Focal Points

Application Note FP-123

UVP BioImaging Systems

DNA Quantitation Using Agarose Electrophoresis Densitometry

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 260nm, 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.

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 along side 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 (Fig. 1).

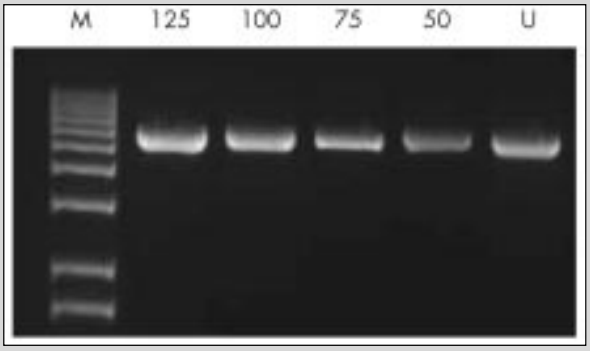


Fig. 1. Agarose Quantitation

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 (Fig. 2), and the intensities of the bands were measured densitometrically. 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 10 and 100 ng.

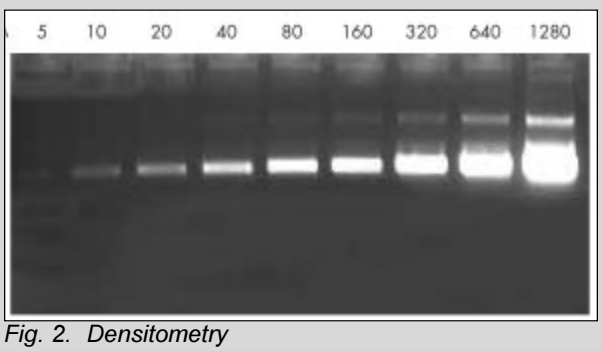


Fig. 2. Densitometry

Typically an A_{260} reading of between 1.0 and 0.1 will be reproducible with standard deviations of 1%. Standard deviations of 0.3% can be achieved with an A_{260} reading of closer to 0.5. When repeatedly reading DNA along the linear range of spectrophotometer, the OD at A_{260} and the standard deviation will increase. Similarly, as repetitive readings are made at decreasing ODs of A_{260} the standard deviation will increase. Graphically this is a parabolic function indicating the linear range (the vertex) as the most reproducible (stable).

Note that double stranded DNA (dsDNA) has a concentration of 50 µg mL^{-} at 1 A_{260} unit or 50 ng $µL^{-}$. Also note that the concentration at an A_{260} reading is then 25 ng uL^{-} which is well within the range of standard agarose gel electrophoresis. In fact a 10-fold increase in concentration to 250 ng is also well within the range of a standard agarose electrophoresis gel as well as a concentration of 12.5 ng. In summary gel densitometry has a larger range than spectrophotometric measurements.

Species	1 A_{260} Unit (µg mL^{-})	0.5 A_{260} Reading (ng $µL^{-}$)	Equiv. Electrophoresis Amount (ng)
dsDNA	50	25.0	25.0
ssDNA	33	16.5	16.5
RNA	40	20.0	20.0
Oligonucleotides	25	12.5	12.5

Effect of pH and ionic Strength

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 used for spectrophotometric measurements (2). Variations in A_{260} measurements of up to 14% had been found when using water due to differences in pH caused by CO_2 from air. Similar measurements in A_{260}/A_{280} ratios were found to be unrealistic until low salt buffer were used. Buffers of with ionic strengths of 10 to 100 mM were found to give reproducible results.

RNA Contamination

Since spectrophotometric measurement does not differentiate between DNA and RNA, RNA contamination can lead to over

quantitation of DNA (Fig. 3). RNA spiking experiments of DNA have shown A_{260} readings to increase. Additionally, an increase in the A_{260}/A_{280} ratio was observed with increasing RNA contamination.

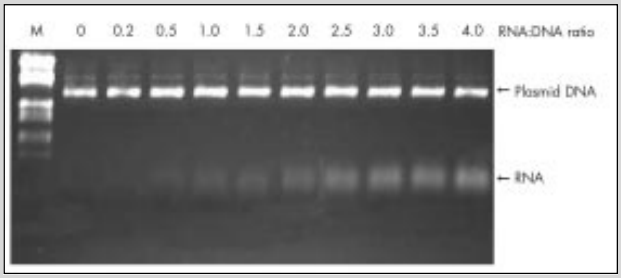


Fig. 3. RNA:DNA Spiking Experiment

Advantages of Densitometery

Along with the increased range of nucleic acid analysis, densitometry resolves effects of buffers and RNA contamination.

- Increased range equates to a greater number of samples that can be tested at differing concentration resulting in a more robust data set.
- Samples are in a consistent buffered environment during gel electrophoresis leading to intra-assay precision.
- RNA contamination is detectable on an electrophoresis gel with ethidium bromide staining and is frequently separated, due to differing electrophoretic mobility, from the analyte (DNA band(s)).

Points to Consider

- Only A_{260} readings between 0.1 and 1.0 are reliable enough to be used for DNA quantitation; while a four (4) step serial dilution of control DNA of known concentration increases quantitation range.
- Low-salt, alkaline buffer should be used as a solvent for spectrophotometric measurement of DNA, in order to achieve reproducible A_{260} values and A_{260}/A_{280} ratios.
- Spectrophotometric quantitation of plasmid DNA is accurate only when the DNA contains no RNA contamination; densitometry will detect and separate RNA contamination.
- The amount of DNA used for densitometric quantitation should fall within the linear range of a standard curve (12.5 – 100 ng R^2 >0.95)

Quantitating DNA Using UVP's Labworks™ Software

- Acquire an image of a gel containing a serial dilution of known and unknowns.
- Perform a DNA analysis by first finding lanes. Hint: (set the number of lanes in the Lanes Menu then click on Find Lanes).
- Next, select all the bands located in the lanes by clicking on the Band button.
- After the bands are selected click on the Background button and select the appropriate background correction.
- If bands are slightly curved you'll need to use the slant correction. Select the Slant tool on the button bar. Click on the "Auto. Slant Lines". You may need to manually adjust the slant lines by clicking on "Add Slant Line". In the example (Fig. 4), the r3 labeled bands are corrected to r2 bands in figure (Fig. 5).
- Click on the Results button to bring up the results table.

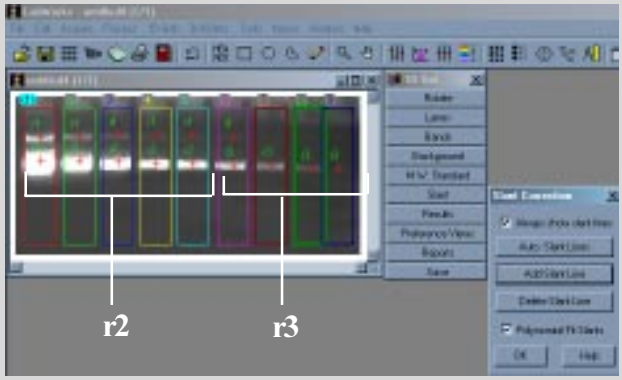


Fig. 4. Slant Line Correction

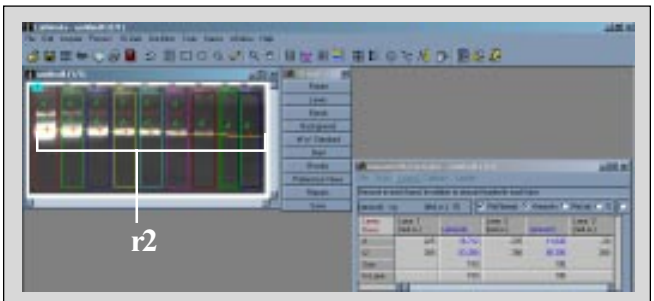


Fig. 5. Results

- In order to correctly separate and identify the bands of interest, select the Loads button from the results table menu (Fig. 6). Enter the amount of known sample for each lane. Click OK.

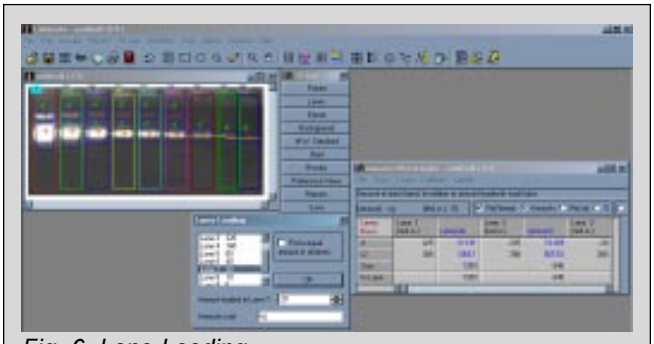


Fig. 6. Lane Loading

- Click on the Show menu of the results table (Fig. 7) check the “Amount or IOD” option; **print** the results table at this point for later use.

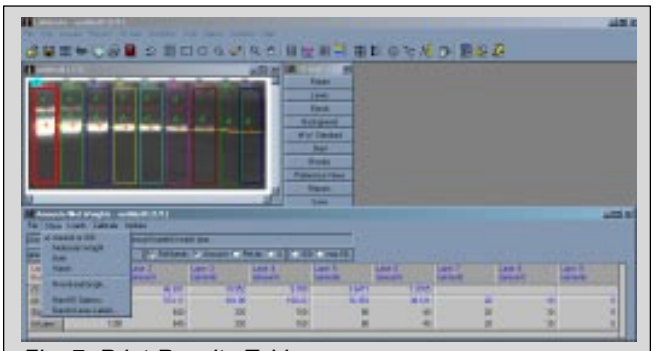


Fig. 7. Print Results Table

- Click on the Calibration menu in the Results Table menu (Fig. 8.). Select the “standard calibration curve” from the menu.

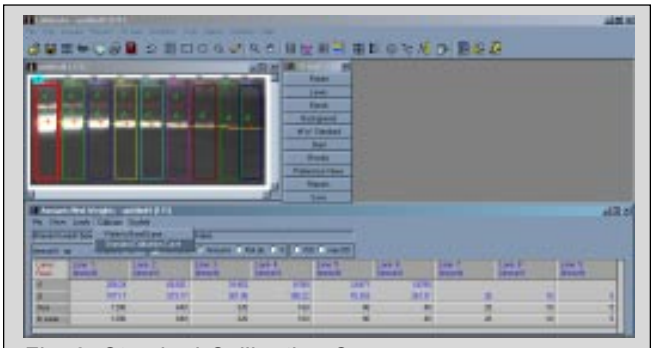


Fig. 8. Standard Calibration Curve

- Click on the brightest band of interest in the gel image and then enter the value from the **printout** into the “Band Amount” in the calibration screen (Fig. 9). Click Add. Repeat this process, select a band and enter its value (concentration) until all of the known lanes have been calibrated (Fig. 10).

- Select a Fitting Method from above the graph. Select a curve fit that has a low standard error.

- Click OK and note the results table. All the calibrated values for knowns and unknowns are listed. Labworks easily solves for the unknown concentrations.

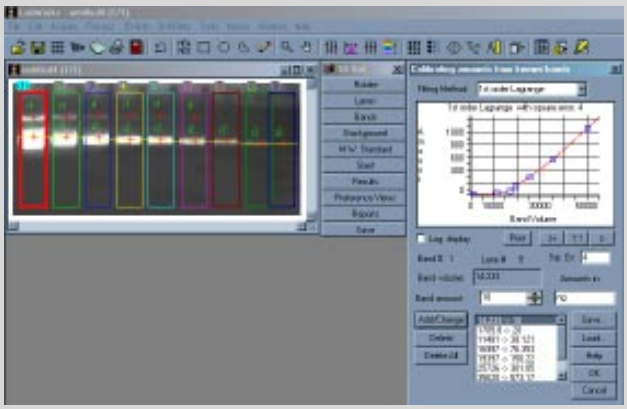


Fig. 9. Calibration Screen

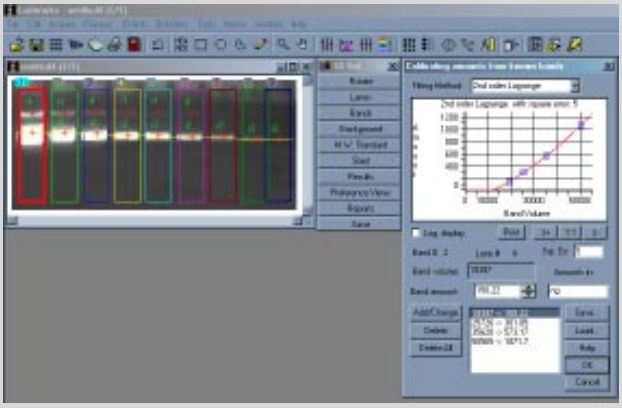
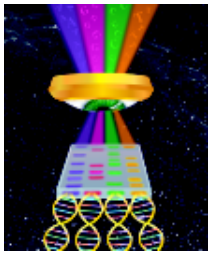


Fig. 10. Calibration

References

1. Effect of pH and ionic strength on the spectroscopic assessment of nucleic acid purity. (1997) BioTechniques 22, 474

2. Quantitation of DNA. Sauer, Philippe; Muller, Markus; Kang, Jie. Qiagen Corporation (1998)



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