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Ethidium Bromide Spot Test Is a Simple Yet Highly Accurate Method in Determining DNA Concentration

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Abstract: A commonly used method to measure the amount of DNA in nucleic acid preparations is to use UV irradiation. However, if the DNA sample contains impurities, measuring UV irradiation is misleading. A quick way to estimate the amount of DNA in such samples is to analyze spot densities created by UV-induced fluorescence emitted by ethidium bromide. The amount of DNA is proportional to the amount of fluorescence. In many references, the spot density analysis is recommended for rough estimation of DNA concentration and is done by visual examination and comparison of the densities of each spot with the densities of DNA standards. Herein, we used a computer-integrated software to test the reliability of such a method, and showed that the analysis is highly reliable and simple.

Key Words: DNA concentration, spot density analysis, ethidium bromide

DNA Konsantrasyonunun Belirlenmesinde Etidyum Bromit Nokta Testi Basit Ama Güvenilir Sonuçlar Veren Bir Metoddur

Özet: UV ışığın absorpsiyonu nükleik asit örneklerindeki DNA konsantrasyonunun belirlenmesinde sıkça kullanılan bir metotdur. Ancak, DNA örneklerinin saf olmadığı durumlarda ölçümler yanıltıcı olabilir. Böyle örneklerle yapılan olçümlerin doğruluğunu kanıtlamanın en kolay yollarından birisi örnekteki DNA miktarını etidyum bromit nokta testi yaparak belirlemektir. Örnek içerisindeki DNA miktarı ile açığa çıkan UV floresan ışığın miktarı arasında doğru bir orantı vardır. Birçok referansta, nokta yoğunluğu analizi DNA konsantrasyonunun kaba olarak belirlenmesi amacı ile tavsiye edilmiştir ve DNA nokta yoğunluklarının göz ile analizi ve standart DNA noktaları ile karşılastırılması sonucu yapılmaktadır. Bu çalışmamızda biz, bilgisayara entegre edilmiş bir program kullanarak bu metodun güvenilirliğini test ettik ve nokta analizinin çok basit ama o derecede güvenilir olduğunu gösterdik.

Anahtar Sözcükler: DNA konsantrasyonu, nokta yoğunluğu analizi, etidyum bromid

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Introduction

In many molecular biology, biotechnology and medical diagnosis applications, knowledge of precise DNA concentration is required for optimization of downstream applications. In the majority of laboratories, the concentration of DNA is assessed by measuring UV at 260 nm, because pyrimidine and purine bases possess the absorption maxima in this region (1). The use of the A_{260}/A_{280} ratio for the qualitative assessment of DNA preparations is also a common practice (2). However, several laboratories raised concerns about the reliability of the measured A_{260} value (3-5), because other factors such as ionic strength, temperature, or pH of the buffer used may affect the determined value (6,7). In addition, the commonly-used phenol-chloroform extraction procedure for removal of proteins from DNA samples leaves traces of phenol in DNA preparations. The strong absorbance of phenol at 270 nm complicates the measurement of DNA concentration (8). In our laboratory, such complications affected our cloning efforts of several genes. Genomic DNA samples that were treated with phenol-chloroform to remove protein contaminants appeared to contain adequate amount of genomic DNA for cloning, when DNA concentration was measured with A_{260}. However, analysis of agarose gels revealed much less DNA. To overcome this difficulty, we optimized a previously known procedure (2) in which DNA concentration is estimated by visual examination of

the ethidium bromide-stained DNA spots. Herein, we used a spot density analysis software and determined the precise DNA concentrations in various DNA samples.

Materials and Methods

Genomic DNA was isolated with CTAB buffer (cetyltrimethylammonium bromide) according to the procedure described (9) in the presence of 0.5 U of RNAse A and cleaned from proteins by phenol-chloroform extraction. Plasmid DNA was isolated either with alkaline lysis method (2) or with commercial kits (Qiagen Inc., Valencia, USA). Lambda DNA (MBI Fermentas Inc., Canada) from a stock solution of 0.5 mg/ml was diluted with water to obtain DNA concentrations of 3.0, 6.0, 12.5, 25, 50, 60, 70, 80 and 100 ng/µL. Ethidium bromide (Sigma Chemical Co., St. Louis, USA) was prepared as 10 mg/ml and diluted 10,000-fold with water to obtain a working concentration of 1 ng/µL. Each DNA sample was appropriately diluted with water and 1 µL of each diluted sample was mixed several times with 10 µL of ethidium bromide, which was placed on a mica tray with the aid of a pipette. The mica tray was placed in the ImageSCI analysis system (Spectronics Corporation, New York, USA), exposed to UV light for 3 seconds, and an image was taken and processed with an integrated image-aid software (ImageSource, Spectronics Corporation, New York, USA).

Results and Discussion

As shown in Figure 1A and B, spot densities increased in parallel to the increase in the amount of DNA. The linear relationship (r=0.99) between spot densities and the amount of DNA continued until the samples reached a concentration of 60 ng/µL. Over this value, a saturation in spot densities was observed. DNA samples containing more than 60 ng/µL of DNA were diluted at least 50 times with high purity water to determine the correct DNA concentration.

When the DNA concentration was measured with UV irradiation, genomic DNA isolated with CTAB buffer appeared to contain more DNA than what was present in spot density analysis (Table 1 versus Figure 1C). In all DNA samples, purity of the DNA preparations was lower than what we expected. In order to confirm and compare reliability of the results, DNA samples listed in Table 1 were diluted according to the concentrations determined with A_{260} and with spot density analysis. A final concentration of 50 ng/µL was targeted in each dilution. Spot densities showed variations when DNA samples were diluted according to the concentrations determined with A_{260}, whereas a uniform spot density was observed among the samples when dilutions were made according to the concentrations determined with spot density analysis, indicating that concentrations determined with spot density analysis are highly reliable (Figure 1 E, F).

Spot density test can be performed without a need for state of the art technology, although our laboratory has a complete gel imaging system. For the purpose of spot analysis, ImageJ, a freely available software, may be used. The software is available at the National Institutes of Health and can be obtained from http://rsb.info.nih.gov/ij/ download.html. ImageJ measures the integrated density of each dot by outlining it and using the *Analyze/Measure* command. The values obtained can be plotted with a simple plotting software and the plot can be used for the measurement of unknown DNA concentrations. In Figure 1 D and G, the peaks representing spot densities were obtained with ImageJ. A linear relationship can be readily observed between peak heights and the amount of DNA. It is thus possible to use spot density analysis routinely in every laboratory for accurate measurement of DNA concentration.

In the literature, spot density analysis was described as a fast but crude method to measure DNA concentration (reference 2 and the references cited therein). On the Internet, similar spot density measurement procedures were also described (http://www.protocol-online.org/prot/General_Laboratory_Techniques/Quantitation/DNA_Quantitation/). Spot density measurement, however, is not commonly used for two reasons: 1. Although this technique provides a rapid way to make a rough but useful estimate of the DNA concentration of a given sample, it is believed to be not highly accurate. 2. It is much simpler to measure A_{260} and thus determine DNA concentration. The measurement of A_{260}, however, is only useful for highly purified DNA samples, since it detects any compound that absorbs significantly at 260 nm. Despite its widespread use, the value of the A_{260} method is questionable. In this study, as an alternative to A_{260}, we optimized conditions for spot density analysis and allowed routine and accurate DNA concentration

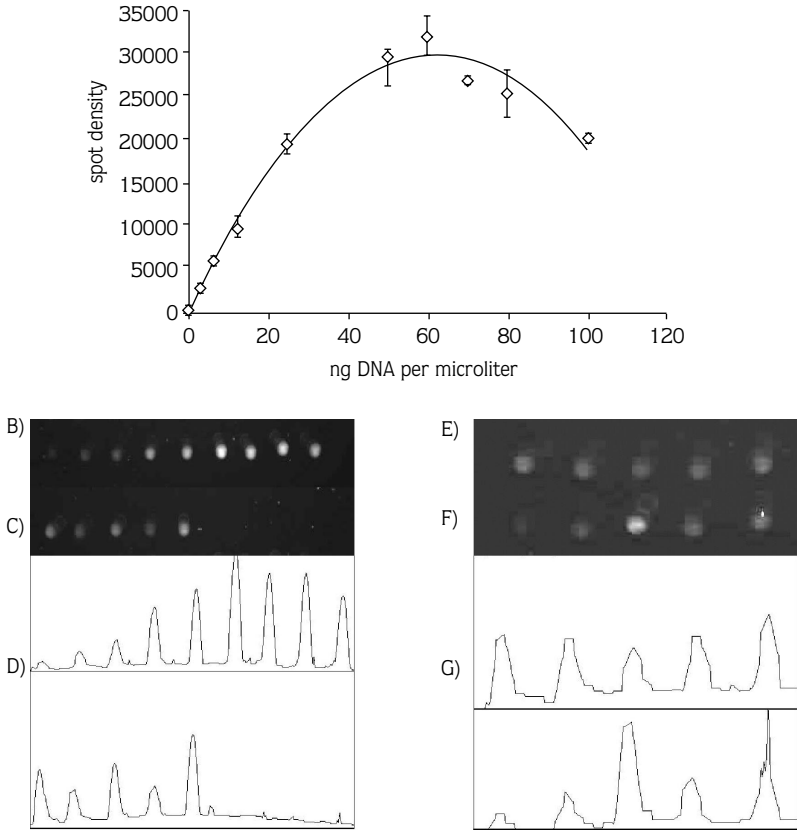

Figure 1. (A) The relationship between DNA concentration and spot densities. Different concentrations of lambda DNA were plotted against corresponding spot densities. (B) Spot densities of DNA standards (3.0, 6.0, 12.5, 25, 50, 60, 70, 80 and 100 ng/µL lambda DNA) (C) Spot density analysis of DNAs listed in Table 1. (D) Peaks represent spot densities shown in B and C, respectively. (E and F) Spot density analysis of the DNA samples that were diluted according to the concentrations listed in Table 1 column 5 and column 3, respectively. (G) Peaks represent spot densities shown in E and F, respectively.

Table 1. Determination of DNA concentration with A_{260} and with spot density analysis

Sample type	Isolation procedure	Amount of DNA spectrophotometrically determined (µg/µL)	Purity A_{260}/A_{280}	Amount of DNA determined from spot density analysis (µg/µL)
Genomic DNA from *Helicobacter pylori*	CTAB method	3.4	1.5	1.8
Genomic DNA from *Acinetobacter baumannii*	CTAB method	2.1	1.4	0.5
Genomic DNA from human blood	CTAB method	1.1	1.2	0.5
Plasmid DNA from *Salmonella typhimurium*	Commercial kit	2.2	1.7	0.9
pBK-CMV recombinant plasmid DNA	Commercial kit	1.8	1.4	1.5

determination. It is our prediction that any study that deals with ng/µL levels of DNA concentration can utilize the advantages of spot density analysis and accurately determine the needed DNA concentration in biological samples.

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