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Factors Affecting Quantification of Total DNA by UV Spectroscopy and PicoGreen Fluorescence

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The total amount of DNA in a preparation extracted from tissues can be measured in several ways, each method offering advantages and disadvantages. For the sake of accuracy in quantitation, it is of interest to compare these methodologies and determine if good correlation can be achieved between them. Different answers can also be clues to the physical state of the DNA. In this study, we investigated the lack of correlation between ultraviolet (UV) absorbance and fluorescent (PicoGreen) measurements of the concentration of DNAs isolated from plant tissues. We found that quantifation based on the absorbance-based method correlated with quantitation based on phosphorus content, while the PicoGreen-based method did not. We also found evidence of the production of single-stranded DNA under conditions where the DNA was not fragmented into small pieces. The PicoGreen fluorescent signal was dependent on DNA fragment size but only if the DNA was in pure water, while DNA in buffer was much less sensitive. Finally, we document the high sensitivity of the PicoGreen assays to the detergent known as CTAB (cetyldimethylethylammonium bromide). The CTAB-based method is highly popular for low-cost DNA extraction with many published variations for plant and other tissues. The removal of residual CTAB is important for accurate quantitation of DNA using PicoGreen.

KEYWORDS: Maize; *Zea mays* L.; soy; *Glycine max*; spinach; *Spinacia oleracea*; wheat; *Triticum aestivum*; CTAB; cetyldimethylethylammonium bromide; DNA; PicoGreen; DNA quantitation

INTRODUCTION

The quality and quantity of DNA extracted from tissues play a role in the subsequent success of downstream reactions such as polymerase chain reaction (PCR), sequencing, and cloning. A particular interest in our laboratory has been the detection of genetically modified plant material using quantitative real-time PCR. The most critical testing involves trace detection to satisfy regulatory requirements (*1*, *2*). Many factors contribute to the accuracy of such testing. The quantity of DNA in an assay is relevant to the limit of detection or the limit of quantification of the methods (*3*). This is more important with grain testing than processed food, where the DNA of several species may be present. The two most common methods of measuring the concentration of DNA in an extract use either the sample's absorbance in the UV range or fluorescent dye binding. UV absorbance measurements are nondestructive, but they do not distinguish between DNA and RNA (*4*). An appropriate, although conservative, concentration range for UV measurements is 5−50 μg/mL (absorbance range of 0.1−1.0). Protein, phenolics, and particulates can interfere between 220 and 340 nm (*4*). PicoGreen, a widely used dye that fluoresces upon binding DNA, is an intercalating dye, so protein does not interfere with the resulting measurements (*5*). The PicoGreen method works with samples with lower concentrations having a linear range of 0.05−1 μg/mL. PicoGreen has very limited binding to RNA and single-stranded DNA (*5*).

Even with pure DNA preparations, there is not always good agreement between the two methods. In this study, we investigate the lack of correlation and some of the possible causes.

MATERIALS AND METHODS

Preparation of DNA. Maize (*Zea mays* L.) and soy (*Glycine max*) DNA were isolated from either ground seed or fresh young leaf tissue. Fresh wheat germ and spinach were purchased from a market. Leaf tissue was chopped and then ground in liquid nitrogen to a fine powder. Calf thymus (Sigma, D-1501, BioRad 170-2480) was purchased. Plasmid DNA (pPCR Script AMP, Stratagene) was propagated in *Escherichia coli* and purified as previously described (*6*).

DNA was isolated from plant tissues using either a variant of the CTAB method, in which the detergent, cetyldimethylethylammonium bromide, is used for cell lysis (*7*), or commercial DNA isolation kits. Two basic variants of the CTAB method were used. Method A (*8*) was modified by the addition of RNase (0.5 mg/100 mg maize powder) and Proteinase K (0.4 mg/100 mg maize powder) during the initial incubation at 65 °C with the CTAB lysis buffer. The RNase was added at the beginning of the 65 °C incubation and the Proteinase K half way through the 1−2 h incubation. Method B (*9*) used the same CTAB lysis buffer but eliminated the CTAB precipitation step. Proteinase K and RNase treatments were included.

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After the initial extraction and alcohol precipitation, small scale size-exclusion columns (Microspin S-300 HR, GE Healthcare) were used (*9*).

Prior to phosphorus measurements, additional enzymatic cleanup of the DNA was conducted. Treatments included additional incubation with RNase (Sigma, Fermentas), α amylase (A-4551, Sigma Chem. Co.), and proteinase K (P-2308, Sigma Chem. Co.). This was followed by extraction with phenol/chloroform/isoamyl alcohol (25/24/1) and/or chloroform and alcoholic precipitation of the DNA. Removal of small molecules was accomplished with dialysis or centrifugal concentrators (Amicon Centripreps).

For sonication experiments, 1 mL aliquots of calf thymus DNA were prepared from solutions of DNA in sterile pure DNase free water (Invitrogen) or in 0.5× TE buffer (5 mmol L^{-1} Trizma base, 0.5 mmol L^{-1} ethylenediamine tetraacetic acid, pH 7.5). The DNA solutions were sonicated for 30, 60, or 120 s using a Misonix XL2020 sonicator equipped with a microtip. The sonication was done at 25% maximum power.

Characterization of DNA. Spectroscopic studies of the absorbance of DNA were conducted on a Beckman DU650 (single beam) or a Perkin-Elmer Lambda 850 (dual beam) spectrophotometer using either quartz or disposable UV cuvets. Data were collected from 220 to 320 nm. The absorbance of denatured DNA was obtained by adding 2 mol L^{-1} NaOH to the DNA solution to a final concentration of 0.2 mol L^{-1} NaOH prior to reading. Determination of DNA hyperchromicity was done by monitoring the absorbance of DNA at temperatures ranging from 25 to 95 °C and calculated by dividing the maximum absorbance value by the absorbance at 25 °C. The maximum absorbance value was achieved between 80 and 95 °C and was dependent on the buffer concentration. The temperature of the DNA solution in a quartz cuvette was achieved with a Peltier controller. Correlation of the Peltier controller and the actual cuvette temperature was validated using a calibrated thermister. Quartz cuvettes with a screw cap with a silicon insert were used. To prevent loss of water during the heating process, Teflon tape was wrapped around the threads. Readings were taken 2−3 min after the cuvette holder block had achieved the correct temperature to allow for the DNA solution to equilibrate at the same temperature. The absorbance was corrected for volume changes related to temperature (*10*).

The fluorescent intercalating dye, PicoGreen (Quant-iT PicoGreen DS DNA assay, Invitrogen P7589), was used to quantitate DNA in solution. λ phage DNA was provided in the Quant It kit to serve as the calibrant. Measurement of the phosphorus content of acid-digested DNA was made using high-performance inductively coupled plasma−optical emission spectrometry (HP-ICP-OES) as previously described (*6*). DNA was electrophoresed in 0.8% (w/v) agarose gels in a buffer of 90 mmol L^{-1} Trizma base, 90 mmol L^{-1} boric acid, and 20 mmol L^{-1} ethylenediamine tetraacetic acid, pH 7.5 (TBE). The gels were stained with ethidium bromide. Two sizing standards (HyperLadder I and HyperLadder VI, Bioline) containing DNA fragments with a range in size from 200 base pairs (bp) to 48500 bp were included in gel runs.

RESULTS AND DISCUSSION

We have isolated DNA primarily from plant material for various studies and often found significant disagreement between the two most popular DNA quantitation methods, absorbance at 260 nm and a fluorescence method utilizing the DNA intercalating dye, PicoGreen. Absorbance in the UV measures double- and single-stranded DNA and RNA (*4*). The extinction coefficient for 1 mg mL^{-1} DNA is generally accepted to be 20 at 260 nm and 10 at 280 nm. Absorbance readings are best done with concentrations from 5 to 50 μg/mL of DNA so as to be in the linear range (absorbance readings of 0.1−1.0 at 260 nm). Proteins also absorb light at both 260 and 280 nm with an extinction coefficient of 57 at 260 nm and 1 at 280 nm for 1 mg mL^{-1} protein. Because protein has a smaller extinction coefficient at 280 nm, significant protein can be hiding in a DNA preparation (*11*). Part of the characterization of a DNA preparation involves the calculation of the 260 nm/280 nm ratio. It follows from the DNA extinction coefficients at 260 and 280 nm that the ratio should be 2, if the DNA preparation has little or no protein contamination (*11*, *12*).

Table 1. Comparison of Absorbance-Based and PicoGreen-Based Quantification of Maize DNA[a]

method	absorbance (μg/mL)	PicoGreen (μg/mL)	PG/ABS (%)	average PG/ABS (SD)
commercial kit #1	55.3 (0.6)	52.3 (10.3)	95	
	68.1 (1.5)	56.0 (4.5)	82	
	64.3 (1.5)	36.5 (8.0)	57	72% (17.2)
	53.5 (0,5)	41.8 (12.9)	78	
	63.9 (0.2)	30.5 (4.6)	48	
	45.8 (0.1)	32.9 (3.0)	72	
CTAB	26.5 (0.5)	16.8 (0.4)	64	
	46.5 (0.4)	31.2 (0.6)	67	
	106.0 (10.9)	71.2 (5.3)	67	67% (3.5)
	86.7 (3.5)	61.7 (4.7)	71	
	47.5 (0.4)	33.1 (1.1)	70	
	94.1 (2.1)	58.3 (0.7)	62	
commercial kit #2	149.6 (2.0	56.2 (6.6)	38	
	219.8 (2.2)	49.4 (4.0)	23	
	88.1 (0.7)	38.7 (4.6)	44	29% (9.5)
	189.7 (5.6)	46.4 (5.2)	25	
	143.6 (0.9)	32.3 (7.4)	23	
	146.1 (7.7)	32.5 (4.0)	22	

[a] Maize DNA was extracted using three different methodologies. Method B was used for CTAB extraction. DNA was quantitated using absorbance at 260 nm and using PicoGreen fluorescence. Numbers in parentheses are expanded uncertainties. The value determined by fluorescence is expressed as a % of that measured by absorbance.

Fluorescent dye measurements require much less DNA than absorbance measurements and are more suitable for dilute solutions of DNA. The fluorescent signal from PicoGreen binding to RNA and single-stranded DNA is only 10% of that of double-stranded DNA (*5*). Protein does not interfere with PicoGreen measurements. Singer et al. (*5*) describe the relative effect that various compounds have on the accuracy of PicoGreen-based measurements. They focused on compounds that could be found in cells as well as DNA isolation components.

Table 1 shows that the PicoGreen quantitation of the total DNA concentration in the preparation always yielded lower values than the UV absorbance-determined quantitation for DNA that was isolated from the same material, ground maize, using all three DNA isolation methodologies, without any additional purification steps. In the case of the CTAB-extracted maize DNA and the DNA extracted using the commercial kit #1, the PicoGreen quantitation was about 30% lower than the concentration calculated from the absorbance measurements. The concentration of the DNA extracted with the commercial kit #2 was 70% lower. An agarose gel of all of the samples showed that high molecular weight DNA was present in all samples. In our experience, agreement between absorbance and PicoGreen quantitation calculations has ranged from excellent (95%+) to very poor (10%), especially with plant DNA and also upon further treatment and extra purification steps. This was true with several types of isolated plant DNA from the leaves or seeds from different species (maize, soy, wheat germ, and spinach). **Figure 1** provides an example of PicoGreen fluorescence signal from multiple types of DNA as compared to λ phage (calibrant supplied with the PicoGreen kit) and calf thymus DNA.

A measurement of phosphorus from acid-digested DNA offers another approach to DNA quantitation. There are now superior measurement tools offering highly accurate and precise measurements traceable to the SI (International System of Units) unit for mass (kilogram) using phosphorus certified reference

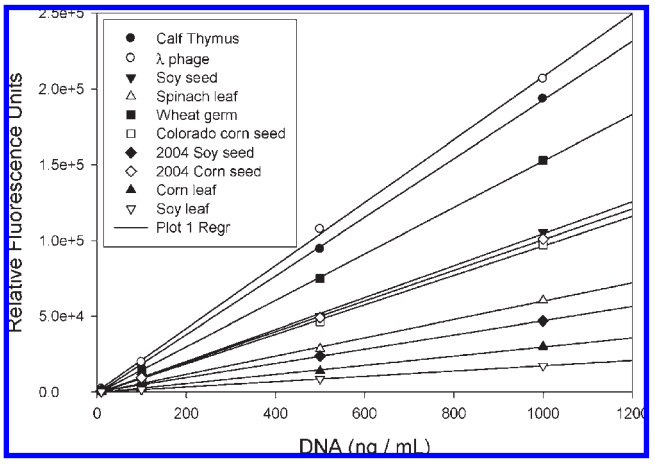

Figure 1. DNA was isolated from ground seed and leaf tissue using the DNeasy Plant mini-prep kit (Qiagen). Samples included two batches of ground maize (recent harvest and several years older), soy seed (two batches), maize and soy leaf tissue from 4 week old plants, spinach leaves, and wheat germ. The concentration of the DNA was determined by UV spectroscopy, all were adjusted to the same concentration, and a series of dilutions made. The fluorescence signals of the plant DNAs were compared to those of commercial calf thymus DNA and lambda (λ) phage DNA, the calibrant in the PicoGreen kit (Invitrogen/Molecular Probes).

Table 2. Maize DNA Comparison of Concentration Based on Phosphorus Mass Fraction, Absorbance, and Fluorescent Dye Binding[a]

	μg DNA/mL			
phosphorus mass fraction (μg DNA/g)	ABS	ABS (NaOH denatured)	Pico Green	hyperchromicity
44.29 (0.05)	49.5 (1.5)	45.6 (0.5)	18	1.32
58.35 (0.07)	52.8 (1.3)	47.3 (0.1)	25	1.32
48.57 (0.06)	45.2 (0.8)	42.1 (0.1)	19	1.35
142.81 (0.14)	139.6 (5.8)		84	
107.60 (0.10)	117.5 (2.7)		16	
108.10 (0.12)	113.4 (7.9)		21	

[a] CTAB methods were used to extract the DNA for these experiments. Method B was used for samples 1−3, and method A was used for samples 4−6. The values in parentheses are expanded uncertainties.

materials (*6*, *13*, *14*). This measurement strategy requires 1−2 mg of material. Recent advances using a demountable direct injection high-efficiency nebulizer (d-DIHEN) have made it possible to reduce the amount of material required for an analysis (to ≈500 μg) (*15*). Nonetheless, this is a technique more suitable for validating and certifying reference materials than for routine measurement. For the phosphorus measurement to accurately estimate the DNA concentration, the purity of the DNA is very important. So, for these measurements, the DNA went through additional treatments to remove non-DNA sources of phosphorus. Such treatments included extra enzymatic treatments for protein, carbohydrates, and RNA and dialysis or buffer washes using centrifugal concentrators. **Table 2** compares the DNA mass fraction [mass fraction is interchangeable with concentration in the range compared in this study (*6*)] in μg DNA/g as calculated from phosphorus content, DNA concentration calculated from absorbance at 260 nm, and PicoGreen dye binding on six different preparations of maize DNA. It is evident that the mass fraction estimate is close to that obtained by spectrophotometry. There are, in some cases, significant differences between the concentration determined by absorbance and the mass fraction determined by phosphorus measurements,

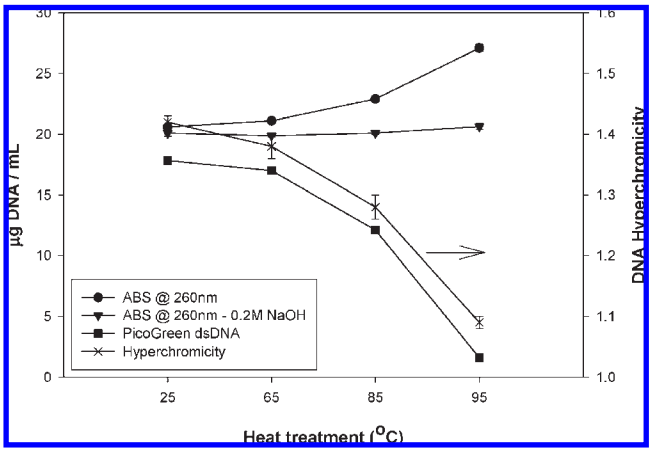

Figure 2. Solution of calf thymus DNA (20 μg/mL) was divided into aliquots, three of which were heated at the indicated temperatures for 2−3 min to create a gradient of denatured (single-stranded) DNA. After they were heated, the aliquots were cooled on ice, and four assays were carried out on each: PicoGreen fluorescence (filled square), absorbance of the DNA in buffer (filled circle), hyperchromicity (×), and absorbance of NaOH-denatured DNA (filled triangle). Three separate assays were made at each temperature point for each sample, and multiple readings were taken for each assay. Error bars for each point (some of which are hidden behind the symbol) are 95% expanded uncertainties.

but the concentration derived from the PicoGreen fluorescence measurements was consistently lower than the absorbance measurements.

PicoGreen fluoresces when bound to double-stranded DNA but has a much lower affinity to single-stranded DNA or RNA (*5*). We considered the idea that isolation and purification of DNA from our plant species produced some single-stranded DNA due to shearing or other forces. So, we compared the absorbance of DNA in buffer to that of DNA denatured by NaOH. At 260 nm, single-stranded DNA has an extinction coefficient of 38 when calculating μg mL^{-1} as compared to 50 for double-stranded DNA (*16*). **Table 2** shows that the calculation based on denatured DNA is lower than the calculation based on the absorbance in buffer by about 10%.

Another way to investigate whether there is a proportion of single-stranded DNA in a preparation is to do a melting curve analysis. The increase in absorbance with heat denaturation is known as hyperchromicity (*17*). The hyperchromicity factor describes the increase in absorbance of denatured/single-stranded DNA over the value prior to denaturation, which is defined as 1. Multiple experiments with calf thymus, λ phage, and plasmid DNA suggest that a hyperchromicity factor of 1.4 is normal for DNA that has been defined as intact (data not shown). Intactness, in this case, was based on agarose gel electrophoresis of the DNA showing appropriate sized stained bands with no observable smearing. The hyperchromicity and concentration measurements shown in **Figure 2** compare aliquots of calf thymus DNA that were heat treated to different temperatures and then plunged into ice so as to restrict DNA renaturation. The heat-treated samples showed an absorbance increase over untreated samples, when read post heat treatment at 260 nm. The higher the heat treatment, the greater the increase in absorbance was seen. The absorbance measurement based on denaturation by NaOH remained the same as would be expected since the total DNA remained the same, with minimal discrepancy, which can likely be attributed to a minor amount of evaporation upon heating. The estimation of DNA by PicoGreen fluorescence for the sample

Table 3. Effect of Sonication on Fragmentation, Absorbance, and PicoGreen Fluorescence Signal of DNA in Water or Buffer[a]

	fluorescense mean	fluorescense % of control	Abs 260 (μg/mL)
	DNA in water		
control	3459	100	47.0
30 s sonication	1496	43	46.4
60s sonication	1357	39	46.5
120s sonication	779	23	46.8
	DNA in buffer		
control	17496	100	57.7
30 s sonication	15996	91	59.4
60s sonication	15524	89	58.8
120s sonication	15001	86	59.4
λ phage	16044		

[a] Calf thymus DNA was added (10μL) to 1 mL of picogreen reagent, and for λ phage, 5 μL was added. This represented approximately 570 ng of DNA in buffer, 470 ng of DNA in water, and 500 ng of λ phage. Fluorescence signals are the means of four readings of each of three separate assays.

heated to 95 °C dropped to 10% of the original concentration. The hyperchromicity factor dropped from 1.41 to 1.08 for the samples originally heated to 25 and 95 °C, respectively. This is reasonable based on the fact that the absorbance of the heat-denatured DNA was greater than the same DNA that had not been heated, before the start of the hyperchromicity assay. Agarose gel electrophoresis of the DNA from the various heat treatments showed a pattern similar to the control with predominately high molecular mass fragments, except for the sample that was heated to 95 °C where there was significant fragmentation. In **Table 2**, there are hyperchromicity values calculated for three of the maize samples, and they show values below 1.4. Taken together, the hyperchromicity values, the absorbance of alkali-denatured DNA, and low PicoGreen readings (**Table 2**) are consistent with the idea that isolation and purification of the maize DNA resulted in the production of some single-stranded DNA in the preparation. Svaren et al. (*18*) described one possible source of single-stranded DNA, the denaturation of DNA during drying after ethanol precipitation. This is often done to eliminate residual ethanol. They demonstrate this denaturation primarily with small fragments of DNA and suggest that it may not be significant with large fragments.

Another issue to consider is whether fragmentation of DNA affects PicoGreen fluorescence. Ahn et al. compared DNA quantification by PicoGreen and absorbance at 260 nm (*19*). They noted the difference between the two types of measurements but concluded that fragment size was not relevant based on comparison of intact DNA versus the same DNA fragmented by restriction endonucleases. Subsequently, Georgiou and Papapstolou proposed that PicoGreen was a very sensitive indicator of fragmentation of DNA and that this property could be used to calculate the % or fragmented DNA (*20*). We investigated their method and noted that their experiments utilized calf thymus DNA in sterile DNase-free water. We ran parallel experiments with DNA in water and DNA in 0.5× TE buffer. Aliquots were sonicated for 30, 60, or 120 s with a microprobe tip. Subsequent gel electrophoresis showed that while the control DNA was composed of mostly large 23000+ base pair DNA, the sonication had resulted in severe fragmentation so that only small fragments (3000 bp, 30 s of sonication, 1500 bp, 120 s of sonication) were seen. The same result was seen for both DNA in water and in buffer. The absorbance readings for all of the treated samples were close to

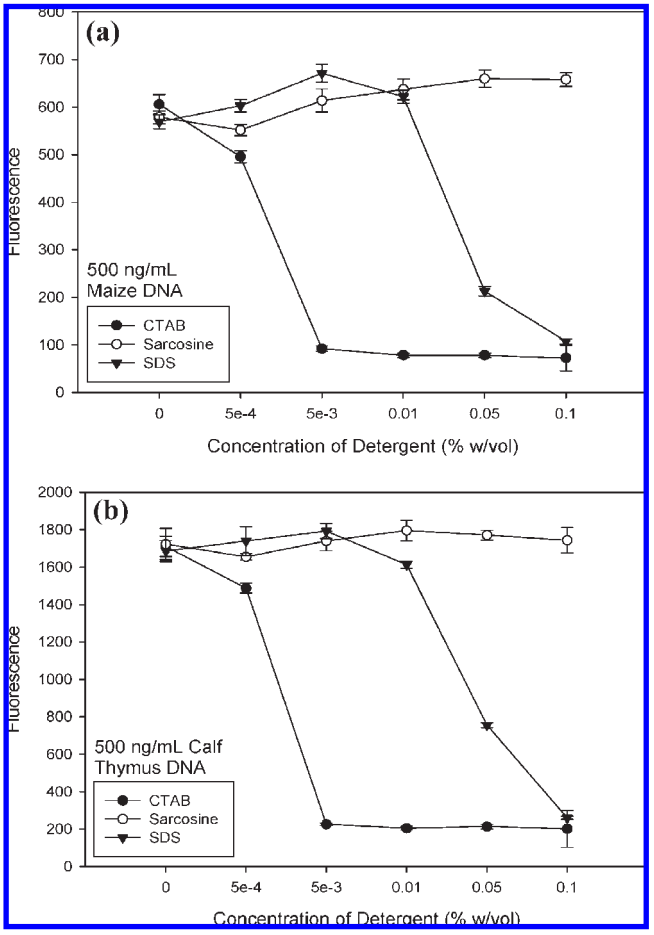

Figure 3. PicoGreen fluorescence measurements were conducted on maize (**a**) and calf thymus (**b**) DNA. Except for the control assays, one of three detergents, CTAB, (filled circle), SDS (filled triangle), or sarcosine (open circle), was added from a concentrate to the assay reagent. Each point represents the mean of three assays, and the error bars are standard deviations.

that of the controls (**Table 3**). The fluorescence signal of the DNA in water decreased with increasing length of sonication time, so that after 120 s the fluorescence was only 23% of the control. The effect of the sonication on the DNA in buffer was smaller (86% of the control at 120). Furthermore, the control DNA in water had a lower fluorescence than would be expected from the amount of DNA added as compared to the DNA in buffer and λ phage calibrant. We can replicate the results of Georgiou and Papapostolou (*20*) as long as the DNA is in water, but the effect is significantly lessened when the DNA is in buffer.

Experiments showed that supercoiled plasmid does not bind PicoGreen to the same degree as linearized plasmid. PicoGreen fluorescence of the supercoiled plasmid was about 60% of the same preparation of plasmid pretreated with the restriction enzyme EcoR1 that has one cutting site in the DNA sequence. The increase in fluorescent signal of the linearized plasmid was likely due to the increased access of PicoGreen to the DNA.

On the basis of the plasmid data, we tested the effect of proteases on PicoGreen binding to ask the question of whether protein−DNA complexes in extracted genomic DNA could reduce accessibility of PicoGreen to the DNA as supercoiling does for plasmids. Proteinase K is effective in disrupting histone protein−DNA complexes (*21*). However, neither proteinase K,

Table 4. Dependence of CTAB Quenching of PicoGreen Fluorescence on DNA (λ Phage) Concentration[a]

	fluorescence			% change		absolute change
λ (μg/mL)	0% CTAB	0.0005% CTAB	0.005% CTAB	0.0005% CTAB	0.005% CTAB	0.0005% CTAB
0.500	2773 (78)	2477 (36)	150 (8)	−10.7	−94.6	−295.9
0.250	1422 (28)	1138 (20)	90 (5)	−20.0	−93.7	−284.0
0.125	704 (24)	437 (20)	47 (6)	−38.0	−93.4	−267.2

[a] Fluorescence values are means (SD) of three assays.

trypsin, nor chymotrypsin had any effect on PicoGreen binding to our plant DNA samples.

Next, we looked specifically at whether detergents have an effect on PicoGreen binding. Singer et al. (*5*) tested sodium dodecyl sulfate (SDS) on PicoGreen fluorescence and showed that 0.01% (w/v) had little effect, but 0.1% completely quenched the dye. **Figure 3** illustrates the results of adding quantities (0−0.1%) of three detergents to maize (**a**) and calf thymus (**b**) DNA. PicoGreen fluorescence was very sensitive to the presence of CTAB, while much less sensitive to sodium SDS and not sensitive to sarcosine up to a concentration of 0.1%. The results with SDS are the same as those of Singer et al. (*5*). Given the ubiquitousness of CTAB methods used for plant DNA isolation, the virtual elimination of fluorescent signal by a concentration of 0.005% is significant. The experiments in **Figure 3a**,**b** were conducted with 500 ng/mL DNA. **Table 4** shows that the magnitude of the effect of CTAB is in inverse proportion to the amount of DNA in the assay. This is because the absolute fluorescent signal was reduced or quenched to the same degree with the addition of the same quantity of CTAB regardless of the amount of DNA in the assay.

Isolation and purification of DNA from plant species sometimes result in significant differences in the estimation of DNA concentration, dependent on the measurement approach. This difference can increase with additional purification steps. We have seen evidence that the production of single-stranded DNA may be responsible for some of this, even when the DNA consists primarily of large (25−50 kb) fragments. Measuring the concentration of alkali-denatured DNA and comparing that to the absorbance of DNA in buffer is the easiest and least costly way to determine the extent of single-stranded DNA in a given preparation of DNA and provides a better estimate of the concentration of the DNA. This technique will be useful in the case where there is sufficient quantity of DNA available for the assay and the DNA has been cleaned of protein, RNA, phenolics, and other contaminants that can interfere with accurate measurement.

CTAB isolation methods may also play a role in the lack of correlation between quantitation methods. CTAB is commonly used in various methods of isolating DNA from plant tissues, and such methods are popular because they offer simple reagent preparation and low cost. The lysis and precipitation buffers contain large quantities of CTAB (2% w/v). Quenching of the PicoGreen signal was seen at 0.0005% and was complete at 0.005%. These concentrations are 4000- and 400-fold lower than the concentration used in the extraction procedure. The possibility of residual CTAB in a DNA preparation should therefore be considered. In the process of evaluating a CTAB method for suitability, it would be of interest to ask if additional purification steps, such as a spin column or dialysis, might result in better correlation between absorbance-based and fluorescent dye binding-based total DNA quantitation.

SAFETY

Phenol: Exposure to phenol can occur through ingestion, inhalation, and the skin. Phenol can cause skin and eye irritation, and absorption can lead to kidney, liver, and other systematic damage. Acute toxicity can cause convulsions. Always open bottles containing phenol and work with phenol in a fume hood. Wear protective clothing and gloves.

Chloroform: Exposure to chloroform can occur through inhalation, ingestion, or contact with the skin or eyes. Chloroform is a central nervous system depressant and carcinogen. The precautions are as for phenol.

Ethidium bromide (EtBr): EtBr is suspected of being a carcinogen and mutagen. Use nitrile rubber gloves in place of latex to avoid skin exposure.

All three compounds should be disposed of in hazardous waste. EtBr can be removed from solutions with activated charcoal or Amberlite ion-exchange resin.

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