DNA Quantitation Protocol

Introduction

We are currently estimating the concentration of genomic DNA in solution by use of a spectrophotometric method (a.k.a. “O.D.”, or optical density). The samples are diluted in 1X TE, and spec. readings are taken at wavelengths of 260, 280, and 320 nm against a blank of TE. The 260nm reading is used to calculate the DNA concentration; the ratio of 260 nm to 280 nm values gives an indication of the purity of the DNA (protein or phenol contamination); the 320 nm reading is an indicator of other substances present which may interfere with the 260 and 280 nm readings (such as carbohydrate or chloroform), and is sometimes subtracted from those values (we have chosen not to do this). After readings are recorded, the data is examined to determine if it is valid. If the data is useable, calculations are made, values are recorded in the O.D. book and data base, and the DNA is placed at 4o C in appropriate boxes for long-term storage. If the spec. readings are not valid, the sample must be run again, using a different dilution if necessary. Criteria for determining validity of data are found following the protocol.

Protocol

Before beginning, sign up to use the spec., and turn on uv light source to allow warm-up time of at least 30 min.

1. Mix genomic DNA by inverting tube several times, then spin down briefly in microfuge.
2. Make appropriate dilutions of DNA into 1X TE buffer in a labelled 1.5 ml tube.A total volume of at least 50 ul is needed to get a reading using our cuvette. An appropriate dilution to start with is 1:50--that is, pipet 49 ul TE into the tube and add 1 ul DNA sample. Careful pipetting is important for correct quantitation. Mix contents of tube by pipetting up and down a few times.
3. Select a nucleic acid program on the spec (one that measures all three wavelengths). Warburg-Christian Concentration works well.
4. Pipet 50 ul TE into a clean cuvette, place in spec., and click on 'blank'. The machine will zero itself to the TE (you can check the actual absorbance reading at the bottom of the screen). The number should be close to 0.000, with not too much drift.
5. Remove cuvette, pipet liquid out of the cuvette and discard tip. Rinse with a strong stream of di H2O from a wash bottle, shake out all residual liquid, and dry outside of cuvette with an Accuwipe. Take care to avoid lint or smudges on the transparent surfaces.
6. Mix the DNA sample gently by pipetting up and down once or twice. Pipet 50 ul into the cuvette, and check to see that no bubbles are present. Click on “Sample” at the top of the screen. The spec. will take readings at all 3 wavelengths, and display them (along with calculations) on the screen.
7. Repeat steps 5 and 6 until finished. It is a good idea to occasionally do a reading using just TE, to check if the instrument is still zeroed correctly, especially if readings do not appear correct.
8. When you are finished with all the samples, click on 'Print' to obtain a hard copy of the results. Turn off uv light. Clean up.

What it all means:

DNA absorbs light at certain wavelengths, so that the amount of light passing through the cuvette is decreased according to how much DNA there is in solution. It has been determined empirically how much DNA it takes to absorb a certain amount of light (the extinction coefficient). In the case of DNA, 1 O.D. unit (an absorbence of 1.0) is equivalent to a DNA concentration of 50 ug/ml. However, there is a limited range over which the absorbance readings are valid. An absorbance reading of between 0.100 and 0.800 is ideal. We have obtained results using readings from 0.050 to 0.900, but samples with O.D.'s outside of this range should definitely be redone at a different dilution. Likewise, samples with negative numbers or other implausible data should be redone. The A260/A280 ratio for pure DNA is 1.8. A range of about 1.6 to 2.0 may be considered normal, with samples falling outside this range being evaluated to see if further processing is needed (ie. phenol:chloroform clean-up, etc.).

Calculations

To calculate the concentration of DNA in mg/ml (same as ug/ul), multiply the reading at 260 nm by the dilution factor (this gives you the actual O.D.) Take that number and multiply it by 50 (because 1 O.D. unit equals 50 ug/ml DNA). Divide that product by 1000 (this converts the number from ug/ml to mg/ml).

((A260)(D.F.)(50))/1000 = mg DNA/ml =ug DNA/ul

ex. a sample was diluted 1:25, and the A260 was .200

((.200)(25)(50))/1000 = 0.25 mg/ml