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7.13 Experimental Microbial Genetics

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UV spectrophotometric measurement of DNA concentration and purity

DNA itself, and most of the common contaminants found in DNA preps, have absorbances in the region 230nm to 320nm so measurement of the absorbances in this region allows measurement of the DNA concentration and provides information about the contaminant levels. The measurements can be performed on a conventional spectrophotometer, and the most important wavelengths to note are:

230nm: Guanidium salts (used to facilitate DNA binding to silica columns) and phenol (used in phenol/chloroform extractions) absorb strongly at 230nm, therefore high absorbances at this wavelength can be indicative of carry-over of either of these compounds into the sample.

260nm: DNA absorbs light most strongly at 260nm so the absorbance value at this wavelength (called A_{260}) can be used to estimate the DNA concentration using an equation derived from **Beer's Law** (see below).

280nm: Since tyrosine residues absorb strongly at this wavelength, the absorbance at 280nm is used as an indicator of protein contamination.

320nm: A_{320} provides a general measurement of the turbidity of the sample and is normally subtracted from the A_{260} value as a background reading for the calculation of DNA concentration, but excessive values may indicate non-specific contamination.

Estimating DNA concentration: measure the absorbance at 260nm (A_{260}), adjust the A_{260} measurement for turbidity (measured by absorbance at 320nm), multiply by the dilution factor, and use the relationship that an A_{260} of 1.0 = 50 μ g/ml pure DNA to calculate the estimated DNA concentration.

$$\text{Concentration } (\mu\text{g/ml}) = (A_{260} \text{ reading} - A_{320} \text{ reading}) \times \text{dilution factor} \times 50\mu\text{g/ml}$$

Total yield is obtained by multiplying the DNA concentration by the final total purified sample volume.

$$\text{DNA yield } (\mu\text{g}) = \text{DNA concentration} \times \text{total sample volume (ml)}$$

DNA purity can be estimated from the A_{260}/A_{280} ratio.

An A_{260}/A_{280} ratio between 1.7 and 2.0 and an A_{260}/A_{230} ratio of greater than 1.5 generally represents a high-quality DNA sample, but since the sensitivity of different techniques to these contaminants varies, these values should only be taken as a guide to the purity of your sample.

The ratio can be calculated after correcting for turbidity (absorbance at 320nm).

$$\text{DNA Purity } (A_{260}/A_{280}) = (A_{260} \text{ reading} - A_{320} \text{ reading}) \div (A_{280} \text{ reading} - A_{320} \text{ reading})$$

$$\text{DNA Purity } (A_{260}/A_{230}) = (A_{260} \text{ reading} - A_{320} \text{ reading}) \div (A_{280} \text{ reading} - A_{230} \text{ reading})$$

Note: For an accurate measurement, the A_{260} value must lie between 0.1 and 1, so dilution of concentrated samples may be required.