INDIANA CTSI Clinical and Translational Sciences Institute

**STANDARD OPERATING PROCEDURE**
**Indiana CTSI Specimen Storage Facility**

TITLE: STANDARD OPERATING PROCEDURE FOR DNA QUALITY CONTROL USING THE NANODROP LITE

CHAPTER: 4-Specimen Processing

SOP #: SF-4-23.01

SUPERSEDES SOP #: SF-4-16.03

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1. REVISION
   1.1. Not Applicable. Initial version.

2. PURPOSE
   2.1. This Standard Operating Procedure (SOP) defines the procedures used in the Indiana CTSI Specimen Storage Facility (SSF) to ensure that Quality Control (QC) of DNA is performed in a compliant and uniform manner.

3. PRINCIPLE
   3.1. Quality Control of DNA is performed by various methods. The SSF performs QC of DNA by using the Nanodrop Lite, a spectrophotometer emitting UV light and measuring absorbance at two different wavelengths to determine the concentration and purity (260/280 ratio) of the DNA. A sample is pipetted directly onto the lower pedestal. A fiber optic cable (i.e., the source fiber) is embedded within the lower pedestal. The UV light is emitted from the LEDs in the fiber optic cable, traverses the sample, and the absorbance is measured using a silicone photodiode embedded in the upper pedestal and the arm (i.e., receiving fiber).

4. SCOPE
   4.1. This SOP applies to all SSF personnel performing QC of DNA. It defines the process of obtaining the concentration and the 260/280 ratio for DNA samples using the Nanodrop Lite spectrophotometer.
   4.2. All SSF processing SOPs may be superseded by specific directives from the investigator as directed in SF-4-1. Initial entry into the worksheet will define whether there are specific processing directives applicable to a specimen.

5. MATERIALS
   5.1. Reagents

5.1.1. DI water (or RO water from lab sink)
5.1.2. FG3 Hydration Buffer, stored at room temp (found in Flexigene DNA Extraction Kit, Qiagen Cat. # 51206), or another hydration buffer that was used to rehydrate the DNA.

5.2. Supplies
5.2.1. Kimwipes® (or similar product)
5.2.2. Pipette tips

5.3. Equipment
5.3.1. Nanodrop Lite Spectrophotometer, appropriate for measuring absorbance at a wavelength range of 260 through 280 (SF-3-17)
5.3.2. Pipette(s) (or other suitable dispensing unit) capable of dispensing 2µL of a liquid sample (SF-3-3)
5.3.3. Vortex
5.3.4. Thermal mixer (e.g., Fisherbrand Thermal Mixer, Eppendorf Thermomixer®)

6. PROCEDURE

NOTE: Record on the applicable extraction worksheet or the QC data printout derived per Sections 6.2.2 and 6.2.3 if exceptions to this SOP per SF-4-1 are applicable.

NOTE: Store DNA samples in -80°C for long term storage unless otherwise stated per SOP SF-4-1 Management Requests for Sample Processing Support. Resolve all discrepancies for sample receipt per SOP SF-4-1.

6.1. Operation of the Nanodrop Lite Spectrophotometer for DNA QC

**NOTES BEFORE BEGINNING:** The Nanodrop Lite cannot accept Sample ID inputs during the analysis process. The machine simply numbers the samples as they are measured (i.e., the Nanodrop-assigned measurement number, or Nano-assigned number). Additionally, these numbers can be reset to "1" at any time by the user and automatically resets if the machine is turned off (i.e., unplugged). In order to accurately identify sample results on the analysis data after the analysis is complete, it is important for the technician to document 1.) The date/time in which the analysis begins and 2.) The Nano-assigned numbers next to the corresponding study defined Sample IDs on the applicable worksheet or some other document during the process.

6.1.1. Obtain DNA and applicable processing worksheet for the DNA extraction batch being analyzed.
6.1.1.1. If analyzing a random group of samples not associated with a specific DNA extraction batch (e.g., previously processed samples which have already been frozen and require re-analysis), obtaining a processing worksheet is not applicable. It will be necessary to obtain and fill out Appendix A for the group of samples in order to document the pertinent information defined in Section 6.1.
6.1.2. If the Nanodrop is in Sleep mode (as evidenced by a black screen and a flashing blue power indicator light, which is located immediately below the 4-button keypad), wake the machine by pressing any button or raising the sample arm.
6.1.3. Establishing a blank measurement
6.1.3.1. From the **Home** screen, select **DNA** and press **Select**.
6.1.3.2. Choose **dsDNA** and press **Select.**

6.1.3.3. Using a fresh pipette tip, establish a blank by pipetting 2µl of FG3 (or other hydration buffer used to hydrate the DNA sample being analyzed) onto the bottom pedestal, lower the sample arm, and press **Blank.**

6.1.3.4. Once the Nanodrop finishes the measurement (~3-5 seconds), raise the sample arm and clean the sample off both pedestals with a dry Kimwipe® (or similar product).

6.1.3.5. Confirm the blank by pipetting a fresh 2µl aliquot of FG3 (or other hydration buffer used), using a fresh pipette tip, onto the bottom pedestal, lower the arm, and press **Blank.**

6.1.3.6. Once the Nanodrop finishes the measurement (~3-5 seconds), raise the sample arm and clean the sample off both pedestals with a dry Kimwipe® (or similar product).

6.1.3.7. Document the date and time the blank was made on the applicable processing sheet (i.e., the time the analysis of the group of samples began). If there is no applicable processing worksheet, document the date/time on Appendix A. Documenting the date and time helps to accurately identify the study defined Sample ID from the Nano-assigned number when formatting the data per Section 6.2.2.

6.1.4. Measuring a DNA sample for concentration and purity

6.1.4.1. Mix sample thoroughly prior to loading by gently vortexing for a few seconds.

6.1.4.2. Using a fresh pipette tip, load 2µL of sample onto the lower sample pedestal with a pipette, lower the sample arm, and press **Measure.**

6.1.4.3. Once the Nanodrop finishes the measurement (~3-5 seconds), raise the sample arm and clean the sample off both pedestals with a dry Kimwipe® (or similar product).

6.1.4.4. Document the Nanodrop-assigned measurement number (displayed with the results on the Nanodrop screen) for the sample that was just analyzed on the processing sheet. If there is no applicable processing worksheet, document the number on Appendix A. The Nano-assigned number will be used to correctly identify the study-defined Sample ID when formatting the data per Section 6.2.2.

6.1.5. Repeat the process outlined in Section 6.1.4 for the rest of the samples until the entirety of the group of samples has been analyzed. If there are any equipment errors during the process, refer to the Nanodrop User Guide (Trouble Shooting) and SF-3-17 as necessary and repeat the reading of the applicable samples, making sure to document the Nano-assigned numbers on the processing worksheet or Appendix A, as applicable.

6.1.6. After all samples have been analyzed, clean the instrument pedestals as defined in SOP SF-3-17.

6.1.7. Transfer the results from the instrument memory to a USB memory drive.

**NOTE:** Sample data is automatically saved on the instrument. The Nanodrop Lite stores the last 500 measurements, including samples, blanks, and blank confirmations, in the internal memory, and identifies each sample measurement with a Nano-assigned number. The data is available to be transferred to a USB memory device at any time. Nanodrop-assigned #501 will replace #1.

6.1.7.1. Insert a USB memory device into the Nanodrop.

6.1.7.2. The **USB Operations** screen automatically appears.

6.1.7.2.1. The **USB Operations** screen may also be accessed from the **Home** screen by choosing **Save** and pressing **Select** (Note: The **Save** option

is only present on the **Home** screen if a USB memory device is currently inserted in the Nanodrop).

6.1.7.3. Choose **Save Data** and press **Select.**

6.1.7.4. The following message will appear on the screen**: Saving…..Do not remove media.** When this message disappears, the USB device can be safely removed from the Nanodrop.

6.1.8. Put the machine in Sleep mode (optional).

6.1.8.1. The Nanodrop can be placed in Sleep mode to conserve energy when not in use.

6.1.8.1.1. From the **Home** screen, choose **Sleep**, and press **Select**.

6.1.8.1.2. The screen will go blank and dark, and the blue power indicator light will begin to slowly flash.

6.1.8.1.3. To exit Sleep mode, simply press any key or raise the sample arm.

6.2. Saving, formatting, analyzing, and recording QC data

6.2.1. Save the data from the USB memory device to the SSF shared drive, in the "ND-Lite Spec Data" folder.

6.2.1.1. The instrument saves the data on the USB memory device in a comma-separated values (CSV) file. Open the file on the USB device, choose "Save As," and save as an Excel Workbook on the shared drive.

6.2.1.2. Name the file to include the SSF-defined DNA Extraction Batch ID and the tech initials (e.g., DNA-17-039 MLT).

6.2.2. Format the data by opening a new sheet in the Excel workbook created in Section 6.2.1 and inputting the data in a table. Include the following information for each sample, at minimum. Most can be copied and pasted from the raw Nanodrop data (See **Figure 1** below for just one example of the formatted data. Variations to the table format are acceptable, as long as the minimum required data is documented):

6.2.2.1. Data Descriptor

6.2.2.1.1. At the top of the table, manually enter the SSF-defined DNA Extraction Batch ID (e.g., DNA-17-037) for the group of samples, along with any other identifying information, if desired. If there is no specific extraction Batch ID associated with the group of samples, document some other appropriate description (e.g., Protocol 1864 DNA samples).

6.2.2.2. Nanodrop-assigned measurement number-derived from raw data

6.2.2.2.1. The Nanodrop-assigned measurement number, or Nano-assigned number, is assigned chronologically by the Nanodrop to each sample measurement (except blank and blank confirmation measurements). It is not unique to any particular sample and can be reset at any time by the user or by powering off the Nanodrop.

6.2.2.3. Sample Time (date/time sample was analyzed)-derived from raw data

6.2.2.4. Type (e.g., Blank, Confirm, dsDNA)-derived from raw data

6.2.2.5. Concentration (ng/µl)-derived from raw data

6.2.2.6. 260/280 value-derived from raw data

6.2.2.7. Sample ID

6.2.2.7.1. The Sample ID is a unique identifier given to each sample and is defined by the study.

6.2.2.7.2. The Sample ID for the blank should include the BLANK ID (per the manufacturer), lot number, and expiration date.

6.2.2.7.3. Manually enter the correct Sample ID next to each Nano-assigned number as documented per Section 6.1.4.4.

6.2.2.8. SSF Sample number

6.2.2.8.1. The SSF sample number is a number assigned by the SSF chronologically to a particular Sample ID in a given DNA extraction batch or other group of samples to be analyzed. The number is only unique within a particular batch/group of samples and can be reused in subsequent batches/groups. It serves solely as a counter for internal use only.

6.2.2.8.2. Enter the sample number defined on the processing worksheet or other document for each Sample ID.

6.2.2.9. Volume

6.2.2.9.1. Manually enter the final volume of the sample, if known.

6.2.2.10. Additionally, make note of any sample that was, or required, a repeated reading.

**Figure 1**

DNA-17-037 MEMOIR/1864

| SSF Sample # | Nano-assigned # | Sample ID (study-defined) | Sample Time | Type | Conc. (ng/ul) | A260/A280 | volume (ul) |
|---|---|---|---|---|---|---|---|
| | | FG3 151039869 exp 3/1/18 | 9/8/2017 8:19 | Blank | | | |
| | | FG3 151039869 exp 3/1/18 | 9/8/2017 8:20 | Confirm | | | |
| 1 | 18 | 1864-11153-D0-DNA-2 | 9/8/2017 8:21 | dsDNA | 466 | 1.84 | 600 |
| 2 | 19 | MEMOIR-HF-031-Scr-DNA-3 | 9/8/2017 8:21 | dsDNA | 356.1 | 1.84 | 400 |

6.2.3. Record the QC data by printing out the formatted data and attaching to the applicable processing worksheet. If there is no processing worksheet, attach the QC data to Appendix A.

6.2.3.1. Document explanations and/or further action taken for samples that needed repeating on the applicable processing worksheet or the QC data printout.

6.2.4. Analyzing data

6.2.3.1. If the 260/280 ratio for any sample is not within SSF-defined acceptable range **(1.7-2.0)** or within the range defined by the PI on Appendix A of SF-4-1 SOP for Managing Requests for Sample Support, alert study personnel and document explanations and/or actions taken on applicable processing sheet or QC data printout If necessary, consult a technical advisor.

6.2.3.2. If the concentration for any sample is **greater than 700 ng/µl**, the sample may require additional rehydration steps.

6.2.3.2.1. Add additional FG3 Hydration Buffer, or other applicable buffer, to the sample. Add enough buffer required to double the original volume of the sample. More buffer may be added to samples of very high concentration.

6.2.3.2.2. Place sample on a thermal mixer overnight or for an equivalent amount of time with the parameters set at 65°C and 1100 rpm.

6.2.3.2.3. Measure concentration and purity again as outlined in Section 6.1.

6.2.3.2.4. Repeat steps outlined in this section as necessary until the concentration is under 700 ng/µl.

6.2.3.2.5. Document explanations and/or actions taken on applicable processing sheet or QC data printout.

6.2.3.3. If the concentration for any sample is **less than 50 ng/µl**, or the required minimum concentration defined by the PI on Appendix A of SF-4-1 SOP for Managing Requests for Sample Support, there may not be enough sample for the study personnel to pursue certain downstream applications. Alert study personnel and document explanations and/or actions taken on applicable processing sheet or QC data printout. If necessary, consult a technical advisor.

6.2.3.4. Additionally, the analysis of a sample may be repeated at the discretion of the SSF technician for any reason he/she may deem necessary. For example, the technician may feel that the technique he/she used to analyze the sample in question was not optimal.

6.2.3.5. Follow Steps 6.2.1-6.2.3 for saving, formatting, and recording any new data obtained.

7. REFERENCES
   7.1. Nanodrop Lite Spectrophotometer User Guide
      7.1.1. Located in the SSF shared folder
      7.1.2. Alternatively, downloadable from the support section of the Nanodrop website (http://nanodrop.com/)

8. DOCUMENTATION
   8.1. Records are maintained per SF-1-6 Controlled Document Management.
   8.2. Deviations are managed per the SF-1-9 Deviation Management SOP. Of special note, per scope of this SOP, complying with investigator specific directives per SF 4-1 is not a deviation to this SOP but is noted on the applicable worksheet or DNA QC data printout.

9. APPENDICES
   9.1. The current version of the following appendices are used to implement this SOP:
   APPENDIX A: Nanodrop Sample Analysis Recording Form (1 page)

# Nanodrop Sample Analysis Recording Form

(For use if no processing worksheet is available for record-keeping) Page ____of ____

Sample Group Description: _____

Date/Time of Analysis: _____

Tech initials/date_____

| SSF Sample # | Sample ID (study defined) | Nano-assigned number (s) | Comments |
|---|---|---|---|
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