	FOOD SAFETY LAB / MILK QUALITY IMPROVEMENT PROGRAM *Standard Operating Procedure*		
Title: Quantification of Purified DNA by NanoDrop			
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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/Milk Quality Improvement Program from external sources (e.g. government agencies, companies, academic institutions) for subsequent DNA fingerprinting techniques or whole genome sequencing.

1.2 Scope

This SOP applies to the Food Safety Lab/Milk Quality Improvement Program 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/Milk Quality Improvement Program and purified by an external source.

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

NanoDrop ND-2000C: Full-spectrum (190-840nm) spectrophotometer with cuvette capability that measures 0.5 – 2 uL 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.

1.4 Safety

DNA, RNA or protein can be derived from BSL-2 pathogens. Appropriate protective measures need to be taken when operating the NanoDrop, including gloves, lab coat and safety glasses.

SECTION 2 MATERIALS

- Kim-wipes
- NanoDrop ND-2000C
- Pipet
- Sterile pipet tips
- dH_20
- Elution buffer, or whatever buffer the molecule of interest (e.g., DNA sample) has been resuspended in (for blanking)

SECTION 3 PROCEDURES

1. Clean the pedestal surface. Apply some ethanol onto a kim-wipe, lift up the sampling arm and wipe the upper and lower pedestals. Apply some dH2O to a fresh kim-wipe and repeat cleaning of upper and lower pedestals. Make sure to leave the sampling arm down after cleaning.

2. Double click on the NanoDrop icon (ND-2000 on computer desktop).

3. From the main menu screen, select the application of interest e.g., nucleic acid button for DNA/RNA quantification.

4. A message will appear prompting you to ensure that the sampling arm is down. Select OK on the wavelength verification window. When the message disappears, the instrument is ready for use.

5. Apply 1.5 ul 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.

6. In the upper right panel is a "sample ID" box, type in BLANK. Apply another 1.5 ul 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 5. 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.

7. Quantify your DNA. The default options, when nucleic acid (see step 3) has been selected, will be DNA for "Type" and measured in ng/uL for "Concentration" which can be changed in the upper right panel.
    a. Before loading the pedestal, enter the name of the DNA sample in the "sample ID" box of the upper right panel.
    b. Flick your sample in the tube, to ensure homogeneity of the sample, before carefully applying 1.5 ul of DNA sample to the lower measurement pedestal. Lower sampling arm and gently press down once.
    c. Select "measure" from the left panel. 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. Cleaning with water in between samples is not necessary. 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

8. When you have completed quantifying your DNA samples, repeat step 1 to prevent sample carryover and contamination of the measurement pedestal. Perform a final cleaning of all surfaces.

9. Retrieve your data. On the upper left panel next to the measure button, click on the "print" option to obtain a print that includes the graph, as well as the numerical results table. You can also click on "reports" on the lower left panel where you can export your data as an excel file that you can save on a USB stick or print the results table without the graph.

10. After you are done hit the red cross on the upper right to exit the program.

SECTION 4 TROUBLESHOOTING

- See section 7 of the NanoDrop ND-2000/2000C user's manual for troubleshooting.

- For decontamination or to ensure that no biologically active material is present, apply 5 ul of a freshly prepared 1:10 dilution of commercial bleach and wipe with a Kim-wipe. Apply 2 ul of water and wipe with a Kim-wipe. Repeat application of water and wipe.

- For more rigorous cleaning of dried on nucleic acids, apply 5 ul 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 5 REFERENCES

NanoDrop 2000/2000C Spectrophotometer V1.0 User's Manual - NanoDrop Technologies, Inc. Wilmington,DE. Thermo Fischer Scientific Inc. 2009

SECTION 6 METHOD VERSION & CHANGES

VERSION	DATE	EDITOR	COMMENTS
Version 1	07/11/2007	Esther D. Fortes	Original SOP
Version 2	05/10/2011	Esther D. Fortes	Records of changes made are unavailable.
Version 3	04/10/2020	Sophia Harrand	Updated SOP for Nanodrop 2000C (prev. ND-1000), added 1.4. Safety