

Principle of Quantification of DNA by Spectrophotometry

The principle of quantification of DNA by spectrophotometry is based on the measurement of the absorbance of DNA at specific wavelengths of light. DNA molecules absorb ultraviolet (UV) light predominantly at a wavelength of 260 nanometers (nm). By measuring the absorbance of DNA at this wavelength, the concentration of DNA in a sample can be determined. Here is a basic outline of the principle and steps involved:

Sample preparation: The DNA sample to be quantified is typically dissolved in a buffer, such as Tris-EDTA (TE) buffer, to provide a stable environment for the DNA and to ensure accurate measurements. The sample should be free from contaminants that may interfere with the spectrophotometric analysis.

Blank preparation: A blank solution is prepared using the same buffer as the sample, without the DNA. This blank solution is used as a reference to compensate for any background absorbance of the buffer or contaminants present.

UV absorbance measurement: The sample and the blank solution are placed in a spectrophotometer, which emits UV light at a wavelength of 260 nm. The spectrophotometer measures the intensity of the transmitted light through the sample and blank. The amount of UV light absorbed by the DNA in the sample is directly proportional to its concentration.

Calculation of DNA concentration: The absorbance readings obtained for the sample and blank are used to calculate the DNA concentration. The absorbance is typically determined using the Beer-Lambert Law, which states that absorbance (A) is equal to the molar absorption coefficient (ϵ) multiplied by the path length (l) through which the light passes and the concentration (c) of the absorbing species. The equation used for DNA quantification is:

$$\text{DNA concentration } (\mu\text{g/mL}) = A_{260} \times \text{dilution factor} \times \text{DNA conversion factor (50 } \mu\text{g/mL)}$$

The dilution factor accounts for any dilution of the DNA sample before measurement, and the DNA conversion factor converts the absorbance readings to DNA concentration based on the specific characteristics of the DNA (e.g., extinction coefficient and molecular weight).

It is important to note that spectrophotometry can measure the total amount of DNA in a sample, including both single-stranded and double-stranded DNA. However, it does not differentiate between DNA and RNA or provide information about the purity or quality of the DNA sample. For more detailed analysis, additional methods such as agarose gel electrophoresis or fluorescence-based assays may be necessary.