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MOLECULAR DIAGNOSTICS

For the Clinical Laboratorian

Second Edition

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3 Extraction of Nucleic Acids

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1. INTRODUCTION

The utility of DNA- or RNA-based testing depends, in large part, on the quality and nature of the diagnostic sample. A variety of methods is available to extract nucleic acids for analysis. The choice of technique should consider both the sample source and the nature of the eventual assay. Both DNA and RNA can provide valuable clinical information. Genotype analysis and infectious disease testing represent two of the primary clinical uses of DNA, but it can also be used for less obvious applications like the assessment of bone marrow transplant engraftment.

The ability to purify and test RNA provides additional and important clinical data. Purified mRNA, for example, can reveal gene expression patterns. In the last few years, two distinct types of B-cell lymphoma have been separated on the basis of their respective gene expression patterns *(1)*. Indeed, molecular classification of malignancy based on gene expression profiles is a promising and rapidly growing field. On a simpler level, the expression pattern of a single gene might be important. The *BCR/abl* fusion product seen in most cases of chronic myelogenous leukemia (CML) can be identified by reverse transcriptase–polymerase chain reaction (RT-PCR) performed on a purified RNA sample. RNA testing is also important in the detection and quantification (viral load) of retroviral infection. The utility of RNA is, unfortunately, counterbalanced by the inherently labile nature of RNA and seemingly ubiquitous presence of RNase. Nucleic acid degradation is always a concern, and RNA is especially vulnerable.

Each tissue source and extraction method presents its own potential quality assurance issues. Similarly, the needs of the eventual assay could vary. Whereas some techniques require high-molecular-weight nucleic acid (Southern blots, pulsed-field gel electrophoresis), others (including PCR-based protocols) often work well with smaller fragments. In addition to nucleic acid quality, purity and concentration are important factors to consider. Many testing techniques are sensitive to contaminating protein, lipopolysaccharide, or tissue preservative. More obvious is the need for adequate concentrations of nucleic acid. There are numerous methods for detecting and quantifying nucleic acid, with varying degrees of sensitivity and background noise. Testing based on DNA fluorescence, for example, might require higher nucleic acid concentrations than that based on radioimaging. These three parameters—quality, purity, and concentration—can be optimized with careful selection of sample source and technique.

2. SAMPLE SOURCE

There are, essentially, two types of tissue available for nucleic acid analysis: fresh and preserved. The ideal source of nucleic acid is, naturally, fresh tissue. If extraction is not possible immediately, it is critical to rapidly limit the damaging action of tissue endonucleases. Prompt flash-freezing of solid tissue with liquid nitrogen preserves nucleic acids and can facilitate subsequent tissue and cell disruption. Timely freezing is especially important to extract RNA successfully. For RNA purification, fresh tissue can also be placed directly in commercially available reagents that preserve cellular RNA for up to a week at room temperature (Ambion, Austin, TX). RNase and DNase are rapidly denatured in the presence of chaotropic agents like guanidium isothyocyanate (GITC). A minimum concentration of GITC of 5 mol/L is necessary for effective RNA preservation, and GITC-preserved tissue can also be stored at room temperature for almost a week without significant RNA loss *(2)*.

2.1. FRESH TISSUE The utility of fresh tissue is enhanced by the powerful sensitivity of PCR testing. Exfoliated cells obtained by swabbing or rinsing mucous membanes provide enough nucleic acid to allow PCR-based testing of genomic or foreign (infectious) DNA *(3,4)*. Buccal cells, for example, can be obtained noninvasively by swab and air-dried onto a glass slide. Although the cells are unfixed, the DNA is preserved well enough over short periods for transport, extraction, and PCR analysis *(3,5)*. As an alternative, buccal cells are collected with a saline mouthwash and pelleted for immediate analysis.

Cervical cells obtained by swab or brush can also be used for PCR or other DNA testing. This is especially useful in the detection of cervical human papilloma virus (HPV), subtypes of which are associated with increased risk of cervical neoplasia. A commercial system for the detection of HPV DNA has been developed that employs specific RNA probes and chemiluminescent antibody (Digene Corporation, Gaithersburg, MD).

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Table 1
The Effect of Tissue Fixatives on the Purification of Nucleic Acid

Fixative	*Active contents*	*Tissue effect*	*Nucleic acid purification*
Neutral-buffered formalin	Formaldehyde	Nucleic acid base hydroxymethylation Crosslinking of DNA and protein	Reduced high-molecular-weight nucleic acid with increased fixation time Suitable for most testing
B-5 fixative	Mercuric chloride Usually mixed with formaldehyde	Mercury–protein complexes reduce DNA extraction yields	Low molecular weight or no extractable nucleic acid Occasionally nucleic acid sufficient for PCR testing
Bouin's fixative	Picric acid Acetic acid Formaldehyde	Acidic DNA Depurination Formaldehyde effects	Low molecular weight or no extractable nucleic acid
Zenker's fixative	Potassium dichromate Mercuric chloride	Metal–protein complexes	Low molecular weight or no extractable nucleic acid
Alcohol	Ethanol or methanol		Good or excellent nucleic acid yields, including high molecular weight Fixation time has no effect

Cervical swabs collected with this kit are useful for up to 2 wk at room temperature, and longer with refrigeration. Fixed cytologic preparations, such as Papanicolaou-stained cervical smears, can also provide useful nucleic acid for PCR testing after many years of storage *(5)*.

Fresh DNA is also available from the hair root. Again, this tissue source combines the advantages of fresh DNA with easy transport and procurement. The robust nature of the sample and PCR assay allow hair root DNA to be used in testing after proteinase K treatment, but without formal nucleic acid extraction *(3)*. This source of DNA is especially useful in forensic testing, when little other tissue might be available.

2.2. FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUE

Although certainly preferable, fresh tissue is not always available for diagnostic molecular studies. For many clinical and research laboratories, the logistical limits of specimen collection make fixed solid tissue or blood more likely sources of nucleic acid.

The archival banks of formalin-fixed/paraffin-embedded tissue accumulated by most pathology departments provide a potentially vast source of tissue for both diagnostic and research analysis. Unfortunately, nucleic acid derived from fixed tissue can be less than ideal. There are many tissue fixatives currently employed by health care and research facilities. The most common is neutral-buffered formalin. Exposure of nucleic acid to formalin results in the formylation of free nucleotide amino groups, methylene bridging of bases, and crosslinking of nucleic acid with available protein. The net result is increased nucleic acid fragmentation *(6–9)*. With increased fixation time, the amount of available high-molecular-weight nucleic acid is markedly reduced. Tissue fixation in formalin for longer than 24 h will likely reduce the yield of high molecular-weight nucleic acid.

Even more problematic are fixatives containing mercuric chloride. Such fixatives include B-5 and Zenker's fixatives (both used in hematopathology). Many groups have documented limited success extracting nucleic acid from tissues fixed with these agents *(6,8–10)*. There is evidence that mercury complexes with available protein and speculation that these large complexes inhibit extraction techniques *(8–11)*. This phenomenon can be compounded with Zenker's fixative, which also contains the heavy metal chromium. Acid-containing fixatives (Bouin's fixative and Zenker's pH 2.0 fixative) could cause nucleic acid depurination. By contrast, alcohol-based fixatives allow the purification of high-quality nucleic acid. Alcohol fixation, however, is not routinely used in most applications and might not be appropriate for other clinical uses of tissue. A summary of fixative effect on nucleic extraction is presented in Table 1.

Because it is less important to have high-molecular-weight nucleic acid for most PCR applications, formalin-fixed tissue can be an excellent source of diagnostic material for these assays. Additionally, as with fresh hair or buccal samples, some PCR assays can be performed by direct amplification from formalin-fixed, paraffin-embedded tissue without prior DNA extraction *(12)*.

The problems presented by overfixation of tissue are complicated by the need for tissue without autolysis, necrosis, or inadequate fixation. With proper care and buffered formalin, most tissue specimens can be completely fixed in 24 h, and this amount of time allows for good yields of high-molecular-weight DNA *(10)*. RNA can also be extracted successfully from formalin-fixed tissue, although formalin might modify the bases enough to inhibit subsequent RT-PCR. Heating the purified sample prior to PCR might remove the offending monomethylol groups *(11)*.

2.3. BLOOD Many times, the best available DNA source is blood. Blood samples, however, present a unique problem in that the specimen is mixed with an agent to inhibit coagulation. Heparin, ethylenediaminetetraacetic acid (EDTA), and acid citrate dextrose (ACD) are all used to prevent in vitro blood clot formation. Generally, both EDTA and ACD specimen tubes provide good yields of nucleic acid appropriate for PCR and other assays. Greater than 70% of the original high-molecular-weight DNA (>25 kb) can be recovered from blood stored for 3 d in either of these preservatives, even when stored at room temperature *(13)*. Yields are even better when samples are refrigerated.

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Heparin, on the other hand, is a problem when mixed with samples intended for nucleic acid extraction. Heparin adsorbs to nucleic acid and is not completely removed by standard extraction techniques. Residual heparin in a DNA or RNA sample can inhibit restriction digests, PCR, and other enzyme-based molecular biology assays. The inhibition of PCR depends, to some extent, on the relative concentrations of template and heparin. Heparin concentrations as low as 0.05 U per reaction volume might prevent amplification *(2,14)*. The sensitivity of various commercial polymerases does appear to vary, however, with some functioning normally at higher heparin levels *(14)*.

Ideally, blood samples will be obtained in either ACD or EDTA. Nonetheless, occasionally, a heparinized sample might be the only source of nucleic acid available. Attempts to remove heparin with repeated ethanol precipitation, boiling and filtering, pH modification with gel filtration, or titration with protamine sulfate do not appear to eliminate subsequent heparin assay inhibition *(15,16)*. For PCR requiring only minimal sensitivity, sample dilution might overcome this inhibition. Obviously, if amplification of a low-copy-number template is desired (e.g., infectious disease testing), sample dilution might compromise assay sensitivity. Serial washing of the buffy coat with saline prior to DNA extraction might also prove useful if white blood cells are the source of template DNA *(15)*.

In the event that a heparinized sample must be used and dilution or washes are inadequate or inappropriate, a few options remain. Heparinase treatment of the extracted DNA might allow subsequent use of the sample for high-sensitivity PCR or other testing. Heparinase is costly, however, and the heparinase preparation might be contaminated with small amounts of RNase. The presence of RNase precludes the use of heparinase in RNA purification protocols *(2)*. Alternately, heparin-free RNA can be precipitated out with lithium chloride. The addition of lithium chloride (final concentration 1.8 *M*) to a nucleic acid solution precipitates RNA, leaving inhibiting lipopolysaccharide or heparin in solution *(16)*. This technique is inexpensive and effective.

2.4. FORENSIC SAMPLES Special consideration must be given to the forensic tissue specimen. Often, these samples are neither fresh nor preserved. In general, the quantity and quality of nucleic acid decreases with specimen age. Bloodstains might provide better DNA than bone samples, especially when the specimens are old and poorly preserved *(17)*. Success has also been reported with tooth pulp, various soft tissues, and hair roots.

Complicating forensic nucleic acid degradation is environmental contamination, the unavoidable repercussion of specimen collection from an uncontrolled environment. Bloodstains, for example, can be seen on an essentially infinite variety of surfaces. Certain surface types present specific problems. Fabric dyes, especially indigo dye used in denim, could contaminate nucleic acid extractions and inhibit PCR. Using capillary action, dye can be removed by drawing saline through the fabric. Nucleic acid is transferred by this solution to a nylon membrane while dye remains in the fabric *(18)*. Other surfaces, like varnished wood, could also reduce the quality and quantity of forensic DNA *(19)*. Even on an ideal surface, the stain or sample might have been washed prior to discovery or exposed to forensic reagents like 3-aminophthalhydrazide (known as Luminol; it fluoresces in the presence of heme and is used to detect bloodstains during field investigation). Although Luminol does not appear to affect subsequent PCR analysis of extracted DNA, surface cleaning can destroy DNA evidence *(19)*. A more detailed discussion of forensic specimen collection is beyond the scope of this chapter, but it should be apparent that this is a challenging and interesting endeavor.

3. EXTRACTION

The first steps of any extraction process are tissue isolation, disruption, and cell lysis. Again, the specific protocol required depends on the sample. When a large portion of fresh or fixed solid tissue is available, it is important to select appropriate areas for subsequent harvest of nucleic acid. For collection of genomic DNA or RNA, tissue cannot be autolyzed or necrotic. Focal necrosis is common in many solid tumors, and sampling of these areas provides little or no intact nucleic acid. The histological complexity of a solid tissue sample should also be considered. Many tissues, lesions, and tumors are composed of multiple cell types and morphological areas. It is possible, and can be critical, to isolate nucleic acid from a single one of these areas. Careful dissection, and techniques like selective ultraviolet radiation fractionation *(20)* allow even small cell groups to be isolated and processed.

3.1. PARAFFIN AND BLOOD Paraffin-embedded tissues require deparaffinisation prior to nucleic acid extraction. A variety of methods exist that employ heat or solvents like xylene to remove paraffin *(21)*. Heat-based protocols are simple and require only a microwave or thermal cycler. More precise temperature control might be possible with a thermal cycler, but either system should prove successful. Some direct comparisons suggest that yields from solvent-based techniques are lower than those using heat *(22)*.

Virtually all nucleic acid obtained from blood samples, barring hematologic pathology, is leukocyte derived. Isolating white blood cells by centrifugation, therefore, could optimize purification yield and reduce reagent requirements. A simple method for this employs Ficoll lymphocyte separation medium *(21)*. Other protocols allow successful nucleic acid preparation directly from whole blood *(23)*. These techniques could prove quicker and involve fewer steps.

3.2. LYSIS/MEMBRANE DISRUPTION To purify nucleic acid from tissue samples, it is first necessary to disrupt cellular and nuclear membranes. This is efficiently accomplished with a detergent, often sodium dodecyl sulfate (SDS). The large amount of protein present in cell or nuclear lysates can make DNA or RNA purification difficult, so most methods employ proteolytic agents during this step. Proteinase K is frequently used for this purpose *(6,14,21,24)*.

3.3. ORGANIC EXTRACTION Traditional nucleic acid purification from lysate is accomplished by phenol–chloroform extraction. Variations of this basic protocol rely on the separation of protein into the organic phase and nucleic acid into an aqueous phase. It is important that the phenol pH lie within a range of 7.8–8.0 to prevent nucleic acid from remaining in the organic phase. Even at this pH, RNA with a long poly(A) tail or tract might partition with phenol. The addition of isoamyl

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alcohol to the mixture prevents this, and reduces RNase activity (final ratio of phenol : chloroform : isoamyl alcohol of 25 : 24 : 1).

A key step in the organic extraction is emulsification of organic and aqueous phases. When low-molecular-weight DNA is desired, this emulsion can be achieved by vortexing. Higher-weight nucleic acid (>10 kb), however, is vulnerable to shearing forces and might tolerate only gentle shaking or rotation. The use of large-bore pipets will also reduce shearing during transfer of material, and limiting the number of transfers will also facilitate high-molecular-weight nucleic acid recovery. Extremely high-weight DNA required for pulsed-field gel electrophoresis might require cell lysis and DNA purification within an agarose plug. Digestion and removal of cellular proteins is accomplished over the course of days, leaving large and intact DNA within the agarose. This method does not employ phenol or chloroform as protein solvents.

The requirements for most molecular assays are met by conventional organic extraction, followed by ethanol precipitation. Adequate purity and yields could require serial phenol–chloroform extractions of the aqueous phase. The presence of visible protein at the interface of organic and aqueous materials warrants another round of extraction. Additionally, yield can be optimized by vigorously mixing Tris-EDTA (TE) buffer with the discarded organic phase ("back extraction"). Extra nucleic acid can be taken from the TE after subsequent centrifugation.

It is critical that the final aqueous nucleic acid solution be free of phenol and protein contamination. Care when removing the aqueous phase and repeated cycles of chloroform extraction prior to ethanol precipitation will prevent phenol contamination. In the presence of ethanol and monovalent cations, DNA or RNA precipitates out of solution at temperatures near 0°C. A variety of salts can be used as a cation source. Perhaps the most common is sodium acetate, and this is suitable for most organic extraction protocols. Other salts have unique advantages and disadvantages. Ammonium acetate reduces dNTP coprecipitation but can inhibit subsequent assays requiring nucleic acid phosphorylation. Sodium chloride is useful when samples are contaminated with SDS *(24)*. As discussed earlier, lithium chloride facilitates RNA precipitation, a technique useful in removing heparin contamination.

3.4. INORGANIC EXTRACTION As an alternative to organic purification, inorganic techniques reduce exposure to hazardous reagents while producing purified nucleic acid of comparable quality. Many commercial kits employ inorganic purification methods, including salt precipitation, adsorption to silica surfaces, and anion-exchange chromatography protocols. Many of these principles are also easily applied without commercial kits. Removal of contaminating protein by precipitation and centrifugation prior to ethanol DNA precipitation, for example, gives good yields with excellent purity *(25)*.

Especially popular are purification systems based on the binding of nucleic acid to silica or glass particles in the presence of chaotropic agents. The chaotropic agent GITC is useful for inhibiting troublesome nuclease, but it also promotes nucleic acid binding to silica or glass media *(26,27)*. Nucleic acid elution after washing can be accomplished with a low-salt aqueous buffer. Commercial systems based on this technique produce high-quality, high-purity nucleic acid preparations with improved safety and speed. There are many variations on this theme, depending on the manufacturer, and many laboratories choose to avoid the trouble of organic purification by investing in these standardized and modestly priced kits.

3.5. RNA EXTRACTION In general, variations of these techniques can be applied to both DNA and RNA purification. RNA isolation, however, demands extra care. Most forms of RNA are labile, and RNase is a frustratingly frequent contaminant of laboratory reagents and equipment. A few simple techniques and principles will help prevent degradation problems. First, reagents and equipment used for RNA preparation should be dedicated to that purpose. This becomes especially important if the laboratory also does DNA purification. RNase is frequently used in DNA work, and contamination of reagents and reusable equipment will affect subsequent RNA preparations.

Perhaps the greatest risk of RNase contamination comes from the laboratory worker's skin. It is critical that clean gloves by worn at all times and changed frequently if contact with potentially contaminated equipment is necessary.

Fresh out of the package, most sterile pipet tips and other disposable materials can be considered RNase free. Other tools might require treatment to destroy contaminating RNase. Glassware, reagent spatulas, and other equipment can be pretreated by incubation at 37°C in a solution of 0.1% diethylpyrocarbonate (DEPC). DEPC is a strong RNase inhibitor. In addition to inhibiting RNase, however, DEPC can also carboxymethylate nucleic acid purine residues. After incubation, therefore, the materials must be autoclaved to remove any remaining DEPC before use in RNA preparation. As an alternative, glassware can be baked at 150°C for 4 h or plastic materials can be soaked in 0.5 *M* NaOH for 10 min. Use of NaOH requires subsequent rinsing and autoclave treatment. Any of these methods will reduce RNase activity on reusable equipment *(21)*.

Decontamination of reagent solutions can also be accomplished by adding DEPC to a concentration of 0.1%. Caution should be exercised, however, with solutions containing amines that will react with DEPC. Tris buffer is a common example. Tris from a freshly opened or dedicated container can be added after autoclaving DEPC-treated water or solutions. To make Tris-EDTA for RNA storage, for example, a solution of EDTA can be DEPC treated and autoclaved before adding RNase-free (uncontaminated) Tris. Subsequent pH adjustments must also be made with RNase-free reagents.

3.6. DNA MICROARRAY The recent development of DNA microarray technology is also worth mentioning for its particular demands on nucleic acid extraction. In brief, cDNA transcribed from a pool of cellular mRNA is hybridized to an array of thousands of distinct DNA sequences fixed to a glass slide. Because the cDNA is transcribed with fluorescent or radioactive tags ,the relative quantity of hybridized cDNA, and therefore original mRNA, can be assessed. Generally, the gene expression profile of one cell source is simultaneously compared to a reference cell source (transcribed with a second fluorescent tag) as an internal control. This is a powerful new tool, with a rapidly growing body of literature describing its techniques and applications. One of the primary technical limits of microarray technology is the quality and quantity of RNA

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available from the test and reference cells *(28)*. Contamination of nucleic acid preparations with protein, lipid, or carbohydrate can interfere with reverse transcriptase or mediate nonspecific array hybridization. A variety of mRNA purification procedures are available, and a review of public domain protocols suggests that most laboratories employ commercial kits to purify total RNA and then isolate the mRNA fraction. Total RNA can be obtained efficiently with both silica-based techniques (RNeasy; Qiagen, Valencia, CA) or precipitation procedures (TRIzol; Life Technologies, Rockville, MD). Subsequent mRNA purification is best accomplished via binding to oligo dT fixed on a solid medium or column. A number of representative protocols are available on the World Wide Web (www.microarray.org, www.nhgri.nih.gov/DIR/Microarray, and cmgm.stanford.edu/pbrown).

As genetic testing is increasingly used for clinical work, other technologies for rapid, large-scale genetic analysis will become important. The Invader® System (Third Wave Technologies, Madison, WI), for example, is an automated system that can be used to test for clinically important genetic sequences such as the Prothrombin G20210A and Factor V Leiden mutations. This system employs a combination of proprietary enzymes and specific oligonucleotide probes to generate a fluorescent signal that is amplified in a linear manner from the target DNA. No PCR amplification is required, and as little as 100 ng of target genomic DNA (or total RNA) is sufficient for testing. This method is, however, sensitive to phenol, chloroform, and high-salt concentrations, so a careful inorganic purification method is generally recommended. As more testing and detection technologies become available, the demands on purified DNA or RNA could change and will likely become less stringent.

4. ASSESSMENT OF QUALITY AND QUANTITY

In many cases, purified nucleic acid is used directly in an assay, without evaluation of its purity, concentration, or size. The results of the assay itself often demonstrate the success or failure of the preparation. In cases where assay setup or interpretation requires prior knowledge of the sample's purity and concentration, a number of methods are available.

Perhaps the simplest and fastest approach is spectrophotometry. Although nucleic acid bases show maximal absorption at an approximate wavelength of 260 nm, contaminating proteins absorb well at 280 nm. Protein absorption is primarily the result of the aromatic amino acids phenylalanine, tyrosine, and tryptophan. Testing sample optical density (OD) at 260 nm and 280 nm, therefore, allows assessment of both nucleic acid concentration and purity. An OD_{260} of 1.0 corresponds to approx 50 µg/mL of double-stranded DNA (40 µg/mL for single-stranded DNA or RNA). The $OD_{260/280}$ ratio provides an estimate of nucleic acid purity, with a pure preparation having a ratio between 1.8 and 2.0. Contamination with organic solvents or protein will obviously lower this value and also prevent accurate nucleic acid quantification from the OD_{260} reading.

If the purified sample is too contaminated or low in concentration for spectrophotometric assessment, fluorescent dyes can be used to quantify the nucleic acid present. A variety of dyes that bind nucleic acid are available, including acridine orange, daminoibenzoic acid (DABA), propidium iodide, and ethidium bromide. These dyes might detect nucleic acid in quantities as low as 1–5 ng *(24)*, although, typically, the detection threshold is closer to 5–10 ng *(29)*. Ethidium bromide, most widely used of these compounds, demonstrates fluorescence that increases by a factor of 20 or more when bound to nucleic acid. This fluorescence under ultraviolet (UV) light is directly proportional to the amount of nucleic acid present. The direct relationship between fluorescence and nucleic acid content allows the use of ethidium bromide in quantitative assays. It is worth noting that the degree of fluorescence is dependent on the ratio of ethidium bromide to nucleic acid, with maximal output at a DNA : ethidium bromide ratio of 0.5 to 3.0 (w/w) *(30)*.

Nucleic acid samples and standards (of known concentration) can be spotted with ethidium bromide on a plastic surface, and fluorescence can be captured by digital or conventional camera. Some contaminants might increase or decrease fluorescence, but this can be partly remedied by spotting on a 1% agarose slab. Small, interfering molecules will diffuse away from nucleic acid over the course of a short incubation at room temperature *(31)*.

Further information can be obtained by electrophoretic analysis of the purification product. As discussed earlier, the size of nucleic acid fragments can be important to subsequent work. Small agarose gels, "minigels," can be used easily and quickly to determine both the size and quantity of nucleic acids. For size determination, a molecular-weight ladder provides a reference standard. Likewise, for quantifying nucleic acid, a series of samples of known concentration can be run in parallel with the unknown sample, and a standard curve prepared. Again, a fluorescent dye like ethidium bromide should be used to detect nucleic acid in the gel, so that nucleic acid quantity can be extrapolated from fluorescence data.

5. NUCLEIC ACID STORAGE

When nucleic acid must be stored, either for archival purposes or before assay performance, the key goal is prevention of enzymatic or physical damage to the purified product. Three chief weapons are available for this endeavor: chelating agents, chaotrophic agents, and refrigeration. As discussed earlier, special care is needed when the solution contains RNA or high-molecular-weight DNA.

In general, DNA can be stored effectively for long periods in a Tris-EDTA buffer at 4°C. The chelation of free divalent cations by EDTA or another chelating agent diminishes the damage caused by contaminating nucleases, which require these cations for function. Cold temperatures further reduce enzyme activity and improve nucleic acid stability. RNA, inherently more labile, should be stored at –80°C in a similar buffer. As an alternative, either DNA or RNA can be stored as an ethanol precipitate, with –20°C being the optimal storage temperature.

Should the nucleic acid sample remain contaminated with nuclease after purification attempts, the addition of chaotropic reagents like GITC will deactivate remaining enzyme. GITC will crystallize at temperatures below room temperature, so solutions containing this reagent must be completely warmed before use. Likewise, RNA stored in a GITC-based solution should be kept at room temperature or frozen at –80°C.

Following these basic guidelines, purified DNA or RNA can be stored for long periods of time.

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