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Detection of contamination in DNA and protein samples by photometric measurements

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Abstract

A solution of dsDNA was spiked with different amounts of protein. While the dsDNA concentration was kept constant, the concentration of added protein was increased in a step-wise fashion. The DNA/protein mixtures were then measured in the Eppendorf BioPhotometer® D30. Using the function "Purity Scan", which is available for measurements of nucleic acids and protein, respectively, the influence of increasing protein contamination in a DNA solution was followed. For comparative purposes the changes in absorption behavior of protein samples in the presence of contamination was also recorded.

Introduction

Nucleic acid purification is a major application in a molecular laboratory. Purity and homogeneity of the sample are important considerations for subsequent applications. In practice, nucleic acids are purified with the help of commercially available kits which allow the separation of most other cellular components. However, the presence of proteins or other organic components in the eluate cannot be entirely ruled out. This is also directly related to the quality of the kit used. In the case where no kit is used for purification, additional contamination risks are posed by the chemicals used, e.g. phenol or ethanol from a phenol/chloroform extraction. Furthermore, this purification method typically yields all nucleic acids in a cell, not only the nucleic acid of interest. For example, a classic plasmid preparation without the RNase digestion step will yield approximately 90 % RNA [1]. In order to ensure minimum contamination of the sample it makes sense to verify its purity by spectrophotometric measurements, fluorimetry or agarose gel. After considering the effort involved, the first method is certainly the simplest to perform.

For photometric determination of the concentration of a nucleic acid solution, absorbance is measured at 260 nm. Using a specific conversion factor, the concentration of the nucleic acid solution is calculated from the absorbance value. The following conversion factors (CF) are valid for an absorbance of 1 and for an optical path length of 1 cm:

dsDNA ≙ 50 µg/mL

RNA ≙ 40 µg/mL

ssDNA ≙ 33 µg/mL

The concentration (C) of the respective nucleic acid is determined via the following formula: C = CF x A (A=Absorbance), which results from a conversion of Lambert-Beer's law:
$A=\varepsilon*C*d <> C=1/\varepsilon *A$. The optical path length of 1 cm may be directly integrated into the coefficient of absorbance. The conversion factor CF is derived from the reciprocal value of the coefficient of absorbance ($1/\varepsilon = CF$).

However, the measurement at 260 nm by itself cannot make a statement about the quality, or purity, respectively, of the sample; it can only speak to the quantity. There are other organic components such as proteins, carbohydrates, certain salts or aromatic compounds which absorb in the UV-range, similar to nucleic acids (fig. 1). Additional measurements at 230 nm (detection of organic substances) and 280 nm (detection of proteins and phenols) will yield a more accurate estimation with respect to sample quality. The ratios of the absorbance values at the wavelengths 260 nm / 280 nm and 260 nm / 230 nm, respectively, provide a clear picture of the purity of a nucleic acid sample. The following values represent a pure DNA sample:

A260/A280 = 1,8 -2,0

A260/A230 ≥ 2,0

As evident in fig. 1, these ratios are reduced if the sample is contaminated with proteins or other organic substances. In these cases the spectrum of the DNA solution will also change. In this article, these phenomena are to be illustrated with the help of a simple experiment in which a nucleic acid is spiked with different amounts of protein. A "Purity Scan" of each sample is then recorded in the BioPhotometer D30, followed by visualization of the influence of increasing contamination.

With respect to protein purification there are no set rules for purity grades which are comparable to those for nucleic acids. However, contaminations are easily identified by observing the absorbance spectrum of a protein sample. This phenomenon will be illustrated within this article.

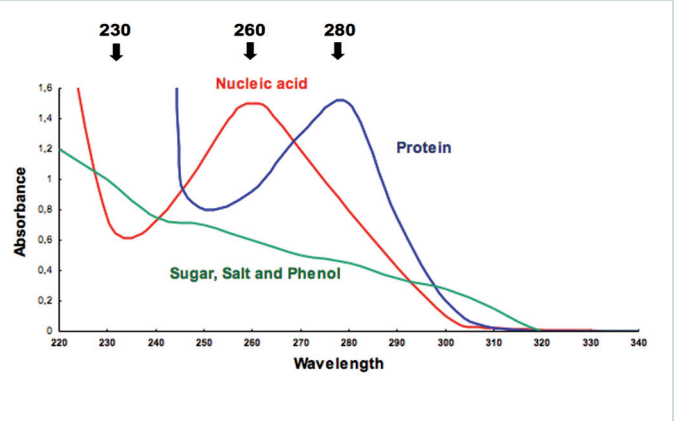

Figure 1: Absorbance scan of a nucleic acid sample and possible contaminants

Experimental Procedures

Materials

λ-DNA (AppliChem®: A5187,1000), Eppendorf µCuvette™ G1.0, Eppendorf BioPhotometer D30, water (AppliChem: A7398,1000), BSA solution in water (2 mg/mL)

100 µg/mL DNA solutions were mixed with an equal volume of a protein solution. The following protein concentrations were used: 0 µg/mL, 250 µg/mL, 500 µg/mL, 1000 µg/mL and 2000 µg/mL, which were produced from a BSA stock solution of 2000 µg/mL. Thus, the DNA was kept at a final concentration of 50 µg/mL in all samples. The final concentrations of the protein solutions were 0, 125, 250, 500 and 1000 µg/mL, respectively. 4 µL of the DNA/Protein mixture were applied to the Eppendorf µCuvette and measured using the method dsDNA_1mm in the Bio-Photometer D30. In order to demonstrate the changes within the absorbance spectrum reflecting the increasing protein concentrations, the "Purity Scan" was activated.

Results and discussion

The DNA/protein mixtures with increasing protein content were measured in succession. The resulting comparative spectra are shown in figure 2.

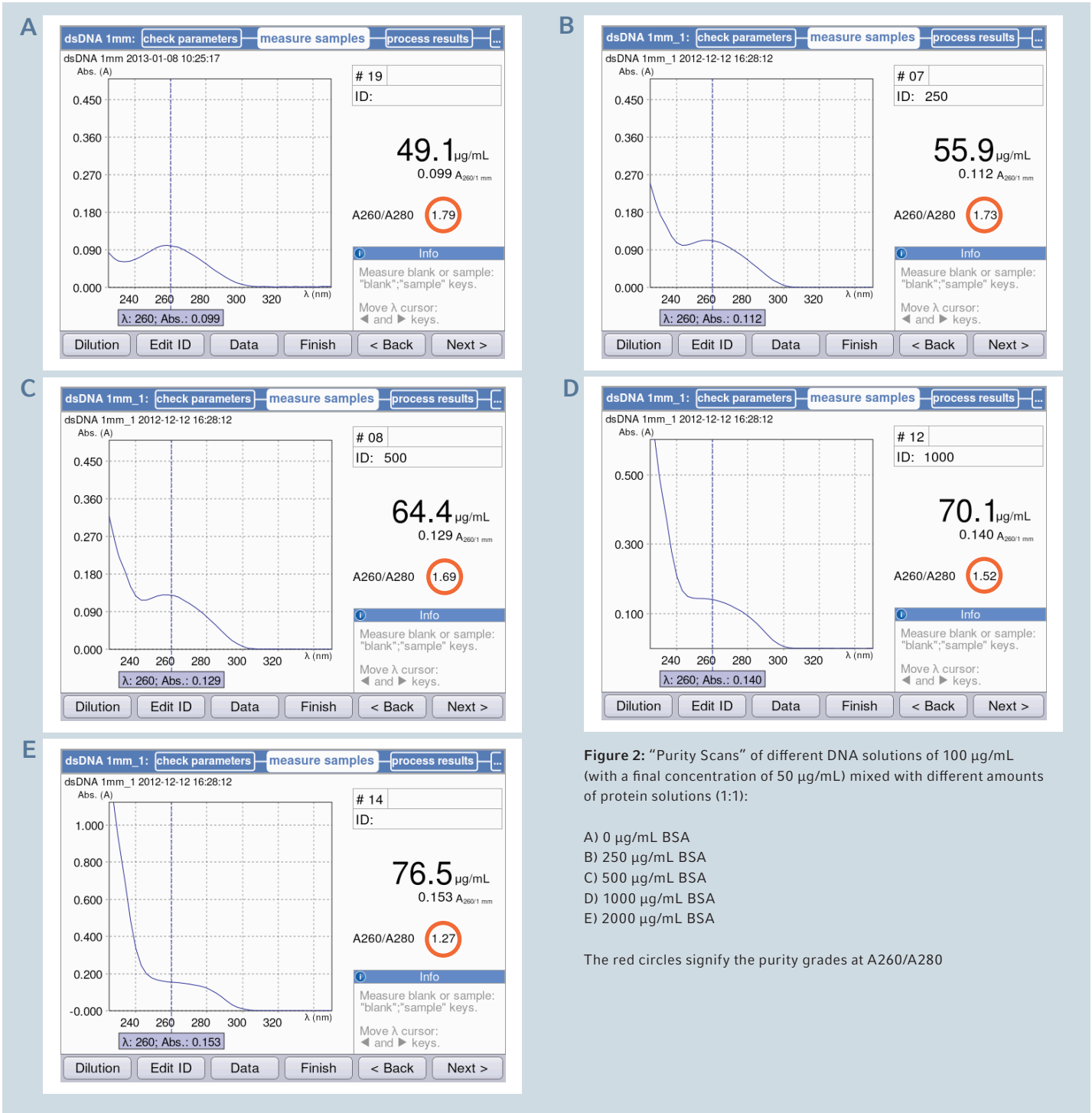

Figure 2: "Purity Scans" of different DNA solutions of 100 µg/mL (with a final concentration of 50 µg/mL) mixed with different amounts of protein solutions (1:1):

A) 0 µg/mL BSA
B) 250 µg/mL BSA
C) 500 µg/mL BSA
D) 1000 µg/mL BSA
E) 2000 µg/mL BSA

The red circles signify the purity grades at A260/A280

Figure 2 clearly shows that the recorded spectra deviate from the typical absorbance spectrum of a DNA solution to a larger extent with increasing protein contamination.

In general, the purity of a DNA solution is easily defined using the purity ratios defined in figure 1. If the purity grades are considered in isolation, a deviation from the control values may also be attributed to other causes. For example, if only the A260/A280 values in figure 2A and 2C are compared, sample contaminations are not immediately detected, since the deviation of both values is not significant (1.79 to 1.69). The contamination only becomes clear, if the scans of both samples are compared.

It is possible that a reduced purity grade may be attributable to the fact that the sample is solubilized in water instead of in buffer. Since water does not have buffer properties, the structure of the molecule to be measured, and therefore the absorbance behavior of the sample, is affected [1].
For this reason, the BioPhotometer D30 is capable of recording a "Purity Scan" which displays the absorbance properties of a sample graphically, thus enabling fast and simple visual quality control. In addition, all important raw data pertaining to the DNA sample are displayed in table format (figure 3). Furthermore, the table optionally lists the purity grade A260/A230, the absorbance at the wavelengths 280 und 230 nm, as well as the background absorbance at 340 nm. Thus, the two display formats complement each other perfectly to allow an evaluation of the quality of a DNA sample.

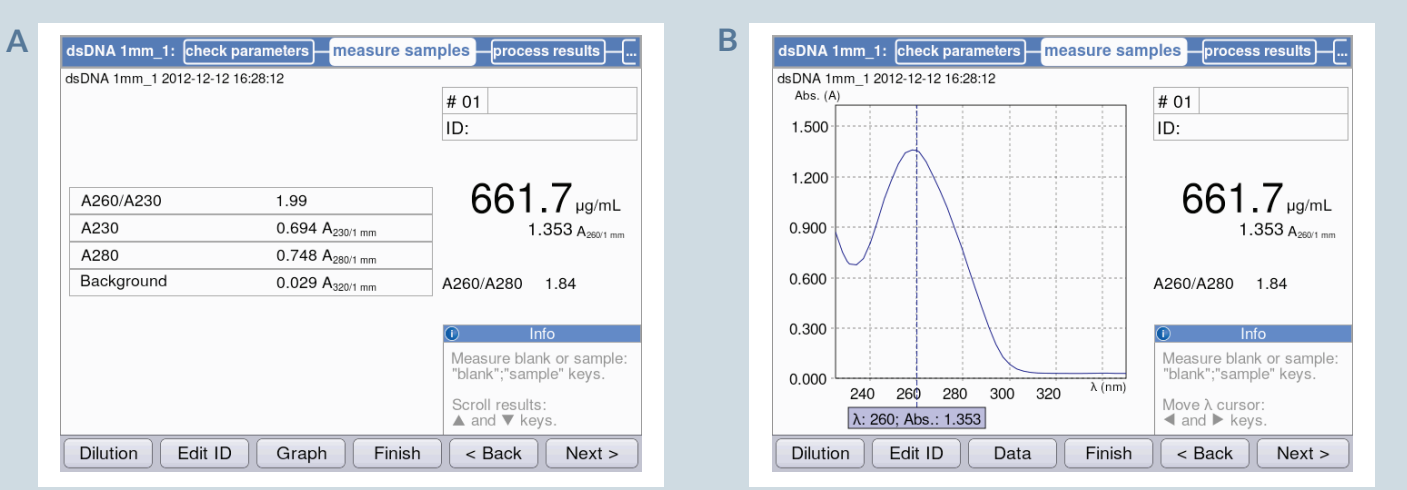

Figure 3: Data for the photometric measurement of a DNA sample displayed in table format as well as graphically:
A) Table format: Concentration in µg/mL, purity grades A260/A280 and A260/A230, absorbance values at 280 nm, 260 nm and 230 nm, as well as background absorbance at 340 nm, and calculation parameter for optical path length (in this case 1 mm)
B) Graphic format: Absorbance behavior of the sample ("Purity-Scan"): Result displayed in µg/mL, purity grade A260/A280, absorbance value at 260 nm, and calculation parameter for optical path length (in this case 1 mm)

In addition, the "Purity-Scan" function is also available for the method "Protein direct", which allows determination of protein concentrations via direct absorbance measurements at 280 nm. In contrast to DNA determination, no standard data for defined purity ratios are available. In this case, contaminations are best identified using a "Purity Scan" (figure 4). In the case of protein purification the absorbance behavior of a sample is an easy and fast indicator of whether additional purification steps need to be undertaken.

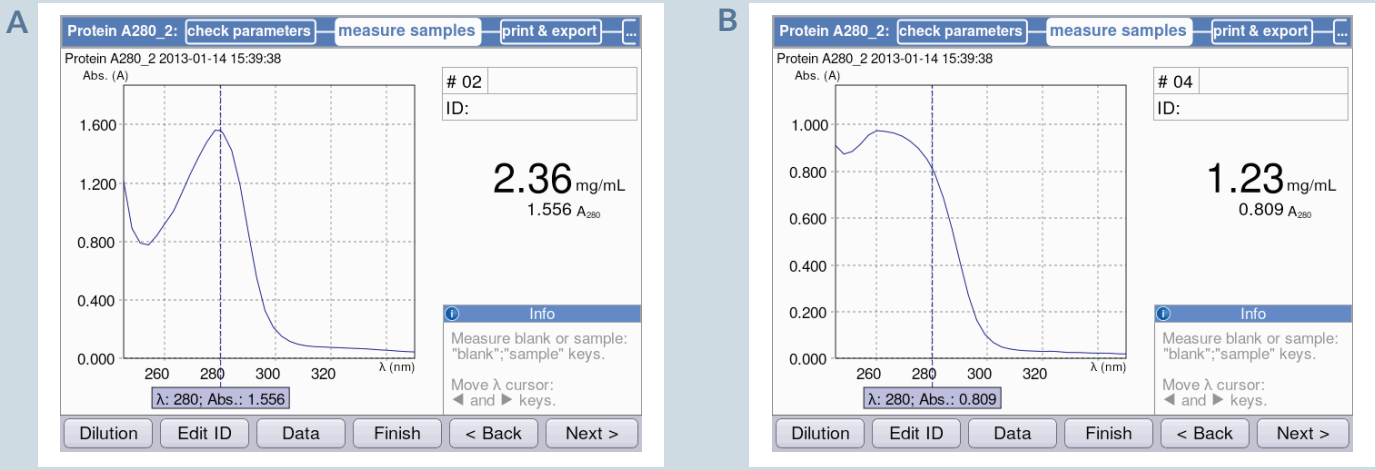

Figure 4: "Purity Scan" of a non-contaminated and a contaminated protein sample.
A) Non-contaminated
B) Contaminated: No clear curve shape typical of proteins, with one absorbance peak, can be discerned.

Conclusion

Protein and DNA samples are simply and easily quantified using the Eppendorf BioPhotometer D30. The results are displayed in a clear manner showing all information pertaining to sample quality and quantity. In addition, the optional "Purity Scan" enables assurance of the data by this format of optical control.

Literature

[1] Mülhardt, C. Der Experimentator, Spektrum – Akademischer Verlag, Berlin (2003) [German]
(The Experimentor, Spektrum Academic Publishers)

Ordering information

Description	Ordering information International	Ordering information North America
Eppendorf BioPhotometer® D30, 230 V/50 – 60 Hz, additional electrical connection variants are available	6133 000.001	
120 V/50-60 Hz, plug North America	6133 000.010	6133000010
Eppendorf µCuvette™ G1.0 & BioPhotometer D30 Eppendorf microvolume measurement cell and BioPhotometer D30		
> 230V/50-60Hz, Plug Europe	6133 000.907	
> 120V/50-60Hz, Plug North America	6133 000.908	6133000940
Eppendorf µCuvette™ G1.0 Eppendorf microvolume measurement cell for Eppendorf BioPhotometer® and Eppendorf BioSpectrometer®	6138 000.018	6138000018
Thermoprinter DPU 414 incl. adapter and printer cable		
230 V, EU	6131 011.006	
115 V/100 V, USA	6131 010.000	952010140
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