Protocol 6: DNA Extraction Quality Control

Goal: Assess the quantity and quality of DNA extracted from biological samples.

During this lab you should learn to:

- Operate a Nanodrop spectrophotometer to measure absorption
- Interpret results of Nanodrop readings

Introduction:

A successful DNA extraction protocol yields a solution containing purified DNA, which can then be used for various downstream applications such as PCR, sequencing, or cloning. This solution ideally consists of intact, high-quality DNA, free of proteins, lipids, and other cellular debris. However, potential sources of contamination can compromise the purity and integrity of the extracted DNA. Common contaminants include residual proteins, RNA, and phenolic compounds from the cell lysis step, as well as chemical residues from reagents used during the extraction process.

Absorption spectroscopy is a valuable technique for assessing both the quantity and quality of a DNA sample. The absorbance of a sample can be determined by directing a specific amount of light through the sample and measuring the amount of light that emerges on the other side. By measuring the absorbance of a DNA solution at specific wavelengths, particularly at 260 nm, the concentration of DNA can be quantified, as DNA absorbs UV light strongly at this wavelength. The absorbance reading at 260 nm is used to calculate the DNA concentration using Beer-Lambert's law. To evaluate the quality of the DNA, the absorbance ratios at 260/280 nm and 260/230 nm are examined. A 260/280 nm ratio of ~1.8 is indicative of pure DNA, as proteins and other contaminants absorb more at 280 nm. The 260/230 nm ratio, ideally between 2.0-2.2, provides additional insight into the purity, with lower values suggesting the presence of contaminants such as phenol or carbohydrates. Thus, absorption measurements offer a quick and reliable method to assess both the concentration and purity of DNA samples.

Protocol:

Materials needed:

Kimwipes
Sample
Distilled water
Elution buffer

Step-by-step Instructions:

1. Raise Nanodrop arm and use the Kimwipe dampened with distilled water to clean the top and bottom of the pedestal.
2. Close the pedestal and start up the Nanodrop for measuring DNA.

3. Once you can take measurements, select the option to add a "Blank". This allows the Nanodrop to account for any absorbance due to chemicals in the elution buffer.
4. Add 1.5 uL of elution buffer to the pedestal and gently close it. Take the blank measurement.
5. Raise Nanodrop arm and use the Kimwipe dampened with distilled water to clean the top and bottom of the pedestal.
6. Add 1.5 uL of your sample to the pedestal and gently close it. Take the measurement of your sample noting concentration, A260, A280, and A230 measurements.
7. Complete steps 5 and 6 for all of your samples. You could also run replicates of your measurements on individual samples.

Sample Source	Conc. (ng/uL)	A260	A280	A230	$\frac{A260}{A280}$	$\frac{A260}{A230}$

Before you leave, enter your quality control data to the class data spreadsheet.