Lab 2

Quantification of DNA Concentration and Purity

The concentrations and purity of extracted DNA are determined by measuring the absorbance of the sample at 260 nm by using a Nanodrop spectrophotometer.

- Moreover, the A260/A280 value is used as an indication of DNA purity.
- The A260/A280 value of pure DNA must occur within a range of 1.7 to 2.0.
- However, the purified DNA samples with A260/A280 ratio of more than 2.0 are re-processed.
- A lower ratio indicates the sample is protein-contaminated. The A260/230 ratio indicates the presence of organic contaminants,

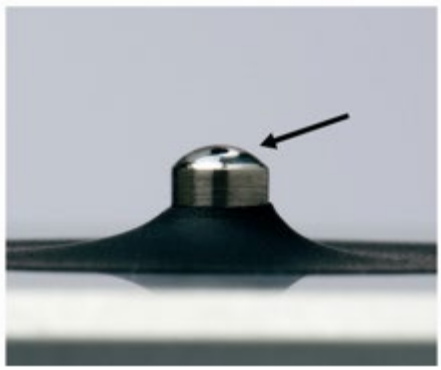
The Experiment

DNA Concentration and Purity

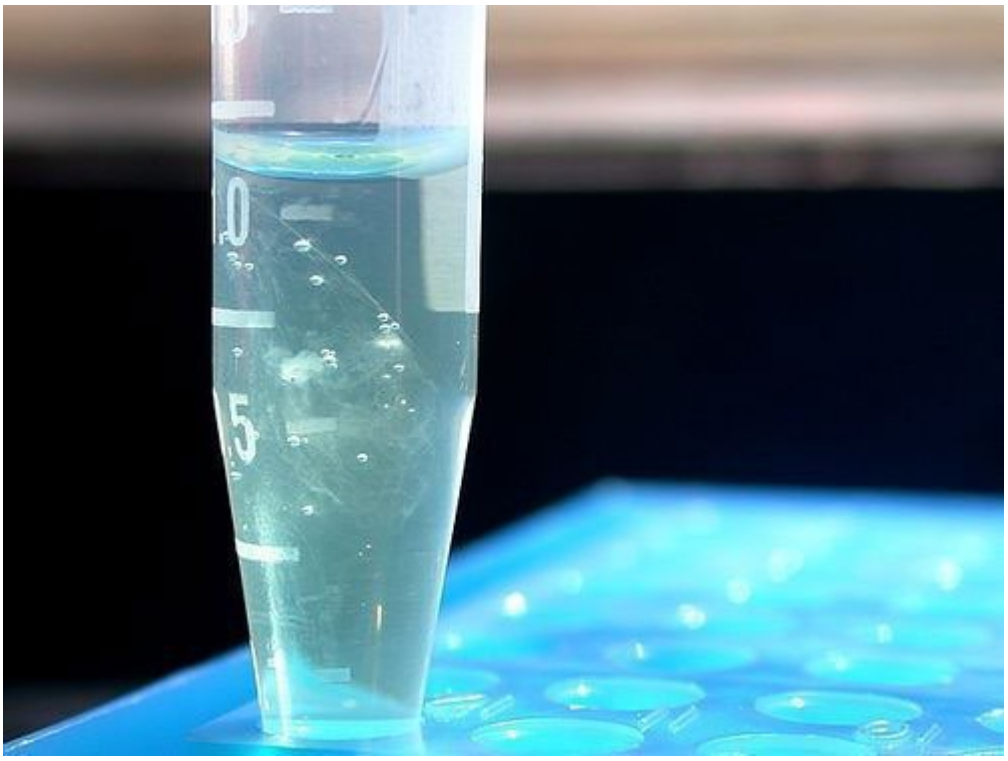
Materials:

Nanodrop spectrophotometer

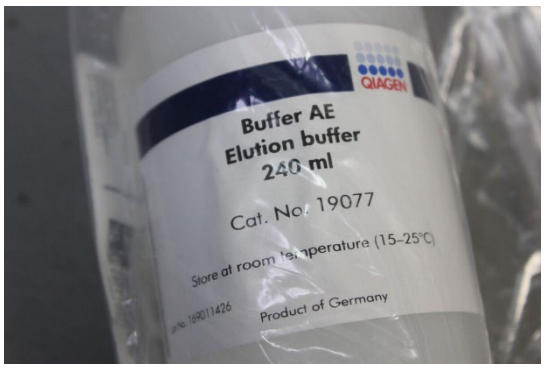
- Measures the concentration and Purity of DNA or protein or RNA which enables the quantification of samples in volumes as low as 0.5-2 µL.
- This capability has become increasingly important as molecular techniques continually evolve to use smaller amounts of material for analysis.



The extracted DNA from bacteria



Elution buffer (AE) or Nuclease-free water



Micropipette

Micropipette tips

Eppendorf tubes

Vortex

70% Ethanol

Methods:

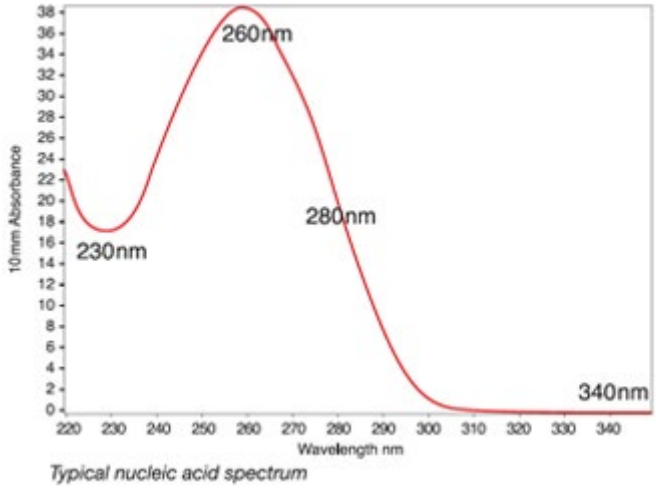
1. Establish a blank using an appropriate buffer (EA).

It is important to use the buffer in which the DNA is suspended. The buffer used should be the same pH and of similar ionic strength as the sample solution

2. Pipette 2 µL of the appropriate blanking solution (EA) onto the bottom pedestal, lower the arm, and click Blank.

Important note: The homogeneity of the sample is extremely important since such a small volume is being measured. To ensure that samples are homogenous, gently but thoroughly mix the samples immediately before taking an aliquot for measurement. Avoid inserting bubbles when mixing and pipetting.

3. Wipe the blank solution from the lower and upper pedestals using a dry laboratory wipe.
4. After all of the Standards have been measured, click on the Samples button. Enter the sample ID. Load 2 µL of sample onto the lower pedestal and click Measure. A fresh 2 µL aliquot of the sample should be used for each measurement.
5. Wipe the sample from the lower and upper pedestals using a dry laboratory wipe.



Typical nucleic acid spectrum