INDIANACTSI
Clinical and Translational Sciences Institute

STANDARD OPERATING PROCEDURE
Indiana CTSI Specimen Storage Facility

TITLE: STANDARD OPERATING PROCEDURE FOR DNA QUALITY CONTROL

CHAPTER: 4-Specimen Processing

SOP #: SF-4-16.01

SUPERSEDES SOP #: N/A

Issue Date: 10-22-10

Effective Date: 10-29-10

AUTHORED BY: Melissa Taylor DATE: Oct 22, 2010
Indiana CTSI SSF Lab Technician

APPROVAL: [signature] DATE: Oct 22, 2010
Indiana CTSI SSF Director

QA APPROVAL: See pg 1a MLT 10/26/10 DATE: _____
Quality Compliance Specialist

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 a spectrophotometer emitting UV light and measuring absorbance at two different wavelengths to determine the concentration and purity (260/280 ratio) of the DNA.

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 Eppendorf model Biophotometer 6131 and/or the Nanodrop model ND-1000 spectrophotometers.
   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 All SSF processing SOPs may be superseded by specific directives from the investigator as directed in SF-4-1.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. DNA Hydration Solution, stored at room temp (Qiagen Cat. # 158916) (for manual DNA extractions only), unless otherwise specified in SF-4-1.
      5.1.2. DI water (from lab sink)

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5.1.3. FG3 Hydration Buffer, stored at room temp (Qiagen Cat. # 1023542) (for DNA extractions via the Autogen Flexstar Method only).

5.2. Supplies

5.2.1. Cuvette applicable for use with Eppendorf spectrophotometer (if using the Eppendorf spectrophotometer)

5.2.2. Kimwipes® (or similar product)

5.3. Equipment

5.3.1. Bar-code Scanner (optional)

5.3.2. Nanodrop ND-1000 Spectrophotometer (alternatively, Eppendorf BioPhotometer 6131 Spectrophotometer), appropriate for measuring absorbance at a wavelength range of 260 through 280 (SF-3-10 and SF-3-11)

5.3.3. Pipette(s) (or other suitable dispensing unit) capable of dispensing 2uL – 60uL of a liquid sample (SF-3-3)

5.3.4. Vortex (optional)

6. PROCEDURE

NOTE: Record on the applicable extraction worksheet if exceptions to this SOP per SF-4-1 are per applicable.

NOTE: Store samples under conditions which sample is received per SOP SF-4-4, Sample Receipt/Log in/Tracking. Resolve all discrepancies for sample receipt per SOP SF-4-4.

6.1. Operation for the Nanodrop ND-1000 Spectrophotometer for DNA QC

6.1.1. Log in and machine preparation

6.1.1.1. Using IU login and password, access the Nanodrop ND-1000 operating software installed on the computer connected to the instrument.

6.1.1.2. Double click on the Nanodrop icon to open the menu screen.

6.1.1.3. Click the Nucleic Acid button which prompts an instruction window to open reading, "*Ensure sample pedestals are clean and then load a water sample. After loading water sample, click OK to initialize instrument.*"

6.1.1.4. Clean lower and upper pedestals on the Nanodrop by dropping distilled (DI) water on them and dry with a Kimwipe® (or similar product).

6.1.1.5. Load 2µL of DI water on the lower pedestal using a pipette.

6.1.1.6. Close the sample arm.

6.1.1.7. Click the OKAY button.

6.1.1.8. Clean the upper and lower pedestals when the Nanodrop finishes analyzing the sample (~3-5 seconds).

6.1.1.8.1. Raise the sample arm and clean the sample off both pedestals with a dry Kimwipe® (or similar product).

6.1.1.9. Load 2µL of the applicable Qiagen Hydration Solution that was used to rehydrate the DNA to the lower pedestal with a pipette, close the sample arm, and click the BLANK button.

6.1.1.9.1. Use the DNA Hydration Solution if the DNA samples were extracted via the extracted via the manual Qiagen method (reference SF-4-12).

6.1.1.9.2. Use the FG3 Hydration Buffer if the DNA samples were extracted via the Auotogen Flexstar method (reference SF-4-18).

6.1.1.10. Clean the upper and lower pedestals when the Nanodrop finishes analyzing the sample (~3-5 seconds).
6.1.1.10.1. Raise the sample arm and clean the sample off both pedestals with a dry Kimwipe® (or similar product).
6.1.1.11. Type the name of the solution used to blank the equipment in the software menu's Sample ID box.
6.1.1.12. Again, Load 2µL of the applicable Qiagen Hydration Solution used to rehydrate the DNA to the lower pedestal with a pipette, close the sample arm, and click the OK button.
6.1.1.13. Clean the upper and lower pedestals when the Nanodrop finishes analyzing the sample (~3-5 seconds).
6.1.1.13.1. Raise the sample arm and clean the sample off both pedestals with a dry Kimwipe® (or similar product).
6.1.1.14. View the resulting spectrum on the screen.
6.1.1.14.1. The result should be a spectrum with a relatively flat baseline.
6.1.1.14.1.1. If this is the case, proceed to section 6.1.2.
6.1.1.14.1.2. If the spectrum does not show a flat baseline, repeat steps 6.1.1.9-6.1.1.14 until it does.
6.1.1.14.1.3. If, after several blanking attempts, the spectrum still does not show a flat baseline, reference The Nanodrop User's Manual (Trouble Shooting; sections 15-1 through 15-7) in SF-3-11.

6.1.2. Measuring DNA samples for concentration and purity
6.1.2.1. Obtain DNA and applicable processing worksheet for recording values.
6.1.2.1.1. Use SOP SF-4-12 (DNA Purification) Appendix A for DNA samples that have been manually extracted.
6.1.2.1.2. Consult the SOP SF-4-18 processing worksheet for DNA extracted via the Autogen Flexstar method.
6.1.2.2. Record the lot number and expiration date of the Hydration Solution used in step 6.1.1.9.
6.1.2.3. Mix sample thoroughly prior to loading by gently inverting the sample tube several times or pipetting the sample up and down multiple times to mix or gently vortexing for a few seconds.
6.1.2.4. In the software menu's Sample ID box, scan (using bar-code scanner) or type in the sample's ID.
6.1.2.5. Load 2µL of sample onto the lower sample pedestal with a pipette, close the sample arm, and click the OKAY button.
6.1.2.6. Clean the upper and lower pedestals when the Nanodrop finishes analyzing the sample (~3-5 seconds).
6.1.2.6.1. Raise the sample arm and clean the sample off both pedestals with a dry Kimwipe® (or similar product).

6.1.3. Repeat the process outlined in steps 6.1.2.3 through 6.1.2.6 for all samples until completion of the batch of samples have been analyzed.
6.1.3.1. If there are any errors, reference The Nanodrop User's Manual (Trouble Shooting; sections 15-1 through 15-7) in SF-3-11 and repeat the reading.
6.1.3.2. If the 260/280 ratio for any sample is less than 1.7, repeat the reading (steps 6.1.2.3 through 6.1.2.6) for that sample.

6.1.3.2.1. If the 260/280 is still less than 1.7, refer to SSF Technical Advisor for advice. Document explanations and/or actions taken on applicable processing sheet.

6.1.4. Naming, saving, and storing the data (Short Term)

6.1.4.1. Click the Show Report button on the menu screen.

6.1.4.2. Click the Report name and type in the Run Identification number found on the applicable processing worksheet defined in 6.1.2.1.

6.1.4.3. Click the Reports tab (top left of window) and use the drop down menu to select Save Report.

6.1.4.4. Click the "Export Report Table Only" in the new window that appears.

6.1.4.4.1. Verify that the filename is the same as the Run Identification number listed in section 6.1.4.2 of this SOP.

6.1.4.5. Save the text file in the Specimen Storage Facility NanoDrop data shared folder.

6.1.4.6. Convert the text file into an Excel document and save in the NanoDrop data shared folder

6.1.5. Naming, saving and storing the data (Long Term)

6.1.5.1. Record QC data (260/280 ratio & DNA concentration) on applicable processing worksheet and/or attach a printout of the Excel document to the applicable worksheet.

6.2. Using the Eppendorf BioPhotometer 6131 Spectrophotometer for DNA QC

6.2.1. Sample Preparation

6.2.1.1. Prepare a BLANK by adding 60uL of DI water to a cuvette (compatible for use with the Eppendorf BioPhotometer 6131) using a pipette.

6.2.1.2. Dilute each DNA sample by combining 2ul of DNA sample with 58uL of DI water into a cuvette.

6.2.2. Machine Preparation

6.2.2.1. Press the Dilution button on the Eppendorf BioPhotometer.

6.2.2.2. Enter the amount of DNA used in the dilution (002) on the key pad and press the Enter button.

6.2.2.3. Enter in the amount of DI water used in the dilution (0058) on the key pad and press the Enter button.

6.2.2.4. Pull the cuvette cover off of the biophotometer and set it aside.

6.2.2.5. Insert the Blank sample cuvette (prepped in section 6.2.1.1 above) into the biophotometer in the proper orientation (see Eppendorf BioPhotometer Users Manual) and press the Blank button.

6.2.2.6. Remove the Blank cuvette and discard it in an appropriate waste container upon completion of the analysis and the result has been printed out.

6.2.3. Measuring DNA Samples for Concentration and Purity

6.2.3.1. Tap the cuvette gently on a hard surface a few times to evenly distribute the sample in the cuvette and to remove any bubbles within the sample.

6.2.3.2. Insert a DNA sample cuvette (prepped in section 6.2.1.2 above) into the biophotometer in the proper orientation (see Eppendorf BioPhotometer Users Manual) and press the Sample button.

6.2.3.3. Remove the sample cuvette and discard it in an appropriate waste container upon the completion of the analysis and the result has been printed out.

6.2.3.4. Repeat the process outlined in steps 6.2.3.1 through 6.2.3.3 until the entire batch of samples is analyzed.

6.2.3.5. Remove the print-out of the results and record on applicable processing worksheet.

6.2.3.6. Make a photocopy of the print-out and attach both the original and the photocopy to the applicable worksheet.

7. REFERENCES

7.1. Nanodrop ND-1000 Spectrophotometer V3.3 Users Manual

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/)

7.2. Eppendorf BioPhotometer 6131 Users Manual

7.2.1. Located in the SSF shared folder

7.2.2. Alternatively, downloadable from the support section of the Eppendorf website (http://www.eppendorfna.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.

9. APPENDICES

9.1. The current version of the following appendices are used to implement this SOP:
None (DNA readings are placed with the applicable DNA extraction worksheet).