PROTOCOL: SPECTROPHOTOMETRIC DETERMINATION OF DNA QUANTITY AND PURITY

(v. 091708)

Purpose: To use spectrophotometric measurements to determine the concentration of DNA

Source/Background Information: The following procedure is adapted from the SmartSpec Plus Spectrophotometer Instruction Manual. This procedure can be used for quantifying genomic DNA, plasmids, and PCR products. When using this procedure to quantitate DNA solutions made in other diluents, the same diluent should be used for blanking the spectrophotometer

Reagents/Materials Required:

- DNA solution
- Buffer AE (form the Qiagen DNeasy Blood & Tissue Kit)*
  *IMPORTANT NOTE: If the DNA is in a diluent other than Buffer AE, then that diluent should be used in its place as described in the procedure below)
- trUView Cuvette (Bio-Rad, Cat. No. 170-2510) NOTE: do not touch the optical window area located near the bottom of the cuvette)
- SmartSpec Plus Spectrophotometer (Bio-Rad)
- Micropipettes and Tips (appropriate volumes)

Procedure:

1) Power up the SmartSpec Plus Spectrophotometer by switching the rear button to the "on" position. Allow the spectrophotometer to 'warm up' for 5 minutes and that 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 keypad, 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 Buffer AE (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. Be sure that the arrow on the frosted side of the cuvette is 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 with the arrow on the frosted side facing forward. Close the chamber lid, then 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 keypad. 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 gauged by the A_{260}/A_{280} ratio. Pure DNA has an 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.