NanoDrop

Overview

This protocol covers use of the NanoDrop spectrophotometer for quantification of nucleic acids. The NanoDrop works off the principle of absorbance, namely that nucleotide absorb at 260nm wavelength light.[i]

Materials

- Kimwipes
- Samples to be quantified
- Appropriate blanks for samples
    - Nanodrop must be blanked with the same buffer that the sample is in (i.e. if your sample is in TE, blank with TE).

Procedure

1. Make sure NanoDrop arm is in down position. Turn on NanoDrop (switch in the back). Allow to start up, do not lift arm until startup has finished (screen with selection of different nucleic acids measure appears).
    - Nanodrop may take some time to turn on. Sometimes screen will be black before screen displays “initializing”. If screen stays black, allow at least 5 minutes before restarting by switching off, waiting 30 seconds and turning on again.
2. Select appropriate sample type for measurement (typically dsDNA).
3. Lift arm, add 1.7µl[ii] of blank onto pedestal. Gently lower arm (do not slam).
    - When loading onto pedestal, remember the following:
      Try to center liquid on black dot on pedestal.
      Try to not make contact between pedestal and pipette tip.
      Only pipette to the first stop, pipetting to the second stop will add air bubbles which will lead to incorrect (artificially bad) quantitation.
    - If blank does not measure automatically, auto blank is turned off. Simply press the blank button to measure.
    - Ensure instrument says Blank Ok. In the upper corner.
4. After blanking/measurement completes, raise arm up, use kimwipe to wipe off pedestal and arm. Add 1.7µl of sample (same guidelines as above), lower arm. If measurement does not proceed automatically, auto measure is off. Simply press measure button to measure.

---

[i] Remember that absorbance will peak at some wavelength of light, but will have a distribution of absorbance to different wave lengths of light (will look similar to Gaussian). Purity can be determined by comparing the absorbance at 260 nm to that of wavelengths further from the absorbance peak. Different contaminants will have different absorbance spectra, but will all deviate from the appropriate pure DNA values. See Thermofisher T042-TECHNICAL BULLETIN for representative spectra and more information.

[ii] Theoretically volumes as small as 1µl can be used. However, our nanodrop has been mistreated and so the recommended volume is now 1.7µl, which will never produce the “broken column” error.

5. Inspect values to ensure that sample was measured correctly (see below for information on purity). Repeat step 4 until all samples are measured.
6. Record information including concentration and purity.
    - Note: While photos may be taken of NanoDrop values, those photos do not constitute sufficient recording. Photos should be transcribed into notebook, preferably in table format.
7. When information is recorded, turn off instrument. Then clean the instrument. (Wipe off the arm and pedestal with kimwipe, 5µL of NFW to the pedestal, lower the arm, wait 2-3 minutes then wipe off.
8. Information on purity.
    - 260/280 represents the primary purity measurement for nucleic acids.[iii] DNA should typically have a value of around 1.8 and RNA a value of around 2.0. These values will fluctuate depending on pH of sample. Any DNA value >1.8 constitutes a "pure" sample.
    - 260/230 represents a secondary purity measurement for nucleic acids.[iv] 260/230 can fluctuate more easily than 260/280. Pure nucleic acids should have values between 2.0-2.2. However, values above 1.8 (preferably 1.9) are considered functionally "pure" for cloning purposes.
    - Note that samples below 1.8 for either 260/280 or 260/230 will have a blue circle with an "*i*" in it. Clicking this bubble will tell you that your values are not indicative of pure nucleic acid (leading to somewhat inaccurate quantitation). It may also tell you "bubble detected" which lets you know that you placed an air bubble in the sample, which led to incorrect measurement of sample. If a bubble is detected it is recommended to measure sample again unless volume is limiting. If bubbles are continually detected, spin down tube (bubbles might be in solution) and try again.

[iii] Low 260/280 is indicative of contamination with (in order of frequency seen): Proteins, Phenol

[iv] Low 260/230 is indicative of contamination with (in order of frequency seen): Ethanol, Guanidine-HCl,, Carbohydrates, Phenol or any other substance that strongly absorbs at 230 nm wavelength.