FOOD SAFETY LABORATORY
CORNELL UNIVERSITY

Title: Quantification of Purified DNA by NanoDrop

FILE NAME: Quantification of Purified DNA by NanoDrop.doc

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Last Modified on: 05-10-2011
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EFFECTIVE DATE: 11-July-2007

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SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this document is to provide a procedure for uniform quantification of *purified* DNA of variable concentrations that is either isolated by our lab or received by the Food Safety Laboratory from external sources (e.g. government agencies, companies, academic institutions) for subsequent DNA fingerprinting techniques.

1.2 Scope

This SOP applies to the Food Safety Lab, including the Laboratory for Food Microbiology and Pathogenesis of Foodborne Diseases and any experimental procedures conducted by laboratory members at other locations.

1.3 Definitions

External purified DNA: DNA that has been isolated from a bacterial sample at a location outside of the Food Safety Lab and purified by an external source.

External source: The location outside of the Food Safety Lab that had the DNA in its possession, likely the location where isolation and purification of the bacterial DNA occurred.

NanoDrop ND-1000: Full-spectrum (220-750nm) spectrophotometer that measures 1ul samples with high accuracy and reproducibility utilizing patented sample retention technology.

260/280 ratio: Ratio of sample absorbance at these wavelengths. A ratio of 1.8 to 2.0 is generally accepted as "pure" for DNA. If the ratio is appreciably lower, it may indicate the presence of protein or other contaminants. See "260/280 Ratio: section of the Troubleshooting section for more details.

260/230 ratio: This is a secondary measure of nucleic acid purity. The ratio is often higher than the respective 260/280 values. When using purification techniques that include salts such as guanidine, a low ratio may indicate carryover of the salt or other contaminants such as phenol-chloroform.

SECTION 2 MATERIALS

Disposable gloves
Kim-wipes
NanoDrop ND-1000
Pipet
Sterile pipet tips
dH_20
Elution buffer, or whatever buffer the DNA sample has been resuspended in (for blanking)

SECTION 3 PROCEDURE

1. Double click on the NanoDrop icon (ND-1000 V3.5.1 on computer desktop).

2. From the main menu screen, select the nucleic acid button for DNA/RNA quantification. The default setting is DNA-50.

3. A message will appear prompting you to perform a brief cleaning to ensure the measurement pedestal surfaces are clean: *Lift sampling arm (do NOT handle by the black cord) and apply 2ul of water to the measurement pedestal. Lower sampling arm and gently press down once. Select OK and the message "Initializing Spectrometer-please wait" will appear. When the message disappears the instrument is ready for use. Lift sampling arm and wipe both the upper and lower measurement pedestals with a Kim-wipe.*

4. Apply 1.5ul of the solution that was used to purify your DNA samples and select "blank". The reading of 0.0 ng/ul will appear at the bottom of the screen (highlighted in green). Clean the blanking solution off the upper and lower measurement pedestals using a Kim-wipe.

5. A cursor will appear in the "sample ID" box, type in BLANK. Apply another 1.5ul of the same blanking solution and select "measure". The reading must be below 1.0 to proceed. You will also see a straight line on the graph.

   Note: A measurement over 1.0 indicates that the pedestal is contaminated by a previous sample. If the solution measures greater than 1.0, you must clean the upper and lower pedestals with water and repeat step 4. Wiping the sample from both the upper and lower pedestals upon completion of each measurement is usually but not always sufficient to prevent sample carryover.

6. Quantifying DNA samples:
   a. Type the name of the DNA sample in the "sample ID" box.
   b. Carefully apply 1.5 ul of DNA sample to the lower measurement pedestal. Lower sampling arm and gently press down once.
   c. Select "measure". The measurement will be displayed both as a numerical result, and as a curve on a plot. Pay special attention to the 260/280 and 260/230 ratios. These will indicate the purity of your DNA. The 260/280 ratio must be 1.8 or above and the 260/230

ratio must be 2.0 or above. If the 260/230 ratio is much less than 2, it indicates that your sample is contaminated with salts or phenolic compounds, and your OD reading will be inaccurate. The sample must then be re-purified.

d. After each measurement, wipe off the upper and lower measurement pedestals and type in the name of your next sample. Repeat as needed.
e. Be aware that due to the small size of the sample, homogeneity is important for an accurate measurement. If you are measuring genomic DNA and you are unsure if it has completely gone into solution, heat your tube to 55°C and vortex first.

7. When you have completed quantifying your DNA samples, repeat step 5 to prevent sample carryover and contamination of the measurement pedestal. Perform a final cleaning of all surfaces with de-ionized water (do not use a squirt bottle) and wipe with a Kim-wipe.

8. Printing results:
    a. Select "show report". All sample measurements from your session will appear in spreadsheet form.
    b. From "File" dropdown list select "print window".

9. Select "Exit" from the report screen.

10. Select "Exit" from the measurement screen.

11. Select "Exit/esc" at the prompt asking "are sure you are done?"

12. Select "Exit" from the ND-1000 main menu screen.

SECTION 4 REPORTING and LABELING

- Quantification results for samples are to be taped or glued into a lab notebook under a dated entry.
- All tubes of purified DNA are to be stored at -20˚C in a well-labeled box.

SECTION 5 TROUBLESHOOTING

- See section 16 of the NanoDrop ND-10000 user's manual for troubleshooting.
- For decontamination or to ensure that no biologically active material is present, apply 5ul of a freshly prepared 1:10 dilution of commercial bleach and wipe with a Kim-wipe. Apply 2ul of water and wipe with a Kim-wipe. Repeat application of water and wipe.
- For more rigorous cleaning of dried on nucleic acids, apply 5ul of water to the lower pedestal, bring down the arm and make the connection, then let the water sit for 2 or 3 minutes. Remove with a Kim-wipe.

- Always blank the NanoDrop with the solution you used to elute/purify your sample. The use of an alternate buffer or solution can drastically alter your quantification results, especially if it contains EDTA (i.e., TE).

- DNA that has not been purified using a kit (i.e., dirty lysate, lysate) cannot be quantified using the Nanodrop.

SECTION 6 REFERENCES

NanoDrop ND-1000 Spectrophotometer V3.5 User's Manual - NanoDrop Technologies, Inc. Wilmington,DE. Copyright 2007