Quantification and Purity of Genomic DNA Using the SmartSpec 3000 Spectrophotometer

The following procedure is adapted from the SmartSpec 3000 Spectrophotometer Instruction Manual. This handbook is available as a downloadable PDF file from the laboratory course web page.

Special Note: This procedure can also be used for quantifying plasmids and PCR products. Consideration only needs to be given to taking measurements by blanking the spectrophotometer using the same solution in which the DNA is suspended.

Reagents/Materials Required:

- Genomic DNA in TE Buffer
- TE Buffer
- trUView Cuvette

Procedure:

1. Power up the SmartSpec 3000 Spectrophotometer by switching the rear button to the "on" position. Wait until the front panel lights begin blinking before proceeding.
2. Press the "DNA:RNA" button.
3. Watching the display, use the blue "Select" button to change the type of nucleic acid to "dsDNA". Once accomplished, press the blue "Enter" button.
4. The display will ask if the conversion factor is correct. Again, press "Enter".
5. The display will indicate that the assay is ready for absorbance readings. At this time, press the button "Dilution Factor". The display will read "enter a dilution factor". Using the key pad, enter "10", then press "Enter".

   NOTE: The present procedure will read a 1:10 dilution of your samples. If you use a different dilution, then enter the appropriate number. If you are not diluting your sample, the dilution factor should be "1".
6. In a trUView cuvette, add 90 µl of TE buffer (or the same solution that is used to suspend the DNA sample).
7. Open the sample chamber lid and place the cuvette in the cuvette holder with the arrow on the frosted side facing forward. Close the lid and press the "Read Blank" button.
8. Once the display indicates that the blank has been read and the assays samples can now be measured, press the right arrow button (">") to continue with the assay.
9. Remove the cuvette and add 10 µl of the DNA solution to the cuvette and mix well by gently pipetting up and down.

10. Replace the cuvette in the holder, close the chamber lid, the press the “Read Sample” button. Allow the measurements to be taken. When they are complete, the data will be placed on the display. You may wish to record this data manually.
11. To read other samples, remove the cuvette and wash it several times with buffer/water, then carefully remove all traces of liquid. Add another diluted sample to the cuvette and repeat steps 9 and 10. There is no need to re-blank the spectrophotometer.
12. When you are finished, press the “Print” button, then press “3” on the key pad. A complete printout of the measurements will appear on a paper tape. Remove and keep this tape for your records.
13. Turn off the spectrophotometer by first pressing the left arrow button (“<”) to exit the assay, then switching the rear power button to the “off” position.

Interpreting Your Data:

The tape print out will give you all the necessary information from your readings.

Typically, A_{260} readings should be between 0.1 and 0.5 to be considered valid. Readings outside these ranges may not accurately reflect the DNA concentration. If higher than 0.5, you may consider performing another measurement on a more dilute sample. If it is lower, prepare a different dilution for measurement. The latter may not be possible with a limited sample volume and the low measurement may be considered acceptable depending upon the procedure for which the DNA will be used (e.g., low concentrations of DNA may be suitable for many types of PCR protocols).

The concentration is given in µg/ml which is equivalent to ng/µl. A concentration >50 ng/µl is sufficient for PCR, but not for other types of procedures (e.g., Southern blots) that require much greater quantities of DNA.

The purity of the DNA sample can be gaged by the A_{260}/A_{280} ratio. Pure DNA has a A_{260}/A_{280} ratio of 1.8-2.0. Readings below 1.6 indicate significant amounts of contamination, mainly protein. The purity can be increased by phenol extractions. Again, however, depending upon the use of the DNA sample, purity may not be a significant issue.