Bio 181: DNA Quantitation

Part One: Quantitation by Spectrophotometry

You will measure the concentration of dsDNA in a sample using a spectrophotometer to measure the sample's absorbance at 260 nm. Sample purity will be evaluated by also measuring absorbance at 280 nm. Pure dsDNA should give a A_{260}:A_{280} ratio of 1.8; ratios significantly less than this indicate the presence of contaminants (especially proteins) which raise the A_{280}. For dsDNA, an optical density (OD) of 1 indicates a concentration of about 50 μg/mL (50 ng/ μL).

1. Obtain 10 μL of bacteriophage λ DNA (pure dsDNA).

2. Add 490 μL of TE buffer (T = Tris-HCl 10 mM; E=EDTA, pH 8; commonly used buffer for storing or diluting DNA). Mix well by pipetting.

3. Carefully clean a quartz cuvette with 95% ethanol; dry. Transfer your sample to this cuvette.

4. (Instructor will have turned on the spectrophotometer 30 minutes ahead to allow UV bulb to warm up). Set spectrophotometer absorbance to 260 nm or engage the program for measuring [dsDNA].

5. Insert a cuvette blank (filled with 500 μL TE) and zero the machine at 260 nm.

6. Insert your sample cuvette and read absorbance at 260 nm.

{Spectrophotometer should already be programmed to perform steps 7, 8, and 9}

7. Change the wavelength to 280 nm and read the absorbance again.
8. Calculate dsDNA concentration:

   A_{260} x dilution x 50 μg/mL
9. Calculate A_{260}:A_{280} ratio to evaluate sample purity.
10. Record results for your notebook.

Part Two: Quantitation by ethidium bromide fluorescence

Spectrophotometry cannot be used to measure DNA concentration if:

- Sample is contaminated (with protein, RNA, solvents, or other UV-absorbing substances)
- Concentration is too low (or too high)
- You don't have enough

A variety of methods can be used to estimate DNA concentration by visually comparing sample fluorescence with a series of known concentration standards. Most simply, DNAs are spotted onto a slide; alternatively, sample & standards can be run out on a gel (for example, to separate fast-running RNA from the DNA).

Ethidium bromide is an intercalating agent which binds DNA and fluoresces under UV illumination. The amount of EtBr bound, and therefore the amount of fluorescence, is basically proportional to the total mass of DNA.

1. Obtain a set of λ DNA concentration standards (0.5, 1, 2.5, 5, 10, 20 μg/mL) and a tube of unknown concentration.

2. Spot 2 μL of each sample onto a slide in an ordered array. In the "dirty" lab space by the UV lamp and camera, add 2 μL diluted EtBr (2 μg/mL in TE) to each spot and carefully mix by pipetting. **Wear gloves. Be sure to change tips each time**.

3. Photograph fluorescence and estimate DNA concentration of unknown sample.