Journal of Chromatography, 618 (1993) 181–201
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6814

Review

Quantitative analysis of enzymic digests of DNA using gel electrophoresis

Eldred A. Ribeiro*

Life Sciences Group, Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547 (USA)

(First received October 30th, 1992; revised manuscript received January 10th, 1993)

ABSTRACT

Quantitative electrophoresis of enzymic digests of DNA and its applications are reviewed. Factors affecting the overall analysis such as DNA length distribution, labels for visualizing DNA, techniques for quantitation, and electrophoresis itself are studied. Methods to analyze restriction fragments and lesions in DNA by gel electrophoresis are presented.

CONTENTS

List of abbreviations . . . 182
1. Introduction . . . 182
2. Electrophoresis . . . 182
   2.1. General considerations . . . 182
   2.2. Dispersion functions . . . 183
   2.3. Resolution in electrophoresis . . . 184
3. DNA length distribution . . . 184
4. Labeling DNA . . . 185
   4.1. Ethidium bromide . . . 185
   4.2. Radioactive labels . . . 186
5. Techniques for detecting and quantitating DNA labels . . . 186
   5.1. Quantitating fluorescent labels using photographic film . . . 186
   5.2. Quantitating fluorescent labels using charge-coupled devices . . . 188
   5.3. Quantitating radioactive labels using X-ray film . . . 189
   5.4. Quantitating radioactive labels using photostimulable phosphors . . . 189
6. Methodology of analysis . . . 190
   6.1. Digest comprised of discrete DNA size distributions: applications to resolving restriction digests . . . 191
      6.1.1. Iterative method using determined point spread function . . . 192
      6.1.2. Mass-constrained deconvolution . . . 192
   6.2. Digest comprised of continuous DNA size distributions: applications to quantitating DNA lesions . . . 196
      6.2.1. Method of intact band depletion . . . 196
      6.2.2. Method of moments . . . 197
7. Conclusions . . . 200
8. Acknowledgements . . . 200
References . . . 200

* Formerly of Brookhaven National Laboratory, Upton, NY 11973, USA.

0378-4347/93/$24.00

LIST OF ABBREVIATIONS

A/D	Analog to digital
CCD	Charge-coupled device
ds	Double stranded
kb	Kilo bases
Mb	Mega bases
PCR	Polymerase chain reaction
ss	Single stranded

1. INTRODUCTION

The quantitative analysis of DNA enzymic digests using gel electrophoresis plays an important role in the study of nucleic acids, and is hence frequently encountered in fields such as molecular biology, photobiology, genetics, medicine, and the forensic sciences. Quantitative electrophoresis of DNA include determination of the copy number of genes and copy number of plasmids, enumeration of various lesions in DNA for use in damage/repair studies, the amount of DNA produced in polymerase chain reactions (PCRs), and mapping/sequencing DNA. Although using electrophoresis for analyzing charged molecules is an established practice, techniques for quantitation in electrophoresis are relatively new. Often, problems arise in analyzing nucleic acids which cannot be solved using a purely qualitative electrophoretic approach, but can easily be solved using quantitation.

Advances in biotechnology and computers have made the routine analysis of DNA digests using electrophoresis accurate, quick and easy. For their part, computers have had a significant role in the development and practice of electrophoresis, and in many laboratories they are used to control the electrophoresis process through interfaces, or to perform numerical analyses too tedious or time-consuming to be done manually. Furthermore, the growth in the understanding of the electrophoresis process in itself generated through the interest in mapping and sequencing the human genome — has led to electrophoretic methods for the separation of DNA sizes from a few bases to over several million long.

Gel electrophoresis is the method of choice for analyzing enzymic digests of DNA because of the ease in separating DNA in a size-dependent manner. However, to perform a thorough quantitative analysis of DNA digests using electrophoresis, the following important factors in the overall process must be considered carefully: (1) the electrophoresis process itself; (2) the nature of the DNA distribution of lengths; (3) the label for visualizing DNA; (4) the technique for detecting and/or quantitating the label; and, finally, (5) the analysis itself. We will address all these factors in order.

2. ELECTROPHORESIS

2.1. General considerations

The electrophoresis itself forms a vital part of the analysis, because it is the fundamental physical process by which length-dependent separation of DNA is achieved through differential mobility in the support matrix. DNA electrophoretic mobility — using gels as a support matrix — depends on several parameters such as the length and tertiary form of the DNA, the pore size and material used to form the gel matrix, the composition of the electrophoresis buffer, the temperature during electrophoresis, and the nature and magnitude of the applied electric field. In rare instances depending on DNA length and base composition — curvature of linear DNA can take place [1]. This is evidenced as an anomaly in the mobility of certain short DNA fragments under electrophoresis.

The simplest manner in which DNA in gels may be electrophoresed is by the application of a steady external uniform electric field. In this arrangement, the field is applied to the gel matrix using two electrodes, one serving as cathode and the other as anode. The gel matrix is itself saturated with a suitable buffer to maintain a stabilizing ionic environment for the DNA. DNA migrates towards the anode because of the electrical driving force on the negative phosphate groups. By varying the pore size of the support material, it is possible to separate effectively DNAs ranging from 1 base to 100 kilo bases (kb) in length. For lengths greater than approximately

100 kb, reptation of DNA occurs, and the length-dependent differential mobility during electrophoresis is lost.

Long DNAs (exceeding 100 kb in length) are effectively separated using modulations in the applied electric field which prevent DNA from undergoing reptation. This extends the upper limit of DNA separation to several mega bases (Mb) in length. A number of schemes have evolved to reduce DNA reptation during electrophoresis by cyclical re-orientation using field modulations. Choice of specific implementation is governed by factors such as the cost, simplicity of operation, resolution, and total run-time. It is beyond the scope of this article to discuss these systems in detail but the reader is directed to the necessary literature in this area [2–6].

There are two fundamentally different modes of obtaining a separation of DNA in electrophoresis: constant time and constant distance. In the first, all the electrophoresed DNA is detected at the same time and the separation in DNA lengths is seen as a spatial one. This would correspond to taking a snap-shot of the electrophoresis process at the end of separation. In the second type, DNAs of varying lengths are detected at a fixed distance by a detector. As a result, the separation of the DNAs is detected temporally, and the physical size of the support matrix can be reduced to some extent. The description of the electrophoresis process in either of these can be readily obtained by transformation of the known for the other. In view of this, only the mathematical formulation for the analysis of DNA dispersed using constant time electrophoresis is discussed.

Determinations of the conditions and schemes required to effectively produce DNA length separation in an enzymic digest of DNA requires knowledge of the lengths present in the digest. After choosing a particular scheme for electrophoresis, a certain amount of trial and error is involved in the final selection of pore size and running conditions. Few refined analytical techniques are worthwhile if the fundamental physical process is experimentally flawed. DNA sample preparation and enzymic digestion, sample loading, preparation of the matrix to insure uniform pore size and gel geometry (excluding deliberately introduced gradients in pore size), electrophoresis running conditions and visualization of the DNA are important to the overall analysis [7].

2.2. Dispersion functions

The purpose of using electrophoresis is to disperse a DNA digest in a length-dependent manner. In practice, the length of the DNA molecules present in the digest are obtained by generating a gel dispersion (or gel calibration) function using suitable co-electrophoresed DNA length standards. A wide range of dispersion functions have been devised to do this. In general these formulae seek to describe the length, L, of DNA as a function of the distance (from the sample well or some arbitrary origin), x, by generating a dispersion function, $L(x)$. These functions are usually based on global or local numerical fits obtained from the co-electrophoresed DNA length standards [8–13].

Dispersion functions are calculated in a parametric form from the locations of the co-electrophoresed DNA length standards. Global dispersion functions are derived by varying the parameters to reduce the difference between the dispersion function and the data from all the DNA size standards. This type of dispersion function is useful when the nature of the electrophoresis process is functionally characterized. Fewer DNA size standards are required for a global dispersion function to be accurate, and it is less sensitive to random error. However, global dispersion functions can lead to systematic errors if applied to a form of electrophoresis where the dispersion of DNA may be different. For example, global dispersion functions which are derived for electrophoresis using a uniform steady external field should not be used to describe electrophoresis under field modulation.

Local dispersion functions are more accurate when dealing with a diversity of electrophoresis field conditions, but are more sensitive to random error. To retain accuracy, these dispersion functions must be generated from a large number of

DNA length standards. A classic example of a local dispersion function is that based on a cubic-spline. Researchers have tried to combine the advantages of both types of dispersion functions — global and local — by creating a moving window consisting of a subset of the DNA size standards, and solving for the dispersion function (within this window) using a global function [10]. These forms effectively combine the advantages of both types of dispersion functions while minimizing the disadvantages.

For an accurate estimate of DNA length, standards should be placed near the DNA which is being analyzed. The effect of variations in electrophoresis over the bulk of the gel can be corrected to some extent by using spatial normalization [14]. However, this approach should be applied only after sufficient control and precautions are used in electrophoresis to ensure uniform migration.

2.3. Resolution in electrophoresis

Resolution is the ability of an electrophoretic system to discriminate between DNA of similar lengths. It is an important parameter to study because the electrophoresis of a homogeneous DNA sample results in bands of finite width (rather than band intensity distributions described by a delta function), which limits the number of DNA species that can be identified at the same time. In electrophoresis, resolution may be expressed [15] using the resolution length limit (R_L), or an extension of the same idea called the resolving power [16]. The resolution length limit takes into account the finite band width of a homogeneous DNA after undergoing electrophoresis and is derived from the closest two bands — corresponding to similar lengths — that can approach one another and yet be distinguished. It is easy to see that in the case of DNA intensity distributions which resemble two identical Gaussians, the separation necessary for resolution is equal to their widths (or full-width at half-maxima), I'. Using the dispersion function, $L(x)$, of the system, R_L can be expressed as $|\mathrm{d}L(x)/\mathrm{d}x| \cdot I'$. The resolving power which is based on the similar idea is the ratio of the length of DNA, L, to the R_L, *i.e.* L/R_L.

Resolving power is a parameter which is dimensionless and, as a result, comparison between different systems is easy. Also, increasing resolving powers correlate well intuitively with higher resolution. From an experimental point of view, the electrophoresis conditions should be chosen with optimal resolution for the range of DNA lengths present in the digest.

Experiments using restriction fragments under steady uniform electric field conditions indicate that the resolving power is a function of the length of DNA, and that it exhibits asymptotic behavior if the electrophoresis distance is increased [10]. Thus, the improvement in resolving power is nominal if the length of the gel used in electrophoresis is increased beyond a certain point. The effect of reducing the well thickness (which is easily done by using thinner combs in forming the gel) for electrophoresis serves to shorten the distance — and hence the time — to achieve the resolving powers approaching asymptotic limits. We will see the important role that the limitations of resolution play in analyzing restriction digests where the length and number of all DNA daughter fragments are required.

3. DNA LENGTH DISTRIBUTION

The suitability of a technique for analyzing a particular enzymic digest of DNA is strongly related to the DNA length distribution present in the digest. Mathematically, DNA length distributions will always be strictly discrete because the length of DNA is an integral number of bases; we can classify them in a different manner because of limited resolution.

For classification, consider a sample of identical single-stranded (ss) or double-stranded (ds) DNA molecules of length L_0. If this sample is enzymatically digested to produce a mixture of DNA molecules of specific lengths, we can treat the final digest as a discrete DNA length distribution. On the other hand, if the original DNA sample is enzymatically digested to produce DNA molecules of any size up to a maximum of L_0, the digest may be treated as a continuous distribution of DNA lengths.

An excellent example of a discrete DNA length distribution is a homogeneous ds DNA sample digested by a type II restriction enzyme. Here, the population of DNA daughter fragments in the digest is comprised of specific DNA lengths, and is dictated by the recognition sequence of the enzyme and the base sequence of the DNA substrate. An electrophoresis of these digests may or may not entirely resolve all the daughter fragments. But since we know the type of DNA length distribution beforehand, we can greatly simplify the analysis of these digests. Discrete DNA length distributions are frequently encountered in molecular biology. They are characterized by distinct bands on an electropherogram in which the DNA has been suitably visualized using a label. These distinct bands are due to the specific lengths of DNA present.

Continuous DNA length distributions arise in DNA damage and repair studies where lesions due to ionizing and non-ionizing radiation are quantitated. If we digested a UV-irradiated homogeneous ds DNA sample with a UV endonuclease [17], and subsequently denatured it into ss DNA, we would generally obtain a continuous distribution of DNA lengths. Distributions of this type are more effectively analyzed using mathematical techniques which employ moments such as the number average or length average of the DNA distribution [18], or the method of intact-band depletion, both of which are readily possible after electrophoresis. Continuous distributions are evident on electrophoresis by the presence of smeared patterns which are skewed in the direction of electrophoresis.

In the laboratory it is difficult to create pure DNA length distribution, and it is not uncommon to observe different degrees of distributions tending to the discrete or continuous categories. In reality, all restriction digests are to some degree a combination of both DNA length distributions and are hence mixed. For example, if the type II restriction enzyme (cited as an example of a discrete distribution) had some residual non-specific enzymic activity (they all have some to one degree or another), this would be apparent as a banded pattern compounded on a background smear. Even though categorization of the DNA length distribution indicates the best line of approach, the overall DNA analysis, from the electropherogram, must always make realistic corrections for nonidealty.

4. LABELING DNA

The object of labeling DNA is to provide a means of visualizing and/or quantitating DNA. Ethidium bromide fluorescent staining and radioactive labels have been in widespread use for visualizing ds or ss DNA. In cases where the sensitivity required is that for detecting nanogram quantities of DNA, ethidium bromide staining will alone suffice. For detecting smaller amounts of DNA, typically in the picogram range, radioactive labeling is the traditional approach. For quantitative use of any DNA label, it is essential to know how the label is incorporated in the DNA.

While chemiluminescent [19,20] and other fluorescent labels such as those used in sequencing applications are not commonly used in the laboratory to analyze enzymic digests of DNA, they are growing in popularity. Although these labeling techniques use specialized equipment for detecting the label, they often provide distinct advantages with regard to sensitivity, personal safety and environmental pollution. The reader is encouraged to explore these. For purposes of analysis we concentrate on the properties of traditional DNA labels. Many of the complications which arise in using traditional labels for quantitative electrophoresis will also be manifested in the newer labeling schemes. Hence, suitable prior tests should be made to assess the overall accuracy for DNA quantitation when using alternative labels.

4.1. Ethidium bromide

Ethidium bromide is a fluorescent dye which intercalates between the bases in DNA. The fluorescence quantum efficiencies (measured at 302 nm) of ds DNA-bound ethidium is about 21 times greater than of the unbound dye, because of

the reduction of fluorescence quenching in the hydrophobic regions present between adjacent bases. For analytical work it is best to stain the DNA with this fluorescent dye after electrophoresis because it can alter DNA mobility in electrophoresis [21]. This alteration results from the increased rigidity and change in effective charge of the DNA dye complex.

In gels which do not denature DNA (pH $\simeq$ 8), typical staining conditions are to soak the gel in a 1 μg/ml solution of ethidium bromide (in distilled water or electrophoresis buffer) for about 5–30 min. During this time the dye permeates the gel matrix and intercalates with the DNA [22]. After the initial staining period, the dye is destained (using distilled water or electrophoresis buffer) for about 30 min to 2 h, during which excess dye that is unbound to DNA diffuses out of the gel, leaving behind the ethidium bromide-intercalated DNA. This results in fluorescently-stained DNA with low background gel fluorescence. More elaborate schemes for destaining ethidium bromide exist. These involve quickly electrophoresing the dye out of the gel so there is no excess, or including activated charcoal in the destaining step to adsorb the excess dye [23].

DNA which is fluorescently-stained using ethidium bromide can be visualized by transilluminating the gel with long- or short-wavelength UV light. The UV light excites fluorescence which has a peak at approximately 590 nm. Photography or charge-coupled devices can be used to record the fluorescence of ethidium-stained DNA [24,25]. The fluorescence from a given band of stained DNA is proportional to its mass. For short DNAs the fluorescence is likely to show marked variation in this depending on base sequence because the dye intercalates with AT and GC regions in different amounts [26]. Also, the tertiary structure of DNA affects the amount of ethidium bromide which intercalates with it. Thus, direct quantitation of DNAs of different tertiary structure on the same ethidium-stained gels cannot be made purely on the basis of the fluorescence.

4.2. Radioactive labels

Radioactive labels for DNA have traditionally been used for visualizing and/or quantitating DNA present in picogram quantities or less. In this method, the label is either incorporated at the ends or essentially throughout the DNA by using one or more labeled (hot) nucleotides. The label itself is usually comprised of unstable nuclei such as ^{14}C, ^{32}P, ^{3}H, or ^{35}S. Since these nuclei have different half-lives, it is important for the DNA incorporating the short-lived ones to be quickly quantitated in order to obtain DNA signals with low background. Methods for recording radioactive labels for DNA in gels include the use of scintillation counters, autoradiography with X-ray film, multi-wire proportional counters and more recently, photostimulable phosphors.

5. TECHNIQUES FOR DETECTING AND QUANTITATING DNA LABELS

5.1. Quantitating fluorescent labels using photographic film

Images of fluorescently-labeled DNA have been recorded for decades on photographic film. The advantages of using film are its simplicity, the lower initial cost and the high resolution obtainable. For quantitative purposes, these are offset by added complications such as non-linearity of the film response to light, reciprocity failure accompanying long exposures to record weak fluorescence, the small dynamic range (typically 50–100), and factors that affect the developed density of the recorded image: development temperature, time, and variations from batch to batch of film.

Recording images of fluorescently labeled DNA without any detailed image processing or calibration is adequate for obtaining the spatial information alone. In the quantitative aspect, however, film is severely limited. With proper techniques and precautions, though, it is possible to quantitate DNA with reasonable accuracy using film [7,24].

The response of photographic film to light

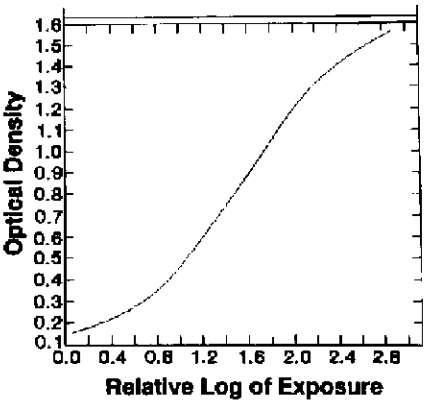


Fig. 1. Optical density as a function of exposure for Polaroid type 55 film (based on manufacturer's data sheet). From ref. 7 with permission.

should be considered before any specific method using film to quantitate DNA is elaborated. The film response may be broadly described in terms of the developed optical density as a function of the logarithm of the light fluence (energy per unit area) at a given wavelength. This behavior is illustrated in Fig. 1 for Polaroid Type 55, a popular instant film used for imaging fluorescently-labeled DNA in gels. The response to visible light is sigmoidal. Important characteristics in this response for quantitation are the toe, the slope of the linear region, and the height of the shoulder of the response curve. The optical density level at the toe determined the lowest amount of fluorescence light which can be quantitated, while the shoulder dictates the highest amount of fluorescence light before saturation sets in. The linear region between the toe and the shoulder is where accurate quantitation is possible.

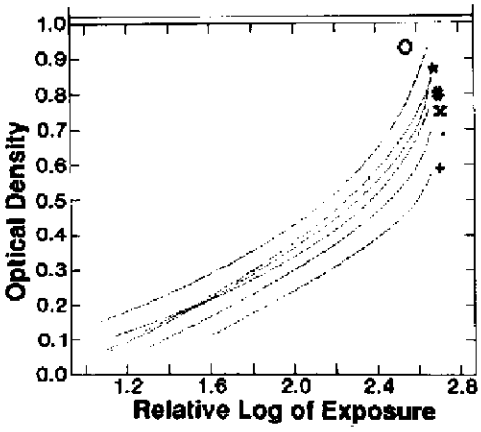


Fig. 2. Optical density as a function of exposure for Polaroid type 55 film. Exposure times used were (○) 10 s, (•) 20 s, (#) 40 s, (×) 80 s, (·) 160 s, and (+) 320 s. The amount of light falling on the film was kept constant. From ref. 7 with permission.

The response of the film is substantially altered by reciprocity failure when long exposures are required to capture weak fluorescence. This is demonstrated in Fig. 2. In situations where reciprocity failure can occur, it is more accurate to calibrate the film *in situ* while the fluorescence is being recorded [7]. This is easily accomplished using a fluorescent object and a calibrated step-density filter. The toe in the response can also be eliminated by pre-fogging the film; this permits accurate quantitation of low-level fluorescence. *In situ* calibration is also effective to reduce the variations in film calibration which arise from different developmental conditions, and can be used to utilize a larger region of the characteristic curve.

Accurate quantitation of low fluorescence is essential in analyzing DNA in gel assays for DNA damage and repair studies. Figs. 3 and 4 show the various steps essential for accurately quantitating ethidium bromide-stained DNA on agarose gels using photographic film. Indicated in these are (i) a filter for permitting transmission of the range of wavelengths associated with ethidium bromide fluorescence, (ii) a step-density filter for *in situ* calibration, and (iii) a fluorescent object for pre-fogging of the film.

The transilluminating source also plays an important role in the overall accuracy in quantitation when using a fluorescent label. This is because the fluorescence excited is proportional to the intensity of transillumination. It is therefore advisable to use either a region of the transilluminator surface that emits a uniform intensity of exciting radiation, or a specially designed transilluminator which emits uniform excitation. Uniformity of excitation can be easily tested (and to some extent corrected) by using an image of a fluorescent object. The intensity of the exciting source should not be attenuated significantly by DNA. This sets an upper limit on the amount of DNA which can be quantitated accurately. It is

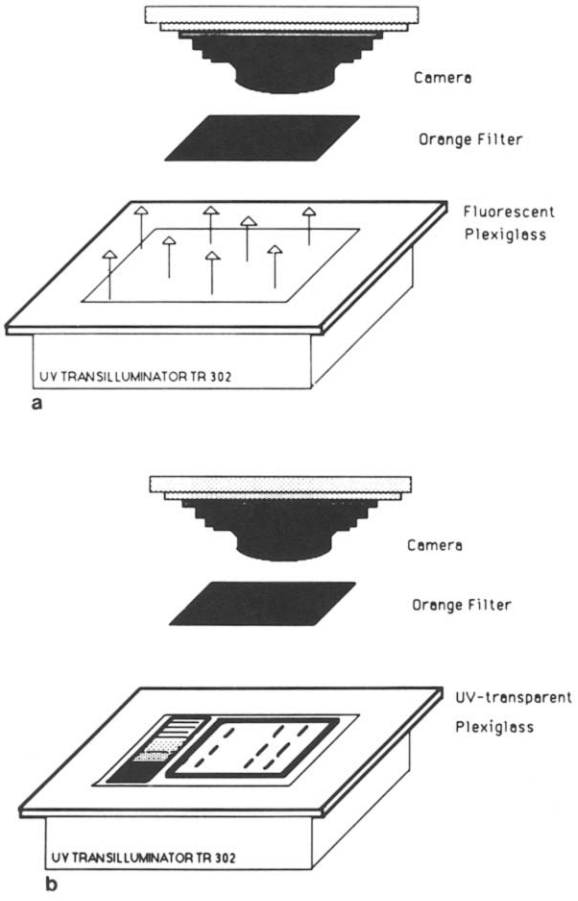


Fig. 3. Steps in recording the fluorescence from a gel: (a) step 1, pre-fogging (5 s × f/32); (b) step 2, superimposing fluorescence of gel on the pre-fogged film. From ref. 7 with permission.

also essential to have properly designed optics. These include a flat-field lens having the requisite uniform light collection efficiency.

5.2. *Quantitating fluorescent labels using charge-coupled devices*

Charge-coupled devices (CCDs) have quickly found application in science and technology, and are excellent for quantitating fluorescence light of ethidium bromide-stained gels [25]. They are currently available for detecting and quantitating light ranging from infrared up to and beyond the ultraviolet. A CCD fundamentally consists of an array of photosensitive elements created and arranged in a one- or two-dimensional array, using integrated circuit technology. Within each element, incident photons create a charge proportional to the incident flux. The linearity between the charge created in an element and the number of incident photons holds over a wide dynamic range (10^4). After exposure to incident photon flux, the charge in each element is read electronically by shifting out the charge into adjacent elements in a coupled fashion. The charge in the light-sensitive elements may then be depleted, making the recording of another image possible. There are a plethora of CCDs available. The best of these are cooled for noise reduction and will

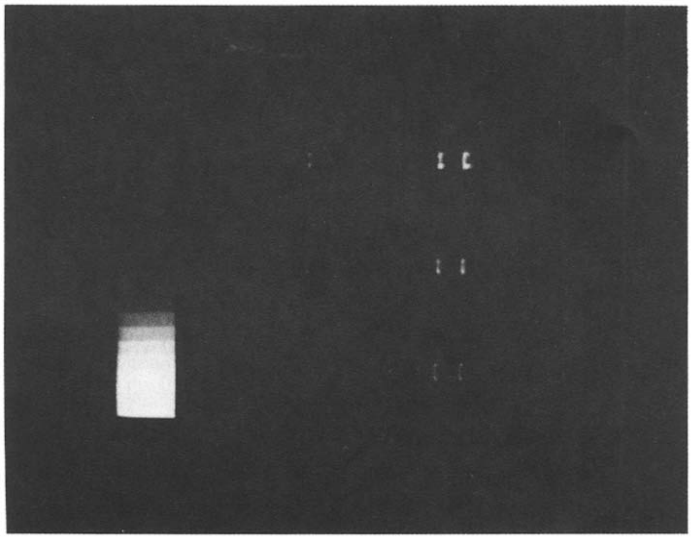


Fig. 4. Photograph with images of the step filter and a gel containing a BglI endonuclease digest of T7 DNA. From ref. 7 with permission.

allow large integration times (time during which the CCD collects light) for quantitating low-level fluorescence in ethidium-stained gels. There are reports in the literature for using these devices to record fluorescent and chemiluminescent labels.

The photometric resolution of CCDs is governed by the number of bits used in the analog-to-digital (A/D) converter for measuring the charge in each photosensitive element. Typically 12–14 bits would be suitable for quantitating fluorescence from stained DNA. The ability to record low-level light is dictated by the electronic noise because it limits the usable integration time.

Advantages of using CCDs for quantitating fluorescence are the rapidity of recording images of fluorescently-stained gels and the wide dynamic range and linearity of these devices. Images produced with these devices can be manipulated electronically and processed with digital computers. These advantages are offset by the higher initial cost incurred and lower resolution obtained than with photographic film. Considering, however, the ease of use, speed, and convenience of electronic imaging, it presents a more attractive solution to analyzing fluorescently-labeled DNA on gels than photographic film.

5.3. *Quantitating radioactive labels using X-ray film*

Visualization of radioactive labels is normally done by autoradiography in the case of electrophoresis (rarely by using scintillation counters). The gel and film are placed in proximity in the dark at low temperatures with an intensifying screen to enhance sensitivity; the duration depends on the label and activity. After the film is developed, regions of the film exposed to radiation label show up as an increase in film optical density. The overall process results in a blurring of the image because of the absence of external focusing elements, and resolution is invariably compromised. The optical density of the developed film is seldom linearly proportional to the amount of label present. This makes corrections an essential part of obtaining quantitative information from the autoradiograms. Also, the dynamic range is severely limited (about 100), making the simultaneous detection of large and small amounts of DNA label difficult.

Since the absorption of α and β particles is low in the film emulsion, X-ray film is coated with photographic emulsion on both sides and used in conjunction with an intensifying screen. Exposures may take hours to several days, depending on the strength of the label. The non-linear response of the film to radioactivity has to be corrected when using this medium to record DNA label. Typical characteristic curves involve plotting the optical density of the developed film as a function of the exposure (total counts) or logarithm of exposure. The advantage of using the logarithm of the exposure is that the response exhibits a greater linear region when plotted this way [27]. Sensitivity of the response can be increased by pre-flashing [28] X-ray film prior to exposure to radioactive labels, and by exposing the film to radioactive label at low temperatures to reduce the effect of reciprocity failure when using an intensifying screen. Care must be taken to develop and fix the film in a controlled manner, and *in situ* calibration standards are highly recommended. Densitometry can be performed by techniques similar to those used for photographic film.

5.4. *Quantitating radioactive labels using photostimulable phosphors*

Due to recent advances in technology, it is now possible to record radioactive labels using photostimulable phosphors [29,30]. These systems have a wide dynamic range and linear response. The initial investment in such a system is considerably higher than that of autoradiography. However, since the images generated from these systems are erasable, the overall working cost, depending on the volume of work, may make it an appealing alternative to autoradiography.

Photostimulable phosphors can be exposed to radioactive label by placing the gels in close contact with them in the dark. The particles emitted from the radioactive label lose their

energy in the phosphor through the creation of color centers in the specially prepared phosphor. The trapped energy is released when the phosphor is stimulated with a light source of suitable wavelength. In commercially available photostimulable phosphor systems, the light used to release the trapped energy is often in the red–infra red region of the spectrum, while the emitted light from the phosphor is in the blue. These systems offer distinct advantages such as reduced time to capture an image of the radioactivity (up to ten times faster than using X-ray film), much higher dynamic range (10^5 as compared to 10^2 in X-ray film) and sensitivity (factor of about 10–100), as well as a reusable (and erasable) medium with resolution better than X-ray film. Some phosphor-imaging systems may even be used to record chemiluminescence with the same phosphor plate, but using specially prepared phosphors which produce color centers from energy in the visible portion of the spectrum.

Multi-wire proportional counters [31] are another alternative to autoradiography for recording hot labels. The spatial resolution of these proportional counters is poor when compared with film or photostimulable phosphors, and these systems can only image a small region because of size limitations of the detector. In comparison to film, however, multi-wire proportional counters offer improved dynamic range and linearity.

6. METHODOLOGY OF ANALYSIS

DNA distribution on slab gels undergoing electrophoresis along one direction can be represented by a mass density function $\rho(x,y,z)$, where ρ is the number of base-pairs or bases per unit volume at the points specified by x, y, and z in the gel. The x-axis is set parallel to the direction of electrophoresis, the y-axis is prependicular to the direction of electrophoresis and in the plane of the gel, and the z-axis is perpendicular to both the direction of electrophoresis and the plane of the gel. With this particular orientation of axis (refer to Fig. 5), the dependence on the spatial coordinates of the mass density function can be further simplified by integrating along the z-axis by w, the thickness of the gel, and between two boundaries y_1 and y_2, chosen to include all the DNA in a specific lane. Under these conditions the DNA density distribution may be written as $\rho(x)$, and is often called the lane profile.

The density distribution of DNA is observed by using labels discussed above. These labels provide a signal which is correlated to the DNA distribution present on the gel. It is important to understand clearly the manner in which a label is incorporated in an enzymic digest of DNA. For example, a radioactive label can be incorporated by end-labeling or by labeling throughout the DNA. In the first case of end-labeling, the signal corresponding to the label would be independent

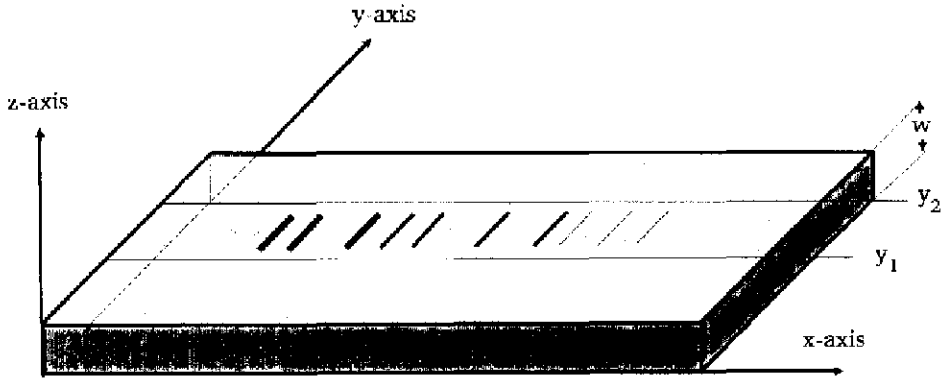


Fig. 5. Orientation of axes to describe electrophoresis. Also shown are boundaries y_1 and y_2 used to create a lane profile of the DNA.

of the length of the molecule, but proportional to the number of DNA molecules, while in the latter, the signal would be proportional to the mass of DNA. The overall analysis must be cognizant of these differences, as well as the effect that the overall response of the system used for detecting the label will have on the quantitation.

6.1. *Digests comprised of discrete DNA size distributions: applications to resolving restriction digests*

Restriction digests of linear, supercoiled, or relaxed DNAs give rise to discrete size distributions of DNA. The interest in these digests has grown considerably in the past decade as a result of the interest in mapping and sequencing DNA. Restriction digestion provides a way to break up long DNAs into shorter pieces which can then be sequenced by conventional methods such as those developed by Sanger *et al.* [32] or Maxam and Gilbert [33]. The process of unravelling the order in which the individual DNA fragments in a digest occur within the intact DNA molecules becomes easier if accurate length measurements of the DNA fragments in the digest are known.

When DNA digest fragments are completely resolved, the lengths of DNAs present on a gel can be obtained easily using a set of suitably chosen size standards, with no need for additional quantitative methods. If a digest remains unresolved despite attempts to change the pore size of the gel matrix or to alter running conditions, a quantitative approach provides a solution to determine the sizes of DNA present. Fig. 6 shows an electropherogram of a restriction digest of Bacteriophage T7 DNA using the following restriction enzymes: *XbaI*, *NsiI*, *AvaI*, and *HaeII* [34]. The overlapped DNA in each electrophoresed digest lane can be identified from the anomaly in band

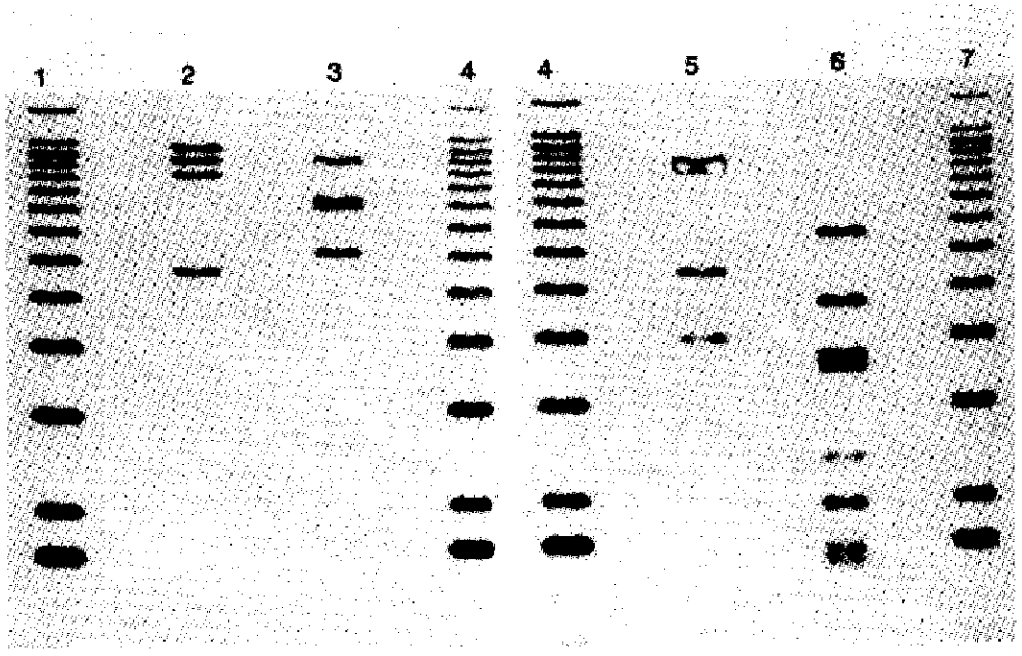


Fig. 6. Photographic prints of two images of overlapping regions of a gel obtained with CCD imaging. Lanes 1, 4, and 7 contain DNA length standards. Lanes 2, 3, 5, and 6 contain DNA from Bacteriophage T7 that has been treated with the restriction endonucleases *XbaI*, *NsiI*, *AvaI*, and *HaeII*, respectively. The images are overlapped so that lane 4 appears in each. The dispersion function used to calculate the length of molecules in bands of "unknown" lanes is always determined from a lane of length standards that appears in the same image as the unknown. The photographic print was made from a color negative that recorded the pseudocolor image on the gel fluorescence displayed on a TV monitor. Apparent curvature in some of the lanes results from photographing the curved surface of the monitor. From ref. 34 with permission.

width; however, the number of fragments in each overlap get progressively difficult to estimate. The *HaeII* digest of Bacteriophage DNA exemplifies this. For resolving these types of DNA band overlaps, two quantitative approaches are presented. The first is a general procedure to recover resolution in electrophoresed DNA, but the second one is more powerful in the specific case of DNA digests by restriction enzymes which have been carried out to completion. The techniques developed for the electrophoretic analysis of enzymic digest of DNA can also be extended to blotting techniques which transfer DNA from the support matrix to a membrane and then highlight certain species of DNA present on the membrane by using probes.

6.1.1. *Iterative method using determined point spread function*

In this approach [35], a smearing/broadening function of electrophoresed DNA bands is computed from standards on the same gel used for analysis of the unknown DNA digest. The observed lane profile $o(x)$ of the digest in a lane may be represented as the convolution of an ideal profile $i(x)$, with a smearing function $s(x)$ which serves to broaden the DNA distribution, thus yielding a distribution similar to that obtained by electrophoresis. Mathematically the convolution may be represented as:

$$o(x) = \int_{-\infty}^{+\infty} i(x-u)\, s(u)\, du \qquad (1)$$

An iterative process is used to yield progressively improved estimates of $i(x)$ by comparing the lane profile $o(x)$, predicted from eqn. 1 to that experimentally observed. When the comparison shows excellent agreement, the iterative process is terminated, and the last trial profile is a resolution of the digest. The iterative method improves resolution obtained from electrophoresis by mathematically reversing the band spreading that takes place during electrophoresis. The following equations outline the algorithm:

$$o^k(x) = i^k(x) * s(x) \qquad (2)$$

$$i^{k+1}(x) = i^k(x) + \gamma(x)\,[o(x) - o^k(x)] \qquad (3)$$

$$k = k + 1 \qquad (4)$$

where $*$ is the convolution above, and

$$\gamma(x) = 1 - A\,|\,o^k(x) - A\,| \qquad (5)$$

is used to apply non-negativity and maximum positivity constraints, with parameter A set to twice some approximation of the maximum peak height. The function $\gamma(x)$ is used to modulate corrections in the iterative process.

Refinement begins with setting $k = 0$, using an initial guess $i^0(x) = o(x)$, and an experimentally derived $s(x)$. Eqns. 2–5 are then used iteratively to yield progressively improved estimates of $i(x)$. When there is no significant difference between $o^k(x)$ and $o(x)$, the iterative process ends, and $i^k(x) = i(x)$. The algorithm can be sped a hundredfold by performing the convolution integrals numerically using Fast Fourier Transforms (FFT). Typical results for obtaining increased resolution are shown in Figs. 7 and 8 for an electrophoresed mixture of *HindIII* restriction fragments from digests of PM2 and SV40 DNA.

6.1.2. *Mass-constrained deconvolution*

The iterative approach will fail to resolve DNA bands which completely overlap one another. In the special case of resolving DNAs which have been digested to completion, the physical constraints relating the amounts of one particular daughter fragment of DNA to another in the digest can help resolve overlapped bands of DNA. For an enzymatic digest of identical DNA molecules made in a site-specific manner, the number of molecules or moles of different daughter fragments will be the same. If, after electrophoresis, we create a lane profile from an image of the labeled DNA (either fluorescent or radioactive), the integrated signal corresponding to the different DNA daughter fragments bears a useful relationship to the length. This relationship, mathematically expressed as a mass constraint, may be used to resolve the digest, and hence determine the total number of components and length of the digest fragments present. A detailed description of this method, including the necessary equations, is outlined below [34].

In the case of ethidium bromide-stained agarose gels, the technique is implemented in the follow-

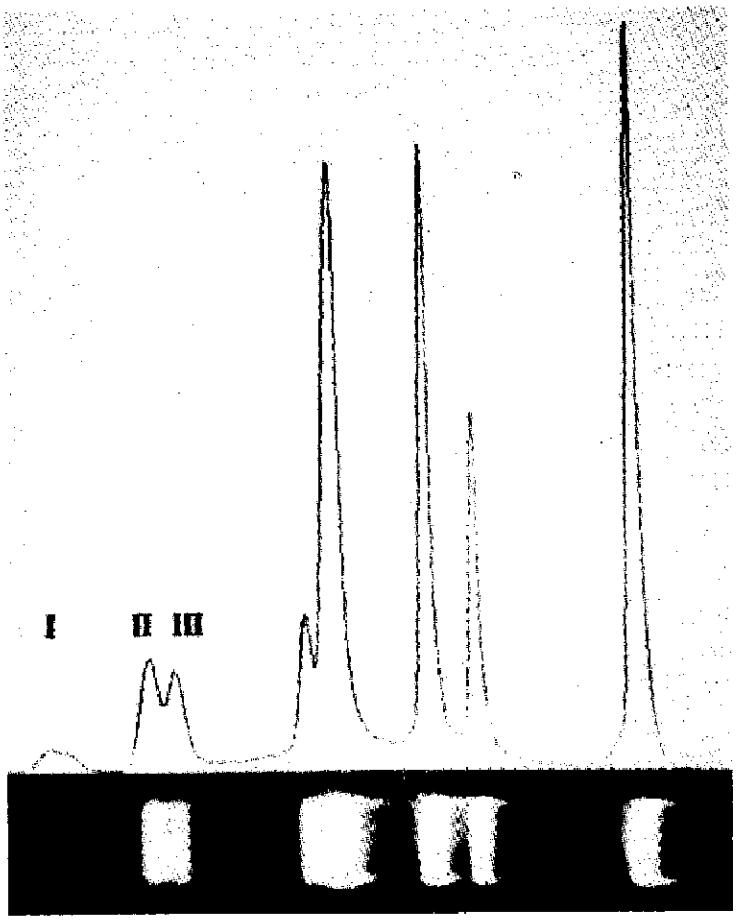


Fig. 7. Gel pattern and densitometer tracing for a mixture of PM2 and SV40 DNA run on an ethidium bromide agarose gel. The labeled peaks were chosen for further analysis. From ref. 35 with permission.

ing manner. Under suitable conditions the fluorescence of ethidium bromide is proportional to the number of base-pairs of DNA. The fluorescence, $F(x,y)$, excited in the electrophoretic gel (under uniform UV transillumination) is related to the DNA mass distribution. In terms of the notation previously introduced,

$$F(x,y) = K \int_{z=0}^{z=w} \rho(x,y,z)dz \quad (6)$$

where the thickness of the gel is represented by w. The constant K includes the effect of numerous experimental parameters such as the fluorescence quantum yield of ethidium bromide, the intensity of light for UV transillumination, the efficiency of the optical system used to record the fluorescence, duration of exposure, etc. It is critical to have a linear relationship between ρ and F. This is achieved easily when using CCDs for recording the ethidium fluorescence. If photographic film is used to record the fluorescence, the necessary corrections as described earlier to obtain $F(x)$ from the recorded optical density should be used.

Since the separation of DNA takes place parallel to the x-axis during electrophoresis, $F(x,y)$ may be replaced with $F(x)$, by integrating along the y-axis between two boundaries y_1 and y_2, chosen so as to include all the DNA (see Fig. 5). The limits y_1 and y_2 may be determined using a cursor on a video screen displaying the fluorescence light intensity in either pseudocolor, grey scale, or contour plots.

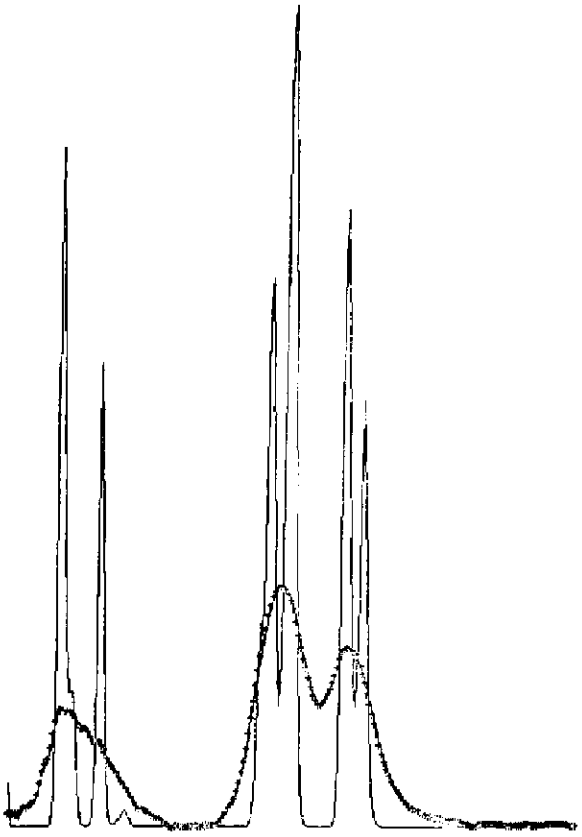


Fig. 8. Profile for the region of Fig. 7 containing bands I, II, and III after correction for increased band broadening as a function of migration distance, shown as a thick line. The same data after 200 cycles of constrained deconvolution are shown superimposed (fine line). The doublets in bands II and III are now resolved. From ref. 35 with permission.

Background fluorescence (non-DNA-specific fluorescence) in the gel may be subtracted from eqn. 6, using the following correction factor:

$$[F(x,y_1) + F(x,y_2)] \cdot \frac{y_1 - y_2}{2} \qquad (7)$$

The method of subtracting the background fluorescence in electrophoresis is related to some extent on how the DNA is quantitated. If photographic film is used for quantitating fluorescence-stained DNA, the method for subtracting background has to be altered slightly. This is because most commercial densitometers for scanning film usually do so in only one dimension. To correct for background fluorescence (non-DNA-specific), it is important to have two empty electrophoresed lanes bordering the lane in which the DNA is located. A densitometric scan of the bordering lanes is used for background subtraction. In the case of two-dimensional scanning of photographic film this requirement is relaxed; nevertheless, the additional space between DNA-containing lanes serves to reduce the spill of fluorescence due to scattering in the gel. This is important for quantitating weak DNA signals accurately.

The fluorescently-stained bands of DNA after electrophoresis may individually contain more than one daughter fragment. Bands comprised of purely one DNA daughter fragment are called singlets, while bands comprised of two daughter fragments are denoted as doublets, and then triplets, etc. The overall analysis proceeds in two major steps: determination of the multiplicity of electrophoresed DNA bands using an unconstrained deconvolution and the subsequent application of the mass-constrained deconvolution to these bands.

For an intact DNA molecule with $n-1$ restriction sites, the number of daughter fragments produced on complete restriction will be n, although only m of these may be resolved through electrophoresis alone. In practice, since some overlap may occur, m is less than or equal to n. The lane profile $F(x)$ may be represented by the following summation

$$F(x) = \sum_{i=1}^{i=m} \alpha_i \cdot \Psi(x,x_i,\sigma_i) \qquad (8)$$

where α_i is the area of the ith band, and Ψ a shape function with unit area and parameters x_i to specify location and σ_i to describe the width. Any fitting procedure can then be used to vary the total of $3m$ parameters in an unconstrained manner to fit the observed lane profile. A Gaussian may be chosen as a suitable shape function. The α_i values obtained from this fitting procedure are then used to determine the multiplicity of the observed band by finding the ratio of the area, α_i of the bands, to the average length, L_i, of the species in bands (from an experimentally constructed dispersion function, $L(x)$, and band locations, x_i). This ratio, called the length-normalized fluorescence, will be

lowest for singlets, roughly twice this for doublets, thrice for triplets, etc. The multiplicity of the bands can be established by these ratios. Should the lowest multiplicity of the electrophoresed bands be suspected to be a doublet, knowledge of the approximate length of the undigested molecule will be adequate for eliminating uncertainty. The presence of residual DNA fluorescence in the lane profile (DNA fluorescence due to non-specific digestion by the enzyme) may be corrected for by incorporating a slowly varying function in summation as described.

After the multiplicity, and hence n, has been determined, the resolution of the DNA digest to get all the daughter fragments on the gel can be rapidly accomplished by fitting the observed lane profile to the mass-constrained form of $F(x)$:

$$F(x) = K \cdot N \sum_{j=1}^{j=n} L(x_j) \cdot \Psi(x, x_j, \sigma(x_j)) \qquad (9)$$

Here N is the number of each distinct daughter fragment in the DNA digest, and the parameters for fitting are the n values of x_j and the product of K and N. The function $\sigma(x_j)$ is obtained from interpolation of the known width of the singlets in the digest lane. The constrained fitting procedure has fewer parameters than the earlier procedure for unconstrained fitting. The optimized x_j values are used in conjunction with the dispersion function to yield a complete resolution of the digest. Full details, including variations of the techniques in cases where $\sigma(x_j)$ is difficult to evaluate from singlets, have been presented in ref. 34. The method is illustrated in Figs. 9–13. It must be

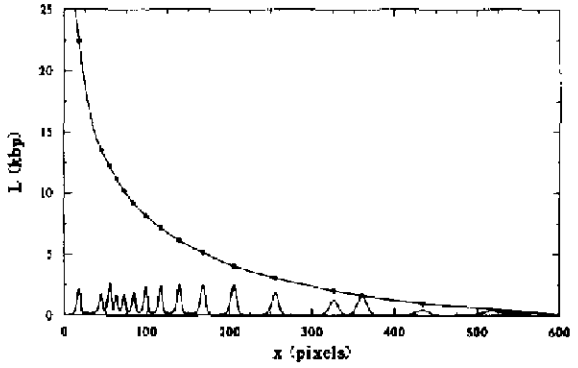


Fig. 9. Integrated lane profile and electrophoretic dispersion function from lane 1 in Fig. 6. The coordinates of the solid circles are determined from the location of the peaks of the lane profile and the known lengths of the molecules in each band. The dispersion function was determined from this set of coordinates by a cubic spline function procedure. The vertical axis is labeled in units of DNA lengths in thousands of base-pairs, which applies only to the dispersion function. The lane profile is expressed in arbitrary units which are not indicated in the figure. The horizontal axis is distance along the direction of electrophoresis, in units of camera pixel. For magnification used to acquire these images, each pixel corresponds to $\simeq 80\ \mu m$ in the gel. From ref. 34 with permission.

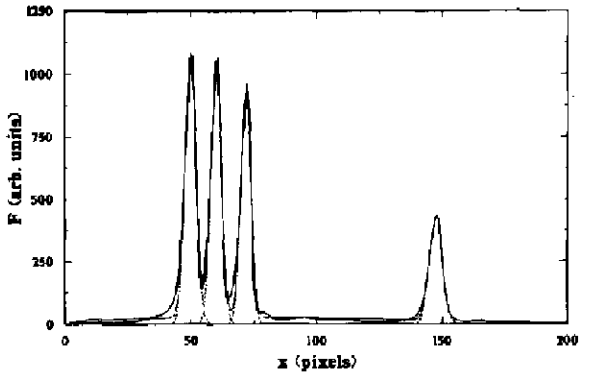


Fig. 10. Lane profile of lane 2 from Fig. 6, which contains T7 DNA digested by *Xbal*. The experimental data are connected by the thick solid line. The dotted line shows the resolved components and the light solid line shows the sum of the resolved components and the background correction. The length-normalized fluorescence of all four resolved bands are nearly equal, confirming that all are singlets. From ref. 34 with permission.

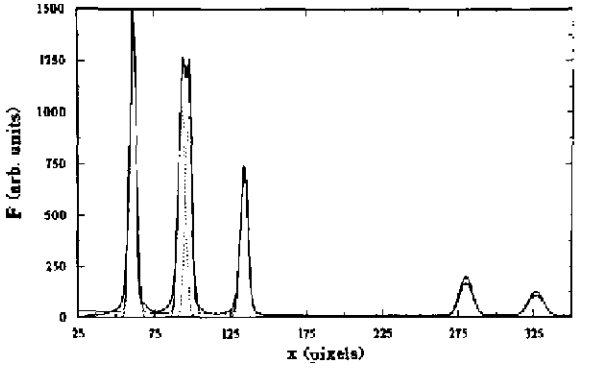


Fig. 11. Lane profile of lane 3 from Fig. 6, which contains T7 DNA digested by *NsiI*. The thick solid line, thin solid line, and dotted line have the same meanings as in Fig. 10. The length-normalized fluorescence of the second band from the left, which is obviously degenerate, indicates that it is a doublet. From ref. 34 with permission.

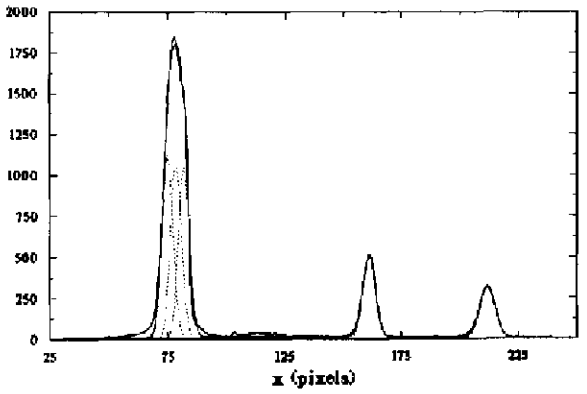


Fig. 12. Lane profile of lane 3 from Fig. 6, which contains T7 DNA digested by *AvaI*. The three types of lines as in Figs. 10 and 11. The left-most band in the figure is degenerated and its length-normalized fluorescence indicates that it is a triplet. From ref. 34 with permission.

pointed out that the mass-constrained deconvolution resolves a digest by using the constraints among the quantities of the DNA daughter fragments, and not by mathematically reversing the effect of band smearing which occurs during electrophoresis.

6.2. *Digests comprised of continuous DNA size distributions: applications to quantitating DNA lesions*

These distributions arise in quantitating a diversity of lesions in DNA that are frequently encountered in damage and repair studies, such as those produced by UV-induced thymine dimers [36], 6-4 photoproducts [37], and γ-ray-induced ss and ds breaks [38]. These lesions are either directly or indirectly (for example through enzymatic cleavage) made to yield a sample of DNA comprised of molecules of varying lengths. There are two major techniques to quantitate lesions from these distributions, both of which are extensions of methods used for centrifugal analysis of DNA [39].

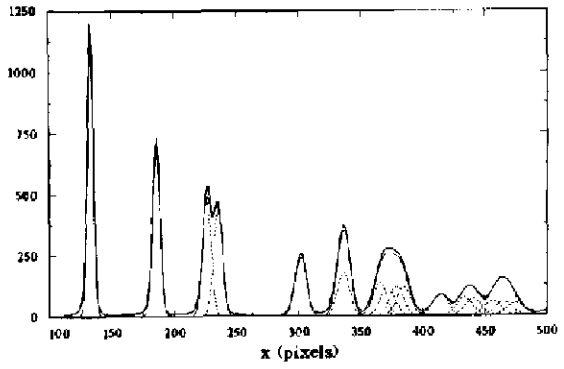


Fig. 13. Lane profile of lane 6 from Fig. 6, which contains T7 DNA digested by *HaeII*. The three types of lines are as those in Figs. 10, 11, and 12. From ref. 34 with permission.

6.2.1. *Method of intact band depletion*

Consider a sample of DNA comprised of a homogeneous population of molecules. If the probability of creating a lesion in a DNA molecule of the sample is small, but the number of molecules is large, the distribution of lesions is governed by Poisson distribution. If $P(n)$ is the probability of having DNA molecules with n lesions in a sample which has an average of μ breaks per DNA molecule,

$$P(n) = \frac{\mu^n e^{-\mu}}{n!} \tag{10}$$

If the original DNA sample without lesions has N molecules, the number of molecules which are intact in the population after introducing lesions is N_0, where:

$$N_0 = N \cdot P(0) = N \cdot e^{-\mu} \tag{11}$$

It is clear from eqn. 11 that, if the ratio of N_0/N is known, μ, the average number of lesions per molecule in the sample is easily obtained. In practice, two samples with the same amount of DNA are used for the determination of μ, one containing the intact DNA and the other one with DNA with breaks in it (depleted DNA). The breaks may be introduced directly by lesions or indirectly through enzymic digestion at the site of the lesions. On electrophoresis of such a sample, the DNA band corresponding to the lesions, will naturally have less DNA, because DNA molecules which have been broken will migrate further down the gel than those which have not been broken. The ratio of the number of DNA molecules in the lane containing the depleted band to

that containing the intact band may be used in eqn. 11 to arrive at the average number of DNA lesions per molecule in the sample.

In case of radioactive or fluorescent labeling, the integrated label signal corresponding to the intact band and the depleted band may be used for quantitation. This is permissible provided there is a linear signal response to the amount of DNA for both labels. In the case of fluorescent labels, CCDs can be used to record the signal of the DNA bands, while in the case of radioactive labels, photostimulable phosphors are excellently suited. The use of photographic film or X-ray film for quantitating labels is suitable only if the response to the labels is calibrated.

A minor variation of the intact band depletion technique is to determine the ratio of the amount of homogeneous DNA (corresponding to DNA of the same length) to the total DNA in an electropherogram in the same lane profile. If the recording medium used for quantitating the DNA label produces a response linearly proportional to the amount of the label, the ratio of the homogeneous DNA to the overall DNA gives N_0/N, and hence μ. In this approach, the requirement for accurate quantitative loading of DNA in samples is relaxed.

The signal corresponding to the intact band can be accurately obtained from the lane profile when a small number of breaks are introduced, but becomes inaccurate when a large number of breaks are introduced in the DNA electrophoresis. This is because the unbroken molecules in the DNA sample (corresponding to the homogeneous population) overlap with those DNA molecules having one or more lesions (corresponding to the heterogeneous DNA population). Numerical methods have been developed to recover the intact band from an entire lane profile. The technique is easily extended by the introduction of Southern blotting and suitable probes for use in specific gene damage and repair studies [40].

The intact band depletion method is useful for quantitating lesions in DNA. It is a good technique to use if a DNA sample contains a reasonably homogeneous population at the start. In addition, it does not require a dispersion function to be experimentally obtained for the electrophoretic process. For quantitating a large number of a certain type of lesions, however, this method gets progressively more inaccurate because the population of DNA molecules corresponding to the homogeneous DNA is difficult to isolate from the heterogeneous population. An alternative approach is to quantitate lesions in DNA using the method of moments, described below.

6.2.2. *Method of moments*

The method of moments may be used to determine the number of DNA lesions by computing the number average length of representative DNA distribution before and after the introduction of lesions. The technique has been successfully applied to DNA separated using centrifugal field. In this technique, no attempts are necessary to isolate the homogeneous and heterogeneous population of DNA, since the analysis is based on the entire electrophoresed DNA. The technique is capable of higher accuracy for quantitating a large number of lesions in DNA than the intact band depletion method, but requires knowledge of the dispersion function, $L(x)$, of the electrophoretic process. It has been shown [41] that the number of breaks per molecule introduced in a sample of DNA can be calculated from a change in the number average length of the DNA distribution by

$$\text{number of lesions} = \langle L_{ni} \rangle \left(\frac{1}{\langle L_{nf} \rangle} - \frac{1}{\langle L_{ni} \rangle} \right) \quad (12)$$

where $\langle L_{ni} \rangle$ and $\langle L_{nf} \rangle$ are the number average lengths of the DNA distributions before and after the introduction of DNA molecular breaks. These are calculated [42] from the DNA distribution itself in the sample from

$$\langle L_n \rangle = \frac{\sum_i (m_i L_i)}{\sum_i m_i} \quad (13)$$

where m_i is the number of moles of the DNA of length L_i in the DNA sample. The summations in eqn. 13 can be easily calculated from a lane profile of the fluorescence of electrophoresed DNA which

is ethidium bromide-stained. Under these conditions, the summations reduce to

$$L_n = \frac{\int_0^\infty \Gamma(x)\,dx}{\int_0^\infty [F(x)/L(x)]dx} \qquad (14)$$

because under suitable conditions, the fluorescence of ethidium bromide is proportional to the mass of DNA with which it intercalates. The technique of moments has been applied to quantitating UV-induced thymine dimers, for quantitating 6-4 photoproducts and for γ-ray-induced ss and ds breaks in DNA.

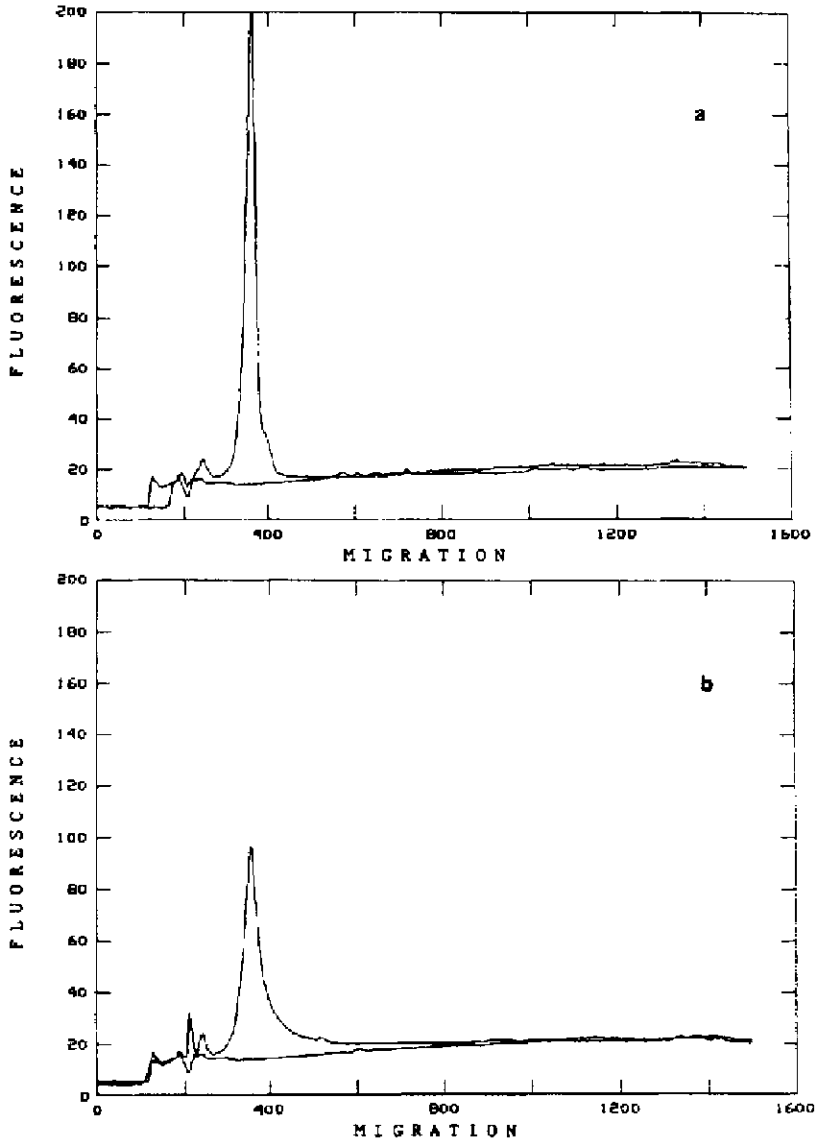
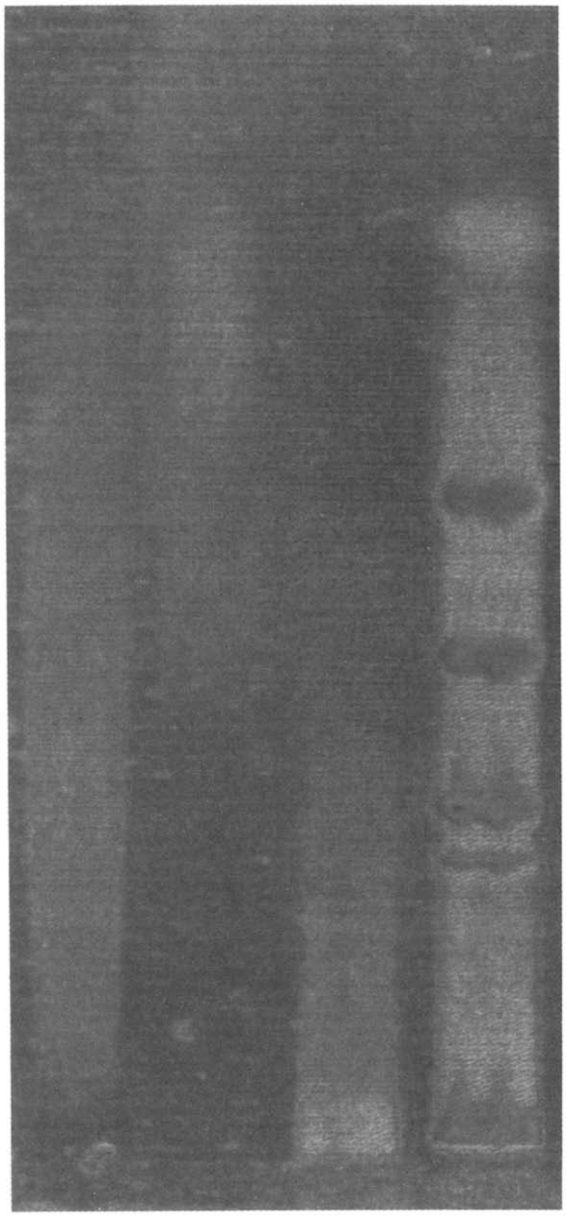


Fig. 14. One-dimensional scans of (a) unirradiated and (b) irradiated phage λ DNA. Purified DNA was exposed to fluence of 40 J/m^2 of 254 nm UV radiation and subsequently digested with *M. luteus* UV endonuclease at 37°C for 45 min. Film was exposed at f/4.5 for 20 s after it had been pre-fogged for 10 s at f/32. When the experimental background was subtracted from the lane profiles, the number of lesions per single strand of DNA were calculated to be 0.466. If an extrapolation from the trailing edges of the lane profile is used to estimate background, the number of lesions calculated are 0.113 per single strand. From ref. 43 with permission.

In practice, the limits for integration in eqn. 14 are determined by noting the locations in the resolving gel where the fluorescence in the sample lane falls to the background level [43], and by performing the integrations after correcting for background signal in the gel. The importance of these is illustrated in Fig. 14 where the number average length of Bacteriophage T7 DNA is calculated using photographic film with and without suitable background corrections. The numerical results are significantly different. The application of the method of moments to enumerating thymine dimers and 6-4 photoproducts in DNA on the same gel is illustrated in Fig. 15.



Instead of directly computing the number average lengths in the DNA sample, an alternative is to calculate the number average length from the length average length (also known as weight average), which is easier to calculate accurately because it is not as sensitive to the tail of the DNA lane profile. If random breaks are introduced in DNA, the length average length can be shown to be twice the number average length. However, the assumption may not be valid when the DNA exhibits hot spots for lesions. Conversely, under the same assumptions, the median of the DNA length distribution may also be used to estimate the number average length.

The accuracy and precision of using the intact band depletion method and the method of moments for quantitating lesions is dictated to a great extent by good sample preparation and careful electrophoresis. Sample overloading, trailing of DNA or DNA loads which produce low signal-to-noise ratio should be avoided. In the case of the methods of moments, the dispersion function should be computed from a suitable set of length standards. If precautions are strictly adhered to, both methods are capable of the necessary accuracy for quantitating lesions in DNA analysis.

Fig. 15. Photograph of an image of a portion of an alkaline agarose gel demonstrating the quantitation of UV light-induced damage in DNA. Lane 1 (extreme left) contains DNA standards of viral origin that are used to determine the dispersion function. They span a range of 170 kb (T4 DNA) to 4 kb (a restriction fragment from the T7 lane). Lanes 2–4 contain DNA from human cells that was exposed to 5 J/m^2 of 254 nm UV. Prior to electrophoresis, the DNA in lane 3 was treated to produce ss breaks adjacent to UV-induced pyrimidine dimers, and the DNA in lane 4 was treated to produce ss breaks adjacent to pyrimidine (6-4) pyrimidone photoproducts. The induction of ss breaks in the two treated lanes results in DNA molecules that are, on average, shorter when separated in the denaturing alkaline gel, and hence migrate further during electrophoresis. Quantitative electrophoresis permits determination of the frequency of lesions induced by a given UV dose. From ref. 37 with permission.

7. CONCLUSIONS

Quantitative electrophoresis of DNA is a rapidly expanding field because of its numerous applications in the life sciences. Problems that cannot be solved using a purely qualitative approach can often be easily solved quantitatively. Recent advances in instrumentation and biotechnology make a diversity of systems available for accurate quantitation in electrophoresis. Such systems will eventually replace photographic and X-ray film for quantitating DNA visualized by fluorescent/chemiluminescent and radioactive labels. Although these systems require a sizable initial investment, the volume of electrophoretic data and the speed at which that data can be accumulated tend to justify such investment for long-term use. Despite the sophistication of the analytic equipment available, it is imperative to guard each step involved in the electrophoresis process, from sample preparation to electrophoresis, if accurate and worthwhile results are to be obtained from the analysis.

8. ACKNOWLEDGEMENTS

The invaluable review and comments of Dr. Lyndon L. Larcom and Vineeta Ribeiro in the preparation of the manuscript are gratefully acknowledged.

REFERENCES

1 C. R. Calladine, H. R. Drew and M. J. McCall, *J. Mol. Biol.*, 201 (1988) 127.
2 G. F. Carle and M. V. Olson, *Nucleic Acids Res.*, 12 (1984) 5647.
3 C. Turmel, E. Brassard, R. Forsyth, K. Hood, G. W. Slater and J. Noolandi, in E. Lai and B. W. Birren (Editors), *Electrophoresis of Large DNA Molecules*, Cold Spring Harbor Laboratory Press, New York, 1990, p. 101.
4 G. Chu, D. Vollrath and R. W. Davis, *Science*, 234 (1986) 1582.
5 D. C. Schwartz and C. R. Cantor, *Cell*, 37 (1984) 67.
6 M. Son, S. J. Hayes and P. Serwer, *Virology*, 162 (1988) 38.
7 E. A. Ribeiro, L. L. Larcom and D. P. Miller, *Anal. Biochem.*, 181 (1989) 197.
8 E. M. Southern, *Anal. Biochem.*, 100 (1979) 319.
9 J. K. Elder and E. M. Southern, *Anal. Biochem.*, 100 (1983) 319.
10 J. K. Elder and E. M. Southern, *Anal. Biochem.*, 128 (1983) 227.
11 H. E. Schaffer and R. R. Sederoff, *Anal. Biochem.*, 115 (1981) 113.
12 J. C. Sutherland, D. C. Monteleone, J. H. Mugavero and J. Trunk, *Anal. Biochem.*, 162 (1987) 511.
13 C. E. Gariepy, M. T. Lomax and L. I. Grossman, *Nucleic Acids Res.*, 14 (1986) 575.
14 H. A. Drury, P. G. Green, B. K. McCauley, M. V. Olsen, D. G. Politte and L. J. Thomas, Jr., *Genome*, 8 (1990) 119.
15 L. S. Lerman and D. Sinha, in E. Lai and B. W. Birren (Editors), *Electrophoresis of Large DNA Molecules*, Cold Spring Harbor Laboratory Press, New York, 1990, p. 1.
16 E. A. Ribeiro and J. C. Sutherland, *Anal. Biochem.*, (1993) in press.
17 W. L. Carrier and R. B. Setlow, *J. Bacteriol.*, 102 (1970) 178.
18 S. E. Freeman, A. D. Blackett, D. C. Monteleone, R. B. Setlow, B. M. Sutherland and J. C Sutherland, *Anal. Biochem.*, 158 (1986) 119.
19 A. K. Campbell, *Methods Biochem. Anal.*, 31 (1985) 317.
20 L. J. Kricka, *Clin. Chem.*, 37 (1991) 1472.
21 J. S. Waye and R. M. Fourney, *Appl. Theor. Electrophor.*, 1 (1990) 193.
22 J. Sambrook, E. F. Fritsch and T. Maniatis, *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, 1989.
23 A. T. Andrews, *Electrophoresis: Theory, Techniques, and Biochemical and Clinical Applications*, Claredon Press, Oxford, 1984.
24 D. E. Pulleyblank, M. Shure and J. Vinograd, *Nucleic Acids Res.*, 4 (1987) 1049.
25 J. C. Sutherland, B. Lin, D. C. Monteleone, J. Mugavero, B. M. Sutherland and J. Trunk, *Anal. Biochem.*, 163 (1987) 446.
26 J. B. LePecq and C. Paoletti, *J. Mol. Biol.*, 27 (1967) 87.
27 J. A. Miller, B. J. Hoffer and N. R. Zahniser, *J. Neurosci Methods*, 22 (1988) 233.
28 R. A. Laskey and A. D. Mills, *FEBS Lett.*, 82 (1977) 314.
29 Y. Amemiya and J. Miyahara, *Nature*, 336 (1988) 89.
30 Y. Amemiya, Y. Satow, T. Masushita, J. Chikawa, K. Wakabayashi and J. Miyahara, *Top. in Curr. Chem.*, 47 (1988) 121.
31 G. Charpak, W. Dominik and N. Zaganidis, *Proc. Natl. Acad. Sci. U.S.A.*, 86 (1989) 1741.
32 F. Sanger, S. Nicklen and A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.*, 74 (1977) 5436.
33 A. M. Maxam and W. Gilbert, *Methods Enzymol.*, 65 (1980) 499.
34 E. A. Ribeiro and J. C. Sutherland, *Anal. Biochem.*, 194 (1991) 174.
35 D. A. Agard, R. A. Steinberg and R. M. Stroud, *Anal. Biochem.*, 111 (1981) 257.
36 S. E. Freeman, R. W. Gange, E. A. Matzinger and B. M. Sutherland, *J. Invest. Dermatol.*, 86 (1986) 34.
37 J. C. Sutherland, B. M. Sutherland, A. Emrick, D. C. Monteleone, E. A. Ribeiro, J. Trunk, M. Son, P. Serwer, S. K. Poddar and J. Maniloff, *BioTechnology*, 10 (1991) 492.

38 C. Z. Chen and J. C. Sutherland, *Electrophoresis*, 10 (1989) 316.
39 J. T. Lett, in E. C. Friedberg and P. C. Hanawalt (Editors), *DNA Repair. A Laboratory Manual of Research Procedures I, Part B*, Marcel Dekker, New York, 1981.
40 P. C. Hanawalt, *Acta Biol. Hung.*, 41 (1990) 77.
41 A. Charlesby, *Proc. R. Soc. Series A*, 224 (1954) 120.
42 C. R. Cantor and P. R. Schimmel, *Biophysical Chemistry*, Freeman, New York, 1979. p. 628.
43 L. L. Larcom, E. A. Ribeiro and J. Sigmon, *Noninvasive Techniques in Biology and Medicine*. San Francisco Press, San Francisco, CA, 1990.