Topic Introduction

Analysis of DNA by Agarose Gel Electrophoresis

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Electrophoresis through agarose or polyacrylamide gels is used to separate, analyze, identify, and purify DNA fragments. The technique is simple, rapid to perform, and capable of resolving fragments of DNA that cannot be separated adequately by other procedures, such as density gradient centrifugation. The location of bands of DNA within the gel can be determined directly by staining with low concentrations of fluorescent intercalating dyes, such as ethidium bromide or SYBR Gold; bands containing as little as 20 pg of double-stranded DNA can then be detected by direct examination of the gel in ultraviolet (UV) light. If necessary, these bands of DNA can be recovered from the gel.

TYPES OF GELS

Agarose and polyacrylamide gels can be poured in a variety of shapes, sizes, and porosities and can be run in several different configurations. The choices within these parameters depend primarily on the sizes of the fragments being separated. Polyacrylamide gels are most effective for separating small fragments of DNA (5–500 bp). Their resolving power is extremely high, and fragments of DNA that differ in size by as little as 1 bp in length or by as little as 0.1% of their mass can be separated from one another. Although they can be run very rapidly and can accommodate comparatively large quantities of DNA, polyacrylamide gels have the disadvantage of being more difficult to prepare and handle than agarose gels. Polyacrylamide gels are almost always run in a vertical configuration in a constant electric field.

Agarose gels have a lower resolving power than polyacrylamide gels, but they have a greater range of separation. DNAs from 50 bp to several megabases in length can be separated on agarose gels of various concentrations and configurations. Small DNA fragments (50–20,000 bp) are best resolved in agarose gels run in a horizontal configuration in an electric field of constant strength and direction. Under these conditions, the velocity of the DNA fragments decreases as their length increases and is proportional to electric field strength (McDonell et al. 1977; Fangman 1978; Calladine et al. 1991). However, this simple relationship breaks down once the size of DNA fragments exceeds a maximum value, which is defined chiefly by the composition of the gel and the strength of the electric field (Hervet and Bean 1987). This limit of resolution is reached when the radius of gyration of the linear DNA duplex exceeds the pore size of the gel. The DNA can then no longer be sieved by the gel according to its size but must instead migrate "end-on" through the matrix as if through a sinuous tube—a process called "reptation" (de Gennes 1971). It is therefore essential to use concentrations of agarose and polyacrylamide that provide optimal separation of DNA fragments over the desired size range (for reviews, see Stellwagen 2009; Stellwagen and Stellwagen 2009). Our protocol for agarose gel electrophoresis can be found in Protocol: **Agarose Gel Electrophoresis** (Green and Sambrook 2019). For a brief history of analyzing DNA by gel electrophoresis, see Box 1.

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BOX 1. EARLY ANALYSIS OF DNA USING ELECTROPHORESIS

The idea of using electrophoresis through a supporting matrix to analyze DNA came from Vin Thorne, a biochemist/virologist who in the mid-1960s was working at the Institute of Virology in Glasgow. Thorne was interested in finding better ways to characterize the multiple forms of DNA that could be extracted from purified particles of polyomavirus. He reasoned that a combination of frictional and electrical forces would allow separation of DNA molecules that differed in shape or size. Using electrophoresis through agar gels, he was able to separate superhelical, nicked, and linear forms of radiolabeled polyomavirus (Thorne 1966, 1967). However, Thorne's work attracted little general interest until the early 1970s, when restriction enzymes opened the possibility of analyzing larger DNAs, and a way was found to detect small quantities of nonradioactive DNA in gels.

The notion of using ethidium bromide to stain unlabeled DNA in gels seems to have occurred independently to two groups. The procedure used by Aaij and Borst (1972) involved immersing the gel in concentrated dye solution and a lengthy destaining process to reduce the background fluorescence. At Cold Spring Harbor Laboratory, a group of investigators had found that *Haemophilus parainfluenzae* contained two restriction activities and were attempting to separate the enzymes by ion-exchange chromatography. Searching for ways to assay column fractions rapidly, they decided to stain agarose gels containing fragments of SV40 DNA with low concentrations of ethidium bromide. They soon realized that the dye could be incorporated into the gel and running buffer without significantly affecting the migration of linear DNA fragments through the gel. The technique described in their paper (Sharp et al. 1973) is still widely used in an essentially unaltered form today.

Between 1972 and 1975, there was a vast increase in the use of agarose gels as investigators mapped cleavage sites on their favorite DNAs with the rapidly expanding suite of restriction enzymes. In those days, gels were cast in sawn-off glass pipettes and were run vertically in electrophoresis tanks attached to homemade power packs. Each DNA sample was analyzed on a separate little cylindrical gel. The first modern electrophoresis apparatus was developed by Walter Schaffner, who was then a graduate student at Zurich. Realizing that the electrical resistance of an agarose gel is essentially the same as that of the surrounding buffer, Schaffner constructed horizontal tanks to hold submerged gels that could accommodate more than a dozen samples. Schaffner distributed the plans for these machines to anyone who asked for them. Once people got over their incredulity that his machines actually worked, cylindrical gels cast in little glass tubes rapidly disappeared, and the newer "submarine" gels took permanent hold.

AGAROSE GEL ELECTROPHORESIS

The Rate of Migration of DNA through Agarose Gels

The rate of movement of DNA through gels is influenced by several factors.

- The electrical current flowing through the gel. According to Ohm's law,

$$V = IR,$$

  where V is the voltage, I is the current (in amperes), and R is the resistance (in ohms). Because buffers commonly used for electrophoresis are slightly alkaline (~pH 7.8–8.0), DNA molecules traveling through the gel carry a negative charge and migrate toward the anode at a rate influenced by the applied current.

- *The molecular size of the DNA*. Molecules of double-stranded DNA migrate through gel matrices at rates that are inversely proportional to the $\log_{10}$ of the number of base pairs (Helling et al. 1974). Larger molecules migrate more slowly because of greater frictional drag and because they worm their way through the pores of the gel less efficiently than smaller molecules.

- *The concentration of agarose*. Agarose gels are spongy hydrocolloids held together by hydrogen bonds and hydrophobic interactions. Under the influence of an electrical current, linear molecules of DNA wriggle through a series of pores whose effective diameters are determined by the concentration of agarose in the gel (see Fig. 1). The linear relationship between the logarithm of the

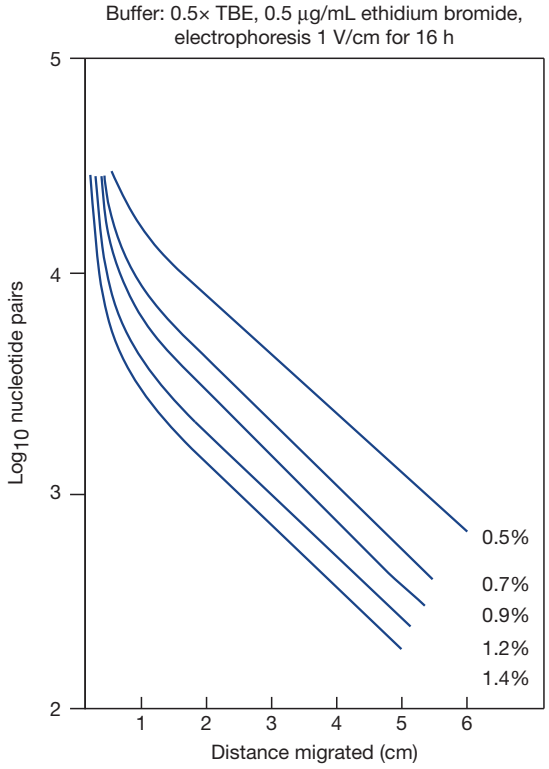

FIGURE 1. The relationship between the size of DNA and its electrophoretic mobility.

electrophoretic mobility of the DNA (μ) and the gel concentration (ι) is described by

$$\log \mu = \log \mu_0 - K_r \iota$$

where μ_0 is the free electrophoretic mobility of DNA and K_r is the retardation coefficient, a constant related to the properties of the gel and the size and shape of the migrating molecules.

- *The conformation of the DNA*. Superhelical circular (form I), nicked circular (form II), and linear (form III) DNAs migrate through agarose gels at different rates (Thorne 1966, 1967). The relative mobilities of the three forms depend primarily on the concentration and type of agarose used to make the gel, but they are also influenced by the strength of the applied current, the ionic strength of the buffer, and the density of superhelical twists in the form I DNA (Johnson and Grossman 1977). Under some conditions, form I DNA migrates faster than form III DNA; under other conditions, the order is reversed. In most cases, the best way to distinguish between the different conformational forms of DNA is simply to include in the gel a sample of untreated circular DNA and a sample of the same DNA that has been linearized by digestion with a restriction enzyme that cleaves the DNA in only one place.
- *The presence of dyes in the gel and electrophoresis buffer*. Intercalation of dyes causes a decrease in the negative charge of the double-stranded DNA and an increase in both its stiffness and length. The rate of migration of the linear DNA–dye complex through gels is consequently retarded by a factor of ~15% (see, e.g., Sharp et al. 1973).
- *The applied voltage*. At low voltages, the rate of migration of linear DNA fragments is proportional to the voltage applied. However, as the strength of the electric field is raised, the mobility of high-molecular-weight fragments increases differentially. Thus, the effective range of separation in agarose gels decreases as the voltage is increased. To obtain maximum resolution of DNA fragments >2 kb in size, agarose gels should be run at no more than 5–8 V/cm.

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TABLE 1. Properties of different types of agaroses

Type of agarose	Gelling temperature (°C)	Melting temperature (°C)	Commercial names
Standard agaroses			
Low EEO Isolated from *Gelidium* spp.	35–38	90–95	SeaKem LE (Lonza Walkersville) Agarose-LE (Affymetrix) Low EEO Agarose (Agilent) Molecular Biology Certified Grade (Bio-Rad)
Standard agaroses			
Low EEO Isolated from *Gracilaria* spp.	40–42	85–90	SeaKem HGT (Lonza Walkersville) Agarose-HGT (Affymetrix)
High-gel-strength agaroses			
	34–43	85–95	FastLane (Lonza Walkersville) SeaKem Gold (Lonza Walkersville) Chromosomal Grade Agarose (Bio-Rad)
Low-melting/gelling-temperature (modified) agaroses			
Low melting	25–35	63–65	SeaPlaque (Lonza Walkersville)
	35	65	NuSieve GTG (Lonza Walkersville)
Ultra-low melting	8–15	40–45	SeaPrep (Lonza Walkersville)
Low-viscosity, low-melting/gelling-temperature agaroses			
	25–30	70	InCert (Lonza Walkersville)
	38	85	NuSieve 3:1 (Lonza Walkersville)
	30	75	Agarose HS (Lonza Walkersville)

- *The type of agarose*. The two major classes of agarose are standard agaroses and low-melting-temperature agaroses (Kirkpatrick 1990). A third and growing class consists of intermediate-melting/gelling-temperature agaroses, showing properties of each of the two major classes. Within each class are various types of agaroses that are used for specialized applications (see Tables 1 and 2 and the discussion on Classes of Agarose and Their Properties, below).
- *The electrophoresis buffer*. The electrophoretic mobility of DNA is affected by the composition and ionic strength of the electrophoresis buffer. In the absence of ions (e.g., if water is substituted for electrophoresis buffer in the gel or in the reservoirs), electrical conductivity is minimal and DNA migrates slowly, or not at all. In buffer of high ionic strength (e.g., if 10× electrophoresis buffer is mistakenly used), electrical conductance is very efficient and significant amounts of heat are generated, even when moderate voltages are applied. In the worst case, the gel melts and the DNA denatures.

Classes of Agarose and Their Properties

Standard (High-Melting Temperature) Agaroses

Standard (high-melting temperature) agaroses are manufactured from two species of seaweed: *Gelidium* and *Gracilaria*. These agaroses differ in their gelling and melting temperatures, but, for practical

TABLE 2. Range of separation of DNA fragments through different types of agaroses

	Size range of DNA fragments resolved by various types of agaroses			
Agarose (%)	Standard	High gel strength	Low gelling/melting temperature	Low gelling/melting temperature low viscosity
0.3				
0.5	700 bp to 25 kb			
0.8	500 bp to 15 kb	800 bp to 10 kb	800 bp to 10 kb	
1.0	250 bp to 12 kb	400 bp to 8 kb	400 bp to 8 kb	
1.2	150 bp to 6 kb	300 bp to 7 kb	300 bp to 7 kb	
1.5	80 bp to 4 kb	200 bp to 4 kb	200 bp to 4 kb	
2.0			100 bp to 3 kb	100 bp to 3 kb
3.0			500 bp to 1 kb	500 bp to 1 kb
4.0				100 bp to 500 bp
6.0				10 bp to 100 bp

purposes, agaroses from either source can be used to analyze and isolate fragments of DNA ranging in size from 1 to 25 kb. Several commercial grades of agaroses have been tested that (1) display minimal background fluorescence after staining with ethidium bromide, (2) are free of DNase and RNase, (3) display minimal inhibition of restriction endonucleases and ligase, and (4) generate modest amounts of electroendo-osmotic flow (EEO) (see below).

Newer types of standard agarose combine high gel strength with low EEO, allowing gels to be cast with agarose concentrations as low as 0.3%. These gels can be used in conventional electrophoresis to separate high-molecular-weight DNA (up to 60 kb). At any concentration of these new agaroses, the speed of migration of the DNA is increased by 10%–20% over that achieved using the former standard agaroses, depending on buffer type and concentration.

Low-Melting/Gelling-Temperature Agaroses

Low-melting/gelling-temperature agaroses have been modified by hydroxyethylation and therefore melt at temperatures lower than those of standard agaroses. The degree of substitution determines the exact melting and gelling temperature. Low-melting/gelling-temperature agaroses are used chiefly for rapid recovery of DNA because most agaroses of this type melt at temperatures (~65°C) that are significantly lower than the melting temperature of duplex DNA. This feature allows for simple purification and enzymatic processing (restriction endonuclease digestion/ligation) of DNA and allows bacterial transformation with nucleic acids directly in the remelted gel. As is the case with standard agaroses, manufacturers provide grades of low-melting-temperature agaroses that have been tested to display minimal background fluorescence after staining with ethidium bromide, to be free of DNase and RNase activity, and to display minimal inhibition of restriction endonucleases and ligase. Low-melting-temperature agaroses not only melt, but also gel at low temperatures. This property allows them to be held as liquids in the 30°C–35°C range, so that cells can be embedded without damage.

Chemically Modified Agarose

Chemically modified agarose has significantly more sieving capacity than an equivalent concentration of standard agarose (see Tables 1 and 2). This finding has been exploited to make agaroses that approach polyacrylamide in their resolving power and are therefore useful for separation of polymerase chain reaction (PCR) products, small DNA fragments, and small RNAs <1 kb. It is now possible to resolve DNA down to 4 bp and to separate DNAs in the 200- to 800-bp range that differ in size by 2% (see Table 2).

Because of the variation in products from manufacturer to manufacturer, it is advisable to read the supplier's catalog to obtain more precise information regarding specific brands of agarose.

Electroendo-Osmosis

In agarose gels, the speed at which nucleic acids migrate toward the positive electrode is affected by *electroendo-osmosis* (EEO). This process is due to ionized acidic groups (usually sulfate) attached to the polysaccharide matrix of the agarose gel. The acidic groups induce positively charged counterions in the buffer that migrate through the gel toward the negative electrode, causing a bulk flow of liquid that migrates in a direction opposite to that of the DNA.

The higher the density of negative charge on the agarose, the greater the EEO flow and the poorer the separation of nucleic acid fragments. Retardation of small DNA fragments (<10 kb) is minor, but larger DNA molecules can be significantly retarded, especially in PFGE. To avoid problems, it is best to purchase agarose from reputable merchants and to use types of agarose that display low levels of EEO. Agaroses that are sold as "zero" EEO are undesirable for two reasons: They have been chemically modified by adding positively charged groups, which neutralize the sulfated polysaccharides in the gel but may inhibit subsequent enzyme reactions; and they have been adulterated by adding locust bean gum, which retards expulsion of water from the gel (Kirkpatrick 1990).

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Electrophoresis Buffers

Several different buffers are available for electrophoresis of native, double-stranded DNA. These contain Tris–acetate and EDTA (pH 8.0; TAE) (also called E buffer), Tris–borate (TBE), or Tris–phosphate (TPE) at a concentration of ~50 mM (pH 7.5–7.8). Electrophoresis buffers are usually made up as concentrated solutions and stored at room temperature. All of these buffers work well, and the choice among them is largely a matter of personal preference. TAE has the lowest buffering capacity of the three and will become exhausted if electrophoresis is performed for prolonged periods of time. When this happens, the anodic portion of the gel becomes acidic and the bromophenol blue migrating through the gel toward the anode changes in color from bluish-purple to yellow. This change begins at pH 4.6 and is complete at pH 3.0. Exhaustion of TAE can be avoided by periodic replacement of the buffer during electrophoresis or by recirculation of the buffer between the two reservoirs. Both TBE and TPE are slightly more expensive than TAE, but they have significantly higher buffering capacity. Double-stranded linear DNA fragments migrate ~10% faster through TAE than through TBE or TPE; the resolving power of TAE is slightly better than TBE or TPE for high-molecular-weight DNAs and worse for low-molecular-weight DNAs. This difference probably explains the observation that electrophoresis in TAE yields better resolution of DNA fragments in highly complex mixtures such as mammalian DNA. For this reason, Southern blots used to analyze complex genomes are generally derived from gels prepared in and run with TAE as the electrophoresis buffer. The resolution of supercoiled DNAs is better in TAE than in TBE.

Gel-Loading Buffers

Gel-loading buffers are mixed with the samples before loading into the slots of the gel. These buffers serve three purposes: They increase the density of the sample, ensuring that the DNA sinks evenly into the well; they add color to the sample, thereby simplifying the loading process; and they contain dyes that, in an electric field, move toward the anode at predictable rates. Bromophenol blue migrates through agarose gels ~2.2-fold faster than xylene cyanol FF, independent of the agarose concentration. Bromophenol blue migrates through agarose gels run in 0.5× TBE at approximately the same rate as linear double-stranded DNA 300 bp in length, whereas xylene cyanol FF migrates at approximately the same rate as linear double-stranded DNA 4 kb in length. These relationships are not significantly affected by the concentration of agarose in the gel over the range of 0.5%–1.4%. Which type of loading dye to use is a matter of personal preference; various recipes are presented in Protocol: **Agarose Gel Electrophoresis** (Green and Sambrook 2019).

ANALYSIS OF DNA FRAGMENTS

DNAs that have been separated by migration through agarose gels may be detected by staining with dyes with low intrinsic fluorescence, a strong affinity for DNA, and a high quantum yield of fluorescence after binding to nucleic acids. The greater the increase in quantum yield, the higher the ratio of signal to noise. Bands of DNA stained with these dyes are visualized by illuminating the gel with UV light at one wavelength and recording at another. Methods are described here for staining and visualization of DNA in gels using three dyes: ethidium bromide, SYBR Gold, and SYBR Green 1. Further information on these and other dyes may be found in Introduction: **Isolation and Quantification of DNA** (Green and Sambrook 2018), in the Introduction to Quantifying DNA.

Note that stains with similar characteristics to SYBR stains are sold by other companies (e.g., GelStar [White et al. 1999] and GelRed [Huang et al. 2010]). Although less sensitive than any of these asymmetric cyanine dyes, ethidium bromide is nevertheless adequate for most routine staining of DNA in gels.

Images of stained gels are captured under UV illumination by a CCD camera equipped with the appropriate converter screen and filter. Sensible advice on setting up a CCD system to capture fluorescently stained images with maximum sensitivity can be found in Oatey (2007).

Staining DNA in Gels with SYBR Gold

SYBR Gold is the trade name of an ultrasensitive dye with high affinity for DNA and a large fluorescence enhancement following binding to nucleic acid. The mode of binding of the dye to nucleic acids is believed to be of a different mechanism than that of the more conventional phenanthridinium intercalator dyes such as ethidium bromide and propidium iodide.

SYBR Gold can be used to stain both DNA and RNA in conventional neutral polyacrylamide and agarose gels and in gels containing denaturants, such as urea, glyoxal, and formaldehyde. The quantum yield of the SYBR Gold–DNA complex is greater than that of the equivalent ethidium bromide–DNA complex and the fluorescence enhancement is more than 1000 times greater. As a result, bands containing <20 pg of double-stranded DNA can be detected in an agarose gel (up to 25 times less than the amount visible after ethidium bromide staining). In addition, staining of agarose or polyacrylamide gels with this dye can reveal as little as 100 pg of single-stranded DNA in a band or 300 pg of RNA. SYBR Gold shows maximum excitation at 495 nm and has a secondary excitation peak at 300 nm. Fluorescent emission occurs at 537 nm.

SYBR Gold is supplied as a 10,000× concentrate in anhydrous dimethyl sulfoxide (DMSO) and should only be handled when using powder-free gloves. The high cost of the dye precludes its use for routine staining of gels. However, the dye may be cost-effective as an alternative to using radiolabeled DNAs in techniques such as single-strand conformation polymorphism (SSCP) and denaturing gradient gel electrophoresis (DGGE).

SYBR Gold should not be added to the molten agarose or to the gel before electrophoresis because its presence in the hardened gel will cause severe distortions in the electrophoretic properties of the DNA and RNA. The dye is sensitive to fluorescent light, and working solutions containing SYBR Gold (1:10,000 dilution of the stock solution supplied by Life Technologies) should be freshly made daily in electrophoresis buffer and stored at room temperature.

SYBR dyes are used to stain DNA by soaking the gel, after separation of the DNA fragments, in a 1:10,000-fold dilution of the stock dye solution. The staining process takes ~30 min (longer if the gel is thick). The level of background fluorescence is so low that no destaining is required.

Images of gels stained with SYBR Gold may be captured by CCD camera under UV illumination with a blue light converter screen and an SG emission filter (Oatey 2007).

Staining DNA in Gels with SYBR Green I

SYBR Green I, a fluorescent nonsymmetrical cyanine dye with a quantum yield of 0.8, binds to double-stranded DNA with an 800-fold to 1000-fold enhancement of fluorescence (Schneeberger et al. 1995). SYBR Green I is maximally excited at 497 nm and has additional broad excitation peaks at 284 and 312 nm, which makes the dye suitable for use with argon ion lasers. The peak of fluorescence emission is at 520 nm.

SYBR Green I has a high affinity for DNA and can be used for a variety of purposes including staining DNA and oligonucleotides in agarose and polyacrylamide gels, detecting the products of amplification in polymerase chain reactions, and as a detector in bandshift and nuclease protection assays.

SYBR Green I binds strongly to double-stranded DNA and with lower affinity to single-stranded DNA and RNA. The dye can detect bands in an agarose gel containing as little as 60 pg of double-stranded DNA with transillumination at 300 nm and 50 pmol of oligonucleotide in a 5% polyacrylamide gel. For greatest sensitivity, SYBR Green I should be used as a postelectrophoretic stain.

SYBR Green I does not interfere with *Taq* DNA polymerase, reverse transcriptase, restriction endonucleases, or bacteriophage T4 DNA ligase and is much less mutagenic than ethidium bromide in Ames tests.

Because SYBR Green I is strongly absorbed onto glass surfaces, staining solutions should be prepared in plastic containers. Like other SYBR dyes, SYBR Green I is supplied in anhydrous DMSO and should be diluted into TE (pH 7.5) or into 1× electrophoresis buffer just before use.

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Images of gels stained with SYBR Green I may be captured by CCD camera under UV illumination with a blue light converter screen and an SG emission filter (Oatey 2007).

Staining DNA in Gels with Ethidium Bromide

A convenient and commonly used method to visualize DNA in agarose gels is staining with the fluorescent dye ethidium bromide (Sharp et al. 1973), which contains a tricyclic planar group that intercalates between the stacked bases of DNA. Ethidium bromide intercalates into double-stranded DNA in a sequence-independent fashion at a maximum stoichiometry of about two dye molecules per turn of the helix (Waring 1965). After insertion into the helix, the dye lies perpendicular to the helical axis and makes van der Waals contacts with the base pairs above and below. The fixed position of the planar group and its close proximity to the bases cause dye bound to DNA to display an increased fluorescent yield compared with that of dye in free solution. UV radiation at 254 nm is absorbed by the DNA and transmitted to the dye; radiation at 302 and 366 nm is absorbed by the bound dye itself. In both cases, the energy is reemitted at 590 nm in the red–orange region of the visible spectrum (LePecq and Paoletti 1967). Because the fluorescent yield of ethidium bromide–DNA complexes is ~20- to 30-fold greater than that of unbound dye, bands containing as little as ~10 ng of DNA can be detected in the presence of free ethidium bromide (0.5 µg/mL) in the gel.

Ethidium bromide can be used to detect both single-stranded and double-stranded nucleic acids (both DNA and RNA). However, the affinity of the dye for single-stranded nucleic acid is relatively low, and the fluorescent yield is comparatively poor. In fact, most fluorescence associated with staining single-stranded DNA or RNA is attributable to binding of the dye to short intrastrand duplexes in the molecules (Waring 1965, 1966). The reaction between DNA and ethidium bromide is reversible, but the dissociation of the complex is very slow and is measured in days rather than minutes or hours.

Ethidium bromide is prepared as a stock solution of 10 mg/mL in H_2O, which is stored at room temperature in dark bottles or bottles wrapped in aluminum foil. The dye is usually incorporated into agarose gels and electrophoresis buffers at a concentration of 0.5 µg/mL for 30–45 min at room temperature. Note that polyacrylamide gels cannot be cast with ethidium bromide because the dye inhibits polymerization of the acrylamide. Polyacrylamide gels are therefore stained with a dilute solution of ethidium bromide (0.5 µg/mL in electrophoresis buffer) after the gel has been run.

Linear DNA molecules saturated with ethidium bromide become stiffer, and their frictional coefficients increase. In consequence, the electrophoretic mobility of linear double-stranded DNA through agarose gels is reduced by ~15% in the presence of saturating concentrations of the dye (Sigmon and Larcom 1996). The ability to examine agarose gels directly under UV illumination during or at the end of the run is a great advantage. However, sharper DNA bands are obtained when electrophoresis is performed in the absence of ethidium bromide. The gel is then immersed in electrophoresis buffer or H_2O containing ethidium bromide (0.5 µg/mL) for 30–45 min at room temperature. Destaining is not usually required. However, detection of very small amounts (<10 ng) of DNA is made easier if the background fluorescence caused by unbound ethidium bromide is reduced by soaking the stained gel in H_2O or 1 mM $MgSO_4$ for 20 min at room temperature.

Images of ethidium bromide-stained gels may be efficiently captured by CDD camera using a UV-emission filter (Oatey 2007).

Disposing of Ethidium Bromide

Most research institutions have developed procedures for the safe handling and disposal of solutions and gels containing ethidium bromide. Researchers should dispose of ethidium bromide according to these procedures.

Kits for safe disposal of ethidium bromide are available from Whatman and MP Biomedicals, among others.

RECOVERING DNA FROM GELS

Purification of DNA from agarose gels is sometimes an essential step in the subcloning of DNA fragments. A plethora of methods to recover bands of DNA from slices of agarose and polyacrylamide gels has been published over the years. Many of these methods probably work to some extent, but the fact that none of them has been widely adopted must indicate a lack of efficiency, reproducibility, or robustness. The familiar problems associated with traditional methods include:

- difficulties in ligating, digesting, or radiolabeling the recovered DNA
- inefficient recovery of large fragments of DNA
- inefficient recovery of small amounts of DNA

Given these problems, it is best wherever possible to design a protocol that does not involve isolation of DNA from gels. PCR, which is quicker and cheaper, is often a better option. However, if PCR is impractical, it is best to use one of the many commercial products that are now available for recovery of DNA from agarose gels. Most of the manufacturers of these kits provide data on the efficiency with which DNA fragments of various sizes can be recovered from gels cast with different concentrations of agarose and on the purity of the recovered DNA, as judged by its ability to be used as a template or substrate. The virtues of these kits—their reproducibility, reliability, efficiency, and speed—are offset to a certain extent by their high cost. But, on balance, kits win hands down over the older traditional methods.

The central step used in many of the commercial kits involves binding the DNA to a silica surface. A segment of an agarose gel containing the DNA band of interest is dissolved in a chaotropic buffer, which disrupts the hydrogen-bonding between the sugar moieties of the agarose polymer. The released DNA is then captured on silica beads or a membrane, recovered by elution in H_2O or a buffer containing a low concentration of salt or ethanol. Examples of kits of this type include: QIAEX II Gel Extraction Kit (QIAGEN), Wizard SV Kit (Promega), Ultra Silica Bead Kit (Thermo Fisher), NucleoSpin Extract IIa (Clontech), and GenElute (Sigma-Aldrich).

In contrast, the Millipore Montage Kit works on a different principle: A slice of an agarose gel is centrifuged at high speed in a device fitted with a built-in nebulizer, so that the gel is both fragmented and compressed. The DNA is then collected from the extruded volume of buffer.

Before using a kit, read the manufacturer's literature carefully because some kits require specially formulated gel running buffers.

Non-Kit-Based Techniques to Recover DNA from Agarose Gels

A common problem with older, non-kit-based techniques is an unfortunate trade-off between the efficiency with which DNA is recovered from the gel and its purity. If DNAs isolated from gels are to be used in enzymatic reactions, it may be best to choose a technique that promises a modest yield but delivers DNA free of significant contamination.

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