Langerhans Lab Protocols

NC STATE UNIVERSITY

Using the NanoDrop 1000 Spectrophotometer to quantify DNA concentration

NanoDrop machine is in room 386 DCL. Melissa (in room 386, 389, or 208) is knowledgeable and very helpful.
Take with you:

- pipettor (1-2 uL)
- tips
- samples to run
- liquid (Qiagen's Buffer AE, water, or other) used for re-suspending DNA

Using the machine:

1. Log onto computer (pw is on wall sign; it is cl373Lab).
2. Double click ND-1000 -> Nucleic Acid (if using DNA) (default user)
3. Clean lenses:
    a. Aliquot 1-2 µL deionized water (there is a tube that stays next to the machine for this) onto the top lens and the pedestal (lower lens). Water should form a column between the upper and lower lenses. Tap the arm gently.
    b. Blot (DON'T WIPE) the water off with a Kimwipe.
4. Initialize:
    a. Aliquot 1 µL water on lower lens.
    b. Close machine arm.
    c. click Initialize
    d. once initialization is complete, raise arm, blot lenses with Kimwipe
5. Blank:
    a. Load 1 µL water or buffer (whatever the DNA is dissolved in) onto bottom lens; close arm.
    b. Click Blank
    c. raise arm, blot lenses with Kimwipe
6. Run sample:
    a. "Finger vortex" sample by gently flicking the base of sample tube with your finger 4-5 times. For greater accuracy, especially with large molecules of DNA (e.g., genomic DNA), heat samples to 55°C before measuring.
    b. Aliquot 1 µL of your DNA sample onto pedestal; close arm.
    c. Type sample ID on computer; **remember to change sample ID** for each sample (data recorder will not overwrite previous data of same sample number).
    d. Choose correct Sample Type: used to select the (color-keyed) type of nucleic acid being measured. The user can select 'DNA-50' for dsDNA, 'RNA-40' for RNA, 'ssDNA-33' for single-stranded DNA, or 'Other' for other nucleic acids. The default is DNA-50.
    e. Click Measure; the program creates a spreadsheet that can be exported to Excel that records your sample #, 260/280 ratio, 260/230 ratio, & ng/ µL.
    f. Optional (recommended by Melissa, not by NanoDrop): repeat measurement (steps 6a – 6e) 2-3 times for each sample, then average the readings for greatest accuracy.
    g. Change pipette tip between each sample.
    h. Blot; rinsing with water between samples is not necessary unless sample is of high concentration. Rinsing will not hurt anything.
    i. If you are running many samples, re-blank with water/buffer after every 15 samples.
    j. When finished with all your samples, clean lenses by aliquotting 2-3 µL water (there is a tube that stays next to the machine for this) onto the top and bottom lenses, then blot with a Kimwipe.
7. To retrieve your data:

a. click Show Report. Click Reports on top menu -> Save Report -> Export Report Table Only -> name the file and save (on Desktop). Exit NanoDrop. Open Mozilla Firefox -> Gmail -> log in. Send e-mail to yourself with your data file Attached. Recycle your file from computer desktop. Turn off the computer when done.

from the on-line User's Manual (saved in Langerhans Lab computer in folder Langerhans Lab Data & Info):

260/280: ratio of sample absorbance at 260 and 280 nm. The ratio of absorbance at 260 and 280 nm is used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as "pure" for DNA; a ratio of ~2.0 is generally accepted as "pure" for RNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm. See "260/280 Ratio" in the Troubleshooting section for more details on factors that can affect this ratio.

260/230: ratio of sample absorbance at 260 and 230 nm. This is a secondary measure of nucleic acid purity. The 260/230 values for "pure" nucleic acid are often higher than the respective 260/280 values. They are commonly in the range of 1.8-2.2. If the ratio is appreciably lower, this may indicate the presence of co-purified contaminants.

ng/uL: sample concentration in ng/uL based on absorbance at 260 nm and the selected analysis constant. See the "Concentration Calculation (Beer's Law)" in the appendix for more details on this calculation.

Check on line for Nano Drop details and how to improve outcomes.

troubleshooting http://www.nanodrop.com/TechResCenter-Home.aspx

protocol: http://nanodrop.com/Library/CPMB-1st.pdf

user's manual: http://www.nanodrop.com/Library/nd-1000-v3.8-users-manual-8%205x11.pdf

user guides: http://www.nanodrop.com/Support.aspx?Type=User%20Guides&Cat=NanoDrop%201000

troubleshooting, interpreting spectra, contaminants (pg 17+): http://www.nanodrop.com/ND1/NucleicAcid-Booklet.html

Alternate Method for Quantification (e.g., for PCR product)

Run sample on agarose gel with ladder. Loading the same amount of ladder and sample, each mixed with the same amount of SYBR Green, allows you to compare intensities for an estimate of PCR product concentration. New England BioLabs' Quick-Load 100 bp DNA ladder has initial conc. of 50 µg/ml. (5 µl = 25 ng)