

CHAPTER 2

Preparation and Analysis of DNA

INTRODUCTION

The ability to prepare and isolate pure DNA from a variety of sources is an important step in many molecular biology protocols. Indeed, the isolation of genomic, plasmid, or DNA fragments from restriction digests and polymerase chain reaction (PCR) products has become a common everyday practice in almost every laboratory. This chapter therefore begins with general protocols for purifying and concentrating DNA (*UNITS 2.1A & 2.1B*), and also specific protocols for purification of genomic DNA from bacteria, plant cells, and mammalian cells (*UNITS 2.3–2.4*). These protocols consist of two parts: a technique to lyse the cells gently and solubilize the DNA, followed by one of several basic enzymatic or chemical methods to remove contaminating proteins, RNA, and other macromolecules. The basic approaches described here are generally applicable to a wide variety of starting materials, but a specialized protocol for preparing DNA from mammalian sperm is also included (*UNIT 2.13*). *UNITS 2.11 & 2.12* describe the chemical synthesis and purification of oligonucleotides.

The last decade has shown a dramatic departure from the use of traditional DNA purification methods outlined in *UNIT 2.2–2.4*, with a concomitant increase in the use of purpose-specific kits for the isolation and purification of DNA. For example, kits for purification of DNA using pre-made anion-exchange columns packaged with all necessary solutions to lyse the cells and solubilize the DNA are available from many molecular biology companies. A variety of kits based on binding of DNA to glass beads are also available. The uses of both types of kits are discussed in *UNIT 2.1B*.

The use of kits has two main advantages: it saves time and makes the process of DNA purification a relatively easy and straightforward process. The purification of DNA by anion-exchange chromatography (*UNIT 2.1B*) is readily becoming the accepted standard for quick and efficient large-scale (more than 100 μg of DNA) production of DNA from bacteria, mammalian tissue, and plant tissue. In most cases, the cell lysis and solubilization of DNA is relatively unchanged compared to traditional methods, with anion-exchange chromatography columns having replaced labor and time-intensive techniques such as cesium chloride centrifugation for the isolation of relatively pure DNA. Purification kits are usually available in several sizes and configurations, allowing the researcher to have variability concerning the processing and purification of their DNA.

A variety of techniques exist for the isolation of small amounts of plasmid DNA from minipreps and for DNA fragments from restriction digests/PCR products from agarose gels (with removal of unincorporated nucleoside triphosphates, reaction products, and small oligonucleotides from PCR reactions). These are detailed in *UNITS 2.1A, 2.1B, 2.6 & 2.7*. Likewise, kits are available from several molecular biology companies, usually based on silica-gel technology, for each of these applications (*UNIT 2.1B*). As with large-scale DNA isolation and purification, these kits provide a quick and efficient means to recover purified DNA that can be used for subsequent cloning or other modifications.

Virtually all protocols in molecular biology require, at some point, fractionation of nucleic acids. Chromatographic techniques are appropriate for some applications and may be used for separation of plasmid from genomic DNA as well as separation of genomic DNA

from debris in a cell lysate (*UNIT 2.1B*). Gel electrophoresis, however, has much greater resolution than alternative methods and is generally the fractionation method of choice. Gel electrophoretic separations can be either analytical or preparative, and can involve fragments with molecular weights ranging from less than 1000 Daltons to more than 10^8 . A variety of electrophoretic systems have been developed to accommodate such a large range of applications.

In general, the use of electrophoresis to separate nucleic acids is simpler than its application to resolve proteins. Nucleic acids are uniformly negatively charged and, for double-stranded DNA, reasonably free of complicating structural effects that affect mobility. A variety of important variables affect migration of nucleic acids on gels. These include the conformation of the nucleic acid, the pore size of the gel, the voltage gradient applied, and the salt concentration of the buffer. The most basic of these variables is the pore size of the gel, which dictates the size of the fragments that can be resolved. In practice, this means that larger-pore agarose gels are used to resolve fragments >500 to 1000 bp (*UNITS 2.5A & 2.6*) and smaller-pore acrylamide or sieving agarose gels (*UNIT 2.7*) are used for fragments <1000 bp. A protocol for resolution of very large pieces of DNA may also be resolved on agarose gels using pulsed-field gel electrophoresis (*UNIT 2.5B*). Finally, the powerful analytical technique of capillary electrophoresis of DNA (*UNIT 2.8*) may be used to assess the purity of synthetic oligonucleotides, analyze quantitative PCR results, and compare DNA fragment lengths from restriction fragment length polymorphism (RFLP) and variable number of tandem repeat (VNTR) analyses.

Frequently it is desirable to identify an individual fragment in a complex mixture that has been resolved by gel electrophoresis. This is accomplished by a technique termed Southern blotting, in which the fragments are transferred from the gel to a nylon or nitrocellulose membrane and the fragment of interest is identified by hybridization with a labeled nucleic acid probe. Section IV of this chapter gives a complete review of methods and materials required for immobilization of fractionated DNA (*UNIT 2.9*) and associated hybridization techniques (*UNIT 2.10*). These methods have greatly contributed to the mapping and identification of single and multicopy sequences in complex genomes, and facilitated the initial eukaryotic cloning experiments.

Other commonly encountered applications of gel electrophoresis include resolution of single-stranded RNA or DNA. Polyacrylamide gels containing high concentrations of urea as a denaturant provide a very powerful system for resolution of short (<500-nucleotide) fragments of single-stranded DNA or RNA. Such gels can resolve fragments differing by only a single nucleotide in length, and are central to all protocols for DNA sequencing (see *UNIT 7.6*). Such gels are used for other applications requiring resolution of single-stranded fragments, particularly including the techniques for analyzing mRNA structure by S1 analysis (*UNIT 4.6*), ribonuclease protection (*UNIT 4.7*), or primer extension (*UNIT 4.8*). Denaturing polyacrylamide gels are also useful for preparative applications, such as small-scale purification of radioactive single-stranded probes and large-scale purification of synthetic oligonucleotides (*UNIT 2.12*).

Resolution of relatively large single-stranded fragments (>500 nucleotides) can be accomplished using denaturing agarose gels. This is of particular importance to the analysis of mRNA populations by northern blotting and hybridization. A protocol for use of agarose gels containing formaldehyde in resolution of single-stranded RNA is presented in *UNIT 4.9*. The use of denaturing alkaline agarose gels for purification of labeled single-stranded DNA probes is described in *UNIT 4.6*.

GELS AND ELECTRIC CIRCUITS

Gel electrophoresis units are almost always simple electric circuits and can be understood using two simple equations. Ohm's law, $V = IR$, states that the electric field, V (measured in volts), is proportional to current, I (measured in milliamps), times resistance, R (measured in ohms). When a given amount of voltage is applied to a simple circuit, a constant amount of current flows through all the elements and the decrease in the total applied voltage that occurs across any element is a direct consequence of its resistance. For a segment of a gel apparatus, resistance is inversely proportional to both the cross-sectional area and the ionic strength of the buffer. Usually the gel itself provides nearly all of the resistance in the circuit, and the voltage applied to the gel will be essentially the same as the total voltage applied to the circuit. For a given current, decreasing either the thickness of the gel (and any overlying buffer) or the ionic strength of the buffer will increase resistance and, consequently, increase the voltage gradient across the gel and the electrophoretic mobility of the sample.

A practical upper limit to the voltage is usually set by the ability of the gel apparatus to dissipate heat. A second useful equation, $P = I^2R$, states that the power produced by the system, P (measured in watts), is proportional to the resistance times the square of the current. The power produced is manifested as heat, and any gel apparatus can dissipate only a particular amount of power without increasing the temperature of the gel. Above this point small increases in voltage can cause significant and potentially disastrous increases in temperature of the gel. It is very important to know how much power a particular gel apparatus can easily dissipate and to carefully monitor the temperature of gels run above that level.

Two practical examples illustrate applications of the two equations. The first involves the fact that the resistance of acrylamide gels increases somewhat during a run as ions related to polymerization are electrophoresed out of the gel. If such a gel is run at constant current, the voltage will increase with time and significant increases in power can occur. If an acrylamide gel is being run at high voltage, the power supply should be set to deliver constant power. The second situation is the case where there is a limitation in number of power supplies, but not gel apparatus. A direct application of the first equation shows that the fraction of total voltage applied to each of two gels hooked up in series (one after another) will be proportional to the fraction of total resistance the gel contributes to the circuit. Two identical gels will each get 50% of the total voltage and power indicated on the power supply.

Finally, it should be noted that some electrophoretic systems employ lethally high voltages, and almost all are potentially hazardous. It is very important to use an adequately shielded apparatus, an appropriately grounded and regulated power supply, and most importantly, common sense when carrying out electrophoresis experiments.

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