Quantitation of DNA

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Reliable measurement of DNA concentration is important for many applications in molecular biology. DNA quantitation is generally performed by spectrophotometric measurement of the absorption at 260 nm, or by agarose gel analysis. In this article, we examine some critical factors for quantitation, such as the effect of solvents and RNA contamination on absorption.

Materials and methods
All spectrophotometric measurements were taken in a Beckman DU7400 or DU640 spectrophotometer using a reference containing the appropriate solvent but without DNA. The solvent used was 10 mM Tris·Cl, pH 8.5 unless otherwise stated. Measurements were repeated 4 times. Experiments concerning the reliability of A260 values and the effects of solvents were performed using plasmid DNA purified with the QIAGEN® Plasmid Maxi Kit. Experiments investigating the effects of RNA contamination were performed with pUC21 plasmid DNA purified using the QIAprep® Spin Miniprep Kit and 5S rRNA (Boehringer Mannheim). Experiments showing agarose gel quantitation were performed with DNA fragments purified with the QIAquick™ PCR Purification Kit.

Reliability of A260 reading
The concentration of nucleic acids in solution can be calculated from the absorbance at 260 nm (Table 1).

Table 1. Spectrophotometric conversions

<table>
<thead>
<tr>
<th>1 A260 unit (1-ml detection path)</th>
<th>Concentration (µg/ml)</th>
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<tbody>
<tr>
<td>dsDNA</td>
<td>50</td>
</tr>
<tr>
<td>ssDNA</td>
<td>33</td>
</tr>
<tr>
<td>RNA</td>
<td>40</td>
</tr>
<tr>
<td>Oligonucleotide</td>
<td>20–30</td>
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Figure 1 shows the A260 measurements for serial dilutions of DNA. A260 values between 0.1 and 1.0 were very reproducible. The standard deviation was below 1% for A260 readings between 0.1 and 1.0, and as little as 0.3% for readings between 0.3 and 0.7.

However, low A260 readings (<0.1) were much less reproducible. The standard deviation was up to 9% for A260 readings of 0.010 and up to 18% for readings of 0.006. High A260
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References

readings (>1.0) also resulted in a high standard deviation, and readings above 3.0 were incorrect potentially leading to underquantitation of DNA.

Therefore, for reliable spectrophotometric DNA quantitation, A\textsubscript{260} readings should lie between 0.1 and 1.0. When working with small amounts of DNA such as purified PCR products or DNA fragments extracted from agarose gels, quantitation via agarose gel may be more effective (see section on page 25).

Effects of solvents
Absorption of nucleic acids depends on the solvent used to dissolve the nucleic acid (1). The effects of solvent on absorption measurements were analyzed by dissolving DNA in various solvents routinely used for spectrophotometric measurement, such as water and low-salt buffers. DNA was dissolved in ultrapure water and stirred for different lengths of time, resulting in increasing solvation of CO\textsubscript{2} and subsequent acidification of the water. A range of low-salt buffers with varied pHs (pH 7.5–9.0) and varied ionic strengths (10–100 mM) were also used. A\textsubscript{260} values were reproducible when using low-salt buffer, but not when using water. Readings in water resulted in a variation of up to 14% (data not shown). This is most likely due to differences in the pH of the water caused by the solvation of CO\textsubscript{2} from air. A\textsubscript{260}/A\textsubscript{280} ratios measured in water also gave rise to a high variability between readings (Figure 2), and ratios obtained were typically <1.8. Since the protein content of the DNA used was below 1%, these A\textsubscript{260}/A\textsubscript{280} ratios were unrealistic. In contrast, A\textsubscript{260}/A\textsubscript{280} ratios measured in low-salt buffer were completely reproducible.

Effect of RNA contamination
Plasmid DNA preparations can contain RNA contamination, for example, when the RNase A treatment during alkaline lysis does not degrade all RNA species. Since spectrophotometric measurement does not differentiate between DNA and RNA, RNA contamination can lead to overquantitation of DNA. This effect was demonstrated by adding 5S rRNA to RNA-free plasmid preparations. The
results in Figure 3A show that increased RNA contamination led to increased $A_{260}$ readings proportional to the amount of RNA added. Only the control RNA-free DNA gave a correct $A_{260}$ measurement. In addition, an increase in the $A_{260}/A_{280}$ ratio was observed with increasing RNA contamination (Figure 3B).

RNA contamination could be detected by agarose gel analysis with routine ethidium bromide staining, although not quantitated effectively. RNA bands appeared faint and smeary and only amounts $\geq 25$ ng (0.5:1 RNA:DNA ratio) were visible (Figure 3C).

RNA contamination of plasmid DNA can be a concern depending on the method used for plasmid preparation. Methods using alkaline lysis with phenol extraction cannot separate RNA from plasmid DNA, leading to high levels of RNA contamination. In contrast, the QIAprep miniprep system uses advanced silica-gel–membrane technology in combination with optimized buffers to ensure that RNA species do not bind to the membrane but pass through (2). Plasmid purification with QIAGEN Anion-Exchange Resin uses efficient chromatographic separation to yield ultrapure plasmid DNA that is completely free of RNA (3, 4).

**Agarose gel quantitation**

Small amounts of DNA such as PCR products can be quickly and easily quantitated by agarose gel analysis. The DNA sample is run on an agarose gel alongside known amounts of DNA of the same size. The amount of sample DNA loaded can be estimated by visual comparison of the band intensity with the standards.

![Figure 3A](image1)

**Effect of RNA Contamination**

![Figure 3B](image2)

![Figure 3C](image3)

Figure 3 - Different amounts of 5S rRNA were added to pUC21 DNA prior to quantitation. The ratio of RNA contamination to DNA for each sample is indicated. A $A_{260}$ measurements of samples containing 500 ng of plasmid DNA and different amounts of RNA. B $A_{260}/A_{280}$ ratios for the same samples as in A. C Agarose gel analysis of samples containing 50 ng of plasmid DNA and different amounts of RNA. M: markers.
Densitometric DNA quantitation

More precise agarose gel quantitation can be achieved by densitometric measurement of band intensity and comparison with a standard curve generated using DNA of a known concentration. Serial dilutions of pUC21 plasmid DNA were run on a 1% TAE agarose gel, and the intensities of the bands were measured densitometrically (Figure 5). Reliable DNA quantitation was achieved when there was a linear correlation between densitometric measurement and the amount of DNA loaded. Since the extent of ethidium bromide staining is relatively weak for DNA quantities ≤10 ng, these small quantities may not give reliable values. In most experiments the effective range for comparative densitometric quantitation is between 20 and 100 ng.

Conclusions

- Only A₂₆₀ readings between 0.1 and 1.0 are reliable enough to be used for DNA quantitation.
- Low-salt, alkaline buffer should be used as a solvent for spectrophotometric measurement of DNA, in order to achieve reproducible A₂₆₀ values and A₂₆₀/A₂₈₀ ratios.
- Spectrophotometric quantitation of plasmid DNA is accurate only when the DNA contains no RNA contamination.
- The amount of DNA used for densitometric quantitation should fall within the linear range of the standard curve.