Experiment Spectrophotometric Analysis of DNA and RNA
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Experiment 5: Spectrophotometric Analysis of DNA and RNA

Objective

To determine the concentration and purity of DNA and RNA samples using UV–Vis spectrophotometry based on absorbance at 260 nm and characteristic purity ratios.

Introduction

Spectrophotometric measurement of nucleic acids remains a fundamental analytical tool in molecular biology. DNA and RNA exhibit a strong absorbance peak at 260 nm due to their aromatic nitrogenous bases. This allows rapid and non-destructive quantification using the Beer–Lambert law and well-established conversion factors. Purity assessment is achieved using absorbance ratios, primarily A260/A280—indicating potential protein contamination—and A260/A230—which reflects the presence of organic or chaotropic impurities such as phenol, guanidinium salts, or carbohydrates. Microvolume spectrophotometers such as the NanoDrop enable direct and accurate readings from very small sample volumes, making this method essential for routine quality control of nucleic-acid preparations.

Principle

Nucleic acids absorb ultraviolet light maximally at 260 nm. The Beer–Lambert law ($A = \varepsilon \times c \times l$) relates absorbance to concentration. Standard conversion factors include:

- 1 A260 = 50 μg/mL for double-stranded DNA

- 1 A260 = 40 μg/mL for RNA

Purity is evaluated using A260/A280 and A260/A230 ratios to identify contaminants that co-absorb at other wavelengths.

Materials and Equipment

Material / Equipment	**Purpose**
Purified DNA samples	Concentration and purity analysis
Purified RNA samples (RNase-free)	Quantification and purity assessment
Nuclease-free water or TE buffer	Blank solution and dilution
Microvolume spectrophotometer (e.g., NanoDrop)	Direct absorbance measurement
UV–Vis spectrophotometer with quartz cuvettes	Conventional absorbance readings
Quartz cuvettes	UV-transparent optical path
Pipettes and RNase-free filtered tips	Accurate, contamination-free handling
Microcentrifuge tubes	Sample preparation and storage
Vortex mixer	Homogenization of samples
Microcentrifuge	Removal of bubbles and droplets
Lint-free wipes	Cleaning of measurement surfaces

Procedure

1. Turn on the spectrophotometer and allow it to stabilize.
2. Clean the pedestal or cuvette using nuclease-free water and a lint-free wipe.
3. Blank the instrument using nuclease-free water or TE buffer.
4. Mix the nucleic-acid sample gently and briefly centrifuge.
5. Load 1–2 μL onto the pedestal or into the cuvette.

6. Measure absorbance across 220–350 nm or specifically at 260 nm, 280 nm, and 230 nm.
7. Record the concentration displayed by the instrument and apply any required dilution factor.
8. Calculate A260/A280 and A260/A230 ratios.
9. Clean the pedestal or cuvette between samples.

References

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2. Watson JD et al. *Molecular Biology of the Gene*. Pearson.
3. Nelson DL, Cox MM. *Lehninger Principles of Biochemistry*. W.H. Freeman.
4. Berg JM, Tymoczko JL, Stryer L. *Biochemistry*. W.H. Freeman.
5. Desjardins P, Conklin D. "NanoDrop microvolume quantitation of nucleic acids." *JoVE*.

Short Questions

1. What factors can reduce the A260/A230 ratio in nucleic-acid samples?
2. Why does RNA often yield a slightly higher A260/A280 ratio compared to DNA?
3. Mention two limitations of UV–Vis spectrophotometric quantification.