Laboratory for Environmental Pathogens Research
Department of Environmental Sciences
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DNA quantification (spectrophotometry)

Materials

DNA standard solutions

We have a standard series of herring sperm DNA solutions that includes DNA concentrations of 500, 100, 50, and 10 ng DNA μl^{-1}. Keep stocks of these solutions by diluting the concentrated herring sperm DNA (10 mg ml^{-1}) accordingly in DNase/RNase-free water.

D.I. water for dilution of DNA prior to reading.

One sterile 1.5 ml microcentrifuge tube for each sample

Plastic (for concentrated samples, >50 μg ml^{-1}) or quartz (for low concentration samples, < 50 μg ml^{-1}) cuvettes.

Spectrophotometer

- Wear gloves throughout the entire protocol.
- Do not cross-contaminate your samples or the solutions. Be aware of your pipette tip.
- Work clean, either on fresh blue bench paper, in the hood, or on a freshly ethanol treated bench top.
- Perform all centrifugations with the hinge of the tube pointing “up”.
- Do not use a vortex at any point in this protocol unless specified.

Begin by turning on the spec to allow the lamp to warm up for approximately 15 minutes before reading samples. Be sure that there is no cuvette in the well as the spec goes through its self-diagnosis.

The protocol in brief

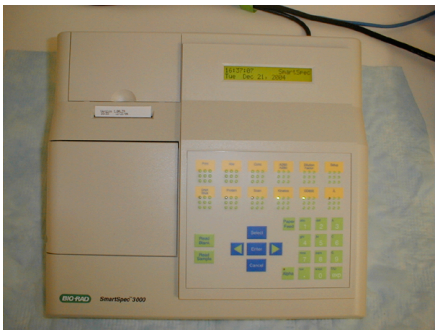
You will quantify DNA in solution by measuring the absorbance of light (260 nm) in a spectrophotometer.

A. Preparing DNA samples

1. Remove the standards and your samples from the freezer and thaw them **on ice or in the refrigerator**. Mix them by tapping the side of the tube with your finger. Do not vortex to mix.

2. In a separate sterile 1.5 ml microcentrifuge tube for each standard/sample, mix 10 μl of DNA with 990 μl of D.I. water. Vortex to mix. Let this solution stand for 10 minutes to ensure the complete diffusion of DNA throughout the solution. This represents a 1:100 dilution of the standards and your DNA samples.

B. Preparing the spectrophotometer



A typical spectrophotometer

3. Select "DNA/RNA", and follow the cues given by the spec to set the machine up for your readings:

We most commonly quantify:	dsDNA
Absorbance of 1 relates to:	50 μg ml^{-1} DNA

4. Press "Enter" to validate these settings.

5. Select "Dilution factor" and enter "100" to account for the DNA concentration in your sample being 1:100 of your original stock.

6. Press "Enter" to validate these settings.

7. Blank the spec by first inspecting the cuvette to make sure that there are no smudges or blemishes that might interfere with the absorbance reading and then inserting a sample of water identical to the water in which you diluted your DNA sample and pressing "Read blank"

8. Return to the assay screen by pressing ">".

   The spec is now set to begin reading standards and samples.

C. Reading standards/samples

9. Inspect the cuvette to make sure that there are no smudges or blemishes that might interfere with the absorbance reading. Briefly vortex the DNA sample again and transfer the solution to the cuvette being careful not to form bubbles along the wall of the cuvette. First read the standards.

10. Insert the cuvette into the spec. Be sure that the correct face of the cuvette is aligned with the direction of the light beam. Close the lid and press "Read sample" to begin reading.

    An absorbance reading will appear on the screen when the reading is complete (approximately 3 seconds).

11. Prepare the next standard as above, and continue reading until all standards have been quantified.

The readout will provide several important pieces of data:

"**A_{260}**" – this is the wavelength of light that is absorbed by DNA. This value is used to determine that concentration of DNA in your sample according to the conversion factor you set previously (A_{260} of 1.0 = 50 μg ml^{-1} DNA, step 3).

"**A_{280}**" – The absorbance generated at 280 nm is used in the ratio A_{260}:A_{280}, which determines the purity of the DNA. Samples are considered of adequate purity if A_{260}:A_{280} >1.5.

"**Conc.**" – Based on the conversion factor and dilution factor you programmed into the spec at start-up, this is the concentration of DNA in your sample (μg ml^{-1}).

The results of the standard readings should be accurate (± 10%) before proceeding with your samples.

12. Continue reading your samples as above until all samples have been quantified.

13. To print your results, press "Print", at which time you will be given three printing options. Choose option "3", which directs the spectrophotometer to print all unprinted sample data. The other two options can also be used but check the manual to determine if these are the best options.

14. To exit the program press "Cancel", then "<" to "Exit assay".

TURN THE SPEC OFF WHEN YOU ARE FINISHED.