SOP: SP014

Quantitation and Quality Assessment of DNA by UV Spectrophotometry

Materials and Reagents:

1. DNA stock, estimated concentration ≥0.25 µg/µl (250 ng/µl)
2. Micropipettors: Pipetman p10/p20 and p100/p200 or equivalent
3. Sterile pipettor tips
4. Dilution buffer options:
   TE (10 mM Tris pH8.0/1 mM EDTA, available from Invitrogen or Pierce through VWR
   EB (10 mM Tris pH 8.5, Qiagen Elution Buffer)
   Sterile water
5. Distilled water for cuvette rinsing (~1.0 – 5.0 ml depending on number of samples)
6. Beckman quartz micro cell (cat #523450) – 8 mm height, required volume 0.1 ml
7. Beckman DU640 spectrophotometer (Microbiology Rm B406 – southeast corner).
8. Printer paper
9. Trace-Klean cuvette cleaner (Beckman-Coulter cat # 598190), available in room B406.

Instructions:

1._____ Dilute DNA stock 1:50 (note 1) by combining 2.0 µl DNA stock with 98 µl TE, EB, or water (note 2).

2._____ Transport DNA, pipettor, tips, water, blank buffer, printer paper to Room B406.

3._____ Click (mouse) UV lamp ON at bottom of screen to ignite lamp.

4._____ Allow ~5 min lamp warm up (note 3). Ignition can be done before step 1.

5._____ Place microcuvette in lidded chamber, in shallow cuvette holder front position (note 4)

6._____ Add 0.1 ml DNA dilution buffer (TE, EB, or water) to cuvette, close lid.

7._____ In Applications window, click DNA/Oligo Quant. (note 5)

8._____ In DNA/Oligo Quant screen, click on white-lettered Concentration Factor (screen upper right). Keypad appears on screen.

9._____ Enter **50** to quantitate DNA. (note 6)

10._____ Click on white-lettered Dilution Factor (screen upper center). Keypad appears on screen.

11._____ Enter **dilution** (10, 20, 50, 100 etc) performed in step 1.

12._____ Click Blank (screen lower left). Wait until blanking process is complete.

13._____ Click Read Sample (screen upper left) to read blank. Blank value will appear on screen.

14._____ Read blank twice to verify stability of background (note 7)

15._____ Remove buffer. Caution – remove entire volume.

16._____ Add 0.1 ml diluted DNA, close lid, and click Read Sample (note 8).

17._____ Rinse cuvette with water after samples of high concentration to avoid cross contamination of following sample.

18._____ Read remaining samples.

19._____ Click Print (top center) to acquire record of A260,A280,A260/280, and DNA concentration on printer at right (check paper supply first). Do this BEFORE leaving DNA/Oligo Quant window.

20._____ Click Quit to leave DNA/Oligo Quant window.

21._____ A non-functioning (apparently) "save changes in method" query will appear. Click ok.

22._____ **TOGGLE UV LAMP OFF.**

23._____ Rinse cuvette 2× with Trace-Klean and 3× with distilled water at sink before returning to storage.

24._____ Evaluate DNA concentration (note 9) and quality (note 10).

Notes:

1. In general, spectrophotometer measurements between A260=0.1−1.0 are most accurate. Since prediction of DNA concentration is tricky before measurement, start the process with a 1:50 or 1:100 dilution. If the resulting A260 is less than 0.1, prepare a less dilute aliquot, eg. 1:20 (5.0 µl DNA added to 95.0 µl buffer). Readings between A260=0.05 and 0.1 may be acceptable as a rough estimate.
2. Avoid diluting DNA stock in buffers containing >1 mM EDTA. EDTA absorbance can interfere with DNA measurement.
3. If the blank reading fails to stabilize after warm-up (see note 7 below), the cuvette may be contaminated with slow-leaching materials. Clean the cuvette with Trace-Klean. Alternatively, the lamp may be aging. In the at-rest screen, click on Diagnostics, then on Status (at top of screen), to view UV source time. Lamps are typically useable up to ~1000 hrs.
4. The cuvette red dot permits proper orientation of cuvette with light path. It also aids consistent orientation between samples if cuvette is removed and replaced. The shallow cuvette holder must be firmly clamped in place. Check nearby drawers if it has been replaced with deep cuvette holder.
5. The DU640 spectrophotometer operates through two mouse-driven screens – (1) Application Selection Screen (at rest) and (2) Data Screen (acquired from the at-rest Applications window). Data Screen presentation depends on the application selected. DNA/Oligo Quant is recommended for DNA quantitation. This mode reports data points A260 (absorbance at 260 nm), A280, A260/280, and DNA concentration.
6. For double-stranded DNA, an A260 of 1.0 is ~ 50 µg/ml (= 0.05 µg/µl = 50 ng/µl). DU640 uses this factor in determining concentration.
7. Blank value should be low, ~ 0.0001-0.0010. If value is high, clean cuvette with Trace-Klean and rinse 3× with water.
8. DNA stock concentration is shown as µg/ml = ng/µl. A (very low) value will be shown for the blank – if it's greater than ~3-5 µg/ml, the cuvette isn't clean.
9. Minipreps (5-20 ml bacterial overnight) of low copy number plasmid DNAs are usually too dilute to measure by spectrophotometer. Determine concentration of dilute or low yield DNA solutions by comparison with commercial DNA standards visualized on an ethidium bromide stained agarose gel (SOP 018 agarose gel electrophoresis)
   Standards: Invitrogen High Mass DNA Ladder cat no. 10496-016
   Invitrogen Low Mass DNA Ladder cat no. 10068-013
10. The A260/A280 ratio indicates DNA quality. Acceptable range is 1.8-2.0. Values outside this range indicate absorbance interference by protein or solvents. DNA stocks with A260/A280 <1.8 cannot be accurately quantified by UV absorbance. Note that contaminants causing a low ratio can interfere with downstream applications.

Reference:

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual (2nd Edition), Vol III, pg E.5