Nucleic Acid Quantification with BioTek Take 3 Adapter

Drafted by Matthew Stasiewicz on 12-09-13 to replace the nanodrop in purity assessment of DNA for high-throughput sequencing. Also works for ssDNA, RNA, and protein.

Initial Setup – Already done, but you can verify if you want

1. Path Length Correction – Spot specific deviation from 0.5 mm nominal length
    a. Take 3 -> Take 3 Plate -> serial 273786 (our device)
    b. Software registers both a successful alignment and site specific path lengths
2. Default Preferences – For MJS created dsDNAw230
    a. Measures 230-300 nm in 2 nm increments
    b. Outputs ng/ul with extinction coefficient of 50, also A260/280 and A260/230 ratios
    c. Expects to average blanks over the chip. *Can be changed to site specific blanks*.

Sample Quantification

1. Turn ON the Synergy H1 Plate Reader
2. Open Gen5 Software. Select Read Now -> Take 3 Application -> Nucleic Acid Quantification
3. Setup the assay
    a. Select sample type. Use **dsDNAw230** for dsDNA if you want the A260/230 ratio.
    b. Check the 'Scan 230-300 nm with 2 mm step' box
4. *Note: Blanks and samples can be assayed simultaneously, but MJS does not recommend. If there is a problem with a blank you will have to wipe the whole plate clean and lose your samples.*
5. Read the blanks
    a. Select at least 3 wells as blanks (light green). Empty the remaining wells (clear)
    b. Pipette 2 ul of the appropriate blank onto the corresponding wells of the Take 3, close the cover
    c. Press 'Read' on the software
    d. Load the Take 3 unit onto the carrier that comes out. Then press OK and wait.
    e. If all blanks are OK, accept them and move to step 6.
        i. If there is a problem with the blanks, identified as a red well and a CV > 10%, wipe the plate clean and go back to 5b.
    f. Watch the software report the blank readings in Excel
6. Clean the plate by blotting the liquid with a kimwipe and use a fresh one to wipe the plate.
7. Read the samples
    a. Select wells for samples (blue). Select all 16 wells if you have > 16 samples.
    b. Pipette 2 ul of your samples onto the corresponding Take 3 well. Remember locations.
    c. Press 'Read' on the software.
    d. Watch the software report the readings on a new worksheet in Excel.
    e. If you have more samples, clean the plate and repeat from 7b.
    f. Once finished, end the batch and watch the software report a summary table
        i. Enter your sample names in the excel sheet before you forget.

8. Clean-up
    a. Blot and wipe the plate one final time. Replace in the black box.
    b. Return the plate holder carriage to inside the plate reader and power down the machine.
    c. Close the software.