Spectrophotometric Determination of DNA or RNA Concentration

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Summary

Read AU of sample at 260 nm and 280 nm in Baker lab. Try 1/100 dilution in cuvette:

Place 495 ul of buffer and blank the cuvette; then add 5ul of sample with long tip and mix well (but gently without making bubbles!) pipetting up and down.

Read absorbance at the two wavelengths using the multiple wavelength setting.

Detailed Instructions

Ask before using the Baker lab Agilent Spectrophotometer.
Sign in the log, select F1 (tasks) (enter) as multi wavelength, using up and down arrows.
Type in 260 and 280. Then done. Make sure lamps are on with m button.
Select lamps on/off (enter) turn on/off with arrows <= or =>.
Wait at least 10 min for lamps to warm up.

Place our cuvette (masked, semi-micro) in place with the 495 ul of buffer (for example, 1X TE) as blank.
Lock in place.
Press Blank Button. Check the blank, by pressing the Sample button to confirm that the reading is > 0.003.
Loosen and remove cuvette, add the sample (5 ul) to the buffer, mix gently, replace cuvette and lock down and read at least 2x.
Print out results.

Absorbance reading at **260nm** allows the calculation of **Concentration of nucleic acid**:

An OD of 1 corresponds to approximately:
* 50 ug/ml of DNA for double-stranded DNA
* 40 ug/ml for single-stranded DNA and RNA
* 20 ug/ml for oligonucleotides.

Ratio of readings at 260nm and 280nm [A_{260}/A_{280}] provides an estimate of **Purity** of the nucleic acid. Pure preparations of DNA and RNA have ratios of 1.8 – 2.0. If there is significant contamination with protein or phenol, the ratio will be lower and accurate quantitation is not possible.

ds DNA OD=1 => 50 ug/ml extinction coefficient = 1/ 50 = 0.02