

6.5 Analysis of Extracted DNA Using an UV Spectrophotometer

DNA quantification is achieved routinely with the use of spectrophotometry. The 230, 260, and 280 wavelengths provide the readings for quantification and purity and the 320 wavelength provides a reading for background compensation.

Standard quantitative conversion factors for nucleic acids are as follows:

- 1 absorbency unit at 260 nm of ds DNA = 50 ng/μL
- 1 absorbency unit at 260 nm of ss DNA = 33 ng/μL
- 1 absorbency unit at 260 nm of ss RNA = 40 ng/μL

Absorbance values for the 260 nm readings need to be in the linear range (between 0.1 and 1) for quantitation to be valid. If the absorbance at 260 nm (A₂₆₀) is above 1.0 the sample needs to be diluted. If the sample is below 0.1 there is negligible DNA in the sample.

Relative purity of the DNA sample can be determined by the 260/A₂₈₀ ratio. If the sample is pure nucleic acid, the ratio should be approximately 1.9. Excess protein in the sample will raise the absorbance at 280 nm thereby reducing the A₂₆₀/A₂₈₀ ratio.

One inexpensive instrument for this purpose is the GeneQuant II (Pharmacia Biotech) although other makes and models are available. The GeneQuant II is a spectrophotometer specific for obtaining concentrations of either double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), or RNA in units of weight, molar fraction, moles of phosphate, and total molecules. The instrument is capable of measuring the RNA or DNA using UV wavelengths at 230nm, 260 nm, 280 nm, and 320 nm simultaneously. If a standard spectrophotometer is all that is available, then conversion to pmoles /μL (i.e. uM) from ng/μL can be accomplished with the following table.

Amount of primer (ng) needed to equal 10 pmol:

<u>Primer Length</u>	<u>ng of Primer Equal to 10 pmol</u>
15	50
16	53
17	56
18	59
19	63
20	66
23	78
24	80