health of the cells at the time of freezing. Cultures to be stored are typically started from "patched out" clones and grown in a suitable medium with agitation overnight.

GLYCEROL STOCKS

Patch out clones on an appropriate plate. Incubate at 30°C for 2 days. Inoculate 6 ml of YPD medium with a "matchhead full" of cells from the plate (this is a large inoculum; the culture will be turbid before incubation). Incubate at 30°C with agitation overnight. Add 2 ml of sterile 80% glycerol and mix thoroughly. Transfer 0.5-ml aliquots into 1-ml freezer vials with O-ring seals. Thoroughly shake the freezer vials and freeze at –60°C or lower (typically –80°C). Yeast tend to die if frozen at temperatures above –55°C.

Yeast strains can be stored at –80°C indefinitely by using this method. Note that strains grown in YPD medium before freezing have better long-term viability than those grown in selective medium.

RETRIEVAL OF FROZEN YEAST STOCKS

Never thaw frozen yeast stocks. Use a sterile loop, sterile wooden stick, or sterile disposable pipette to scratch the surface of the stock. Streak appropriate agar plates (e.g., YPD or selective agar plates) for single colonies. Recap the frozen stock and return it to storage at –80°C. Incubate the plate at 30°C for 2 days. Yeast can be stored at 4°C for approximately 6 months on YPD agar plates or for approximately 2 months on selective plates (i.e., SC plates with added supplements). Plates should be stored upside down at 4°C during this time. For long-term storage, seal the plates or place them in bags to keep them from drying out. Supplementing YPD medium with adenine prevents the toxicity caused by the red pigment produced by *ade2* strains that are stored at 4°C.

SHIPMENT OF YEAST STRAINS BY MAIL

Patch out clones on an appropriate selective plate. Incubate at 30°C for 2 days. Inoculate 6 ml of YPD medium with a "matchhead full" of cells from the plate (this is a large inoculum; the culture will be turbid before incubation). Incubate the culture at 30°C with agitation overnight. Add 2 ml of sterile 80% glycerol and mix thoroughly. Transfer 0.5-ml aliquots into 1-ml freezer vials with O-ring seals. Ship the vials by regular first class mail. Upon receipt, each cell suspension should be streaked on a suitable agar plate and incubated at 30°C for 2–3 days. Alternatively, ship yeast on YPD plates or in tubes or vials (with loosened caps) containing solid YPD medium (YPD slants).

If *un*saturated cultures are prepared (e.g., by inoculating fresh YPD medium with cells and tightly capping the freezer vial) and sent in the mail, the tubes may explode during transit because of the buildup of pressure from actively fermenting yeast in a tightly sealed vessel.

Yeast strains should only be shipped at room temperature in moderate weather conditions. Hot summer weather may prove to be lethal during shipment.

Mammalian Tissue-culture Cells

Typically, mammalian tissue-culture cells are grown in one of a number of media (e.g., RPMI 1640) supplemented with 5–15% FBS/FCS and 1% L-glutamine. Cultures are grown at 37°C in a 5% CO_2 environment. The volume of medium in the flask can affect the growth of cells, since the surface-to-air ratio is important in maintaining the proper pH of the medium. Factors that can affect the growth characteristics of a cell line include a change in incubation temperature, a difference in the lot of FBS/FCS and/or medium, depletion of L-glutamine in the medium, contamination with Mycoplasma, and length of time in continuous culture. To control these factors, medium is warmed to 37°C before it is added to cells, new lots of FBS/FCS and medium are tested for at least 2 weeks with a control cell line before they are accepted for general use, and fresh glutamine is added to the medium as required.

Each cell line has specific growth requirements. The final cell concentration (typically 5×10^6 to 5×10^7 cells/ml) during frozen storage may affect viability. Follow specific handling and storage medium recommendations for each cell line.

DMSO STOCKS

Centrifuge freshly grown, healthy cells at 500*g* at 4°C for 10 minutes. Discard the medium and resuspend the cell pellet in FBS/FCS containing 8–10% DMSO at 4°C. Transfer 0.5–1-ml aliquots of cell suspension into cryotubes and freeze in a −80°C freezer. Samples can be stored at −80°C or transferred into a **liquid nitrogen** storage tank. Cells should be viable at −80°C for up to 1 year or at −185°C in liquid nitrogen for up to 10 years.

Since each aliquot of frozen mammalian cells should be thawed just before use, at least two separate batches of ten aliquots each should be prepared for each cell line. As a precaution in case of freezer failure, store the aliquots of each cell line in at least two different freezers or liquid nitrogen storage tanks.

liquid nitrogen (see Appendix for Caution)

RETRIEVAL OF FROZEN MAMMALIAN TISSUE-CULTURE CELLS

The DMSO must first be removed from the frozen cells and the cells revived. To do this, quickly thaw the frozen aliquot of tissue-culture cells by placing it in a water bath set at 37°C with the top of the vessel above the H_2O line. Clean the outside of the vessel with 70% ethanol. Use a sterile pipette to transfer the cells into a 15-ml centrifuge tube containing appropriate medium (typically, 10 ml of medium containing 10% FBS/FCS). Gently centrifuge the cell suspension at 200*g* at room temperature for 5 minutes. Discard the medium and resuspend the cell pellet in 10 ml of appropriate medium containing FBS/FCS (typically 10%). Transfer the cells into an appropriate-sized flask and incubate at 37°C in a 5% CO_2 environment. Each cell line will recover at a different rate.

SHIPMENT OF MAMMALIAN TISSUE-CULTURE CELLS

Mammalian tissue-culture cell lines can be shipped as frozen stocks or as growing cultures. Frozen stocks should be shipped on dry ice in a Styrofoam container for next-day delivery. To ship growing cultures, inoculate the medium with a small aliquot of cells in a tissue-culture flask so that the cells are approximately a quarter to half confluent on the next day. At the time of shipping, the cells should be in log growth phase but should not be too dense (dense monolayers tend to peel off during transit). Before shipping, fill the flask to the neck with culture medium, cap tightly, and cover the cap with Parafilm M to prevent leaking. Wrap the flask in paper towels or place in a sealable plastic bag and cushion with cotton balls (this prevents the flask from breaking and also absorbs any liquid in case of a leak). Cell lines that grow in suspension can be shipped in centrifuge tubes or flasks filled to the neck and sealed.

Seasonal factors (extreme hot or cold) must be considered in shipping mammalian tissue-culture cells. If extremely warm weather conditions are anticipated, ship live cultures in a Styrofoam container. If extremely cold weather is anticipated, do not ship live cultures. For international shipping, minimize delays at customs by properly stating the value and contents of the package (it is advisable to check with customs officials in advance for additional shipping information). Upon receipt, allow the cells to recover by incubating live cultures at 37°C overnight before unsealing. Additional information about the

shipping of tissue-culture cell lines can be obtained from the Coriell Institute for Medical Research, 401 Haddon Avenue, Camden, New Jersey 08103 (E-mail ccr@arginine.umdnj.edu; phone 609-757-4847; Fax 609-757-9737).

Blood and Tissue Samples

Blood to be used for DNA isolation should be collected in EDTA (purple-top Vacutainers). Blood containing EDTA can be stored at 4°C for 2 months. If the white blood cells are to be immortalized, collect the blood in heparin (green-top Vacutainers) to prevent clotting and store at room temperature for a maximum of 4 days. Blood samples can be shipped on wet ice.

Mouse and **human tissue samples** to be used for DNA preparation should be frozen in **liquid nitrogen** and stored at −80°C for up to 6 months. Do not thaw samples slowly since this allows nucleases to degrade the DNA; immediately place them in a denaturing cell lysis solution at the time of DNA isolation. Frozen tissue samples can be shipped on dry ice.

human blood, blood products, and tissues; liquid nitrogen (see Appendix for Caution)

DNA

Impure DNA containing traces of chemicals used during isolation often does not store well. Contamination with heavy metals, free radicals as chemical breakdown products, and oxidation products of phenol degradation can cause breakage of phosphodiester bonds. UV irradiation causes the production of thymine dimers and cross-links, resulting in loss of biological activity. Ethidium bromide causes photooxidation with visible light and molecular oxygen. Nucleases found on human skin do not generally pose a major problem for DNA. (RNases are very stable, but most DNases are not; however, the use of gloves is always recommended.)

STORAGE OF DNA

As a general rule, the more highly purified the DNA, the longer it can be stored under any conditions. DNA in solution is typically stored in TE (pH 8.0). DNA for long-term storage should contain a high salt concentration (at least 1 M NaCl or other salt) and 10 mM EDTA (to chelate heavy metals). Always dissolve DNA pellets in low-ionic-strength solutions (e.g., TE) and then add more salt if desired. Dried DNA pellets can be stored at −20°C for up to 6 months and DNA precipitated with ethanol can be stored at −20°C indefinitely.

Storage at 4°C is the best condition for routine storage of highly purified DNA. For storage at −20°C, it is generally preferable to use a nonfrost-free freezer. (Single- and double-stranded breaks may occur when the DNA is subjected to frequent freeze/thaw cycles.) For long-term storage, −80°C is recommended.

SHIPMENT OF DNA

Highly purified DNA can be shipped as an aqueous solution at room temperature or 4°C (i.e., on ice) or frozen on dry ice. It can also be precipitated with ethanol and then shipped at room temperature either as a dried DNA pellet or as precipitated DNA in ethanol. When the purity of the DNA is in doubt, do not ship DNA at room temperature as an aqueous solution. Trace contamination with nucleases may result in significant degradation of the DNA during shipment.