**NanoDrop 2000 Instrument Procedure**

1. **PRINCIPLE:**
   1. Reliable determination of nucleic acid concentrations is necessary for the optimum performance of most molecular biologic procedures. This can be achieved using many methods, including chemical, spectrophotometric, and fluorometric. In this laboratory, DNA or RNA samples used for clinical testing are purified and the concentration of DNA or RNA is determined by spectrophotometric determination of UV absorbance at appropriate wavelengths. This method allows measurement of nucleic acid concentrations using a minimal sample volume.
   2. This technique is both accurate and sensitive in the range of DNA and RNA concentrations used in the laboratory. DNA concentration is estimated as 50 ng/ul for each optical density unit (OD260) at 260 nanometers. RNA is estimated as 40 ng/ul for each optical density unit (OD260) at 260 nanometers. This wavelength is close to the absorbance maximum for DNA and RNA (258 nm).
   3. The absorbance of samples is also measured at 280 nm and 230 nm where proteins and other impurities will absorb.

Based on the ratio between the two readings at 260 nm and 280 nm, the purity of the sample is determined. Pure preparations of DNA or RNA should have a ratio between 1.7 and 2.0, while less pure preparations will have a lower value ratio.

1. **SAMPLE:**
   1. DNA or RNA that has been purified and resuspended in AE, TE, or DI water.
2. **REAGENTS:**
   1. Sterile DI water. Store at room temperature.
   2. AE from Qiagen kit (51106). Store at room temperature.
   3. 10 mM Tris, 1 mM EDTA pH8 (TE). Store at room temperature for up to 2 years.
3. **PROCEDURE FOR USING NANODROP:**
   1. The Nanodrop instrument is connected to a computer.
   2. On the computer desktop, click on the Nanodrop 2000 software icon.
   3. Click on nucleic acid. A question will pop up asking ‘Load last workbook and append new data?’ Select ‘YES’ or ‘NO’ based upon your need.
   4. After routine wavelength verification, select type (DNA or RNA) and concentration units (ng/ul).
   5. If you need to change the nucleic acid type in the step above, change to the new type of nucleic acid and perform a blank measurement using the appropriate diluent.
   6. Lift the cover of the Nanodrop. Load 1.5 – 2 ul of appropriate blank (blank should match the diluent used for the sample during nucleic acid extraction).
   7. Lower the cover and click on the ‘blank’ icon or press F3.
   8. After reading, wipe off the droplet of blank on both interfaces using a tissue wet with distilled water. Wipe off the water with a dry tissue.
   9. Scan the product label of the sample to be measured in the Sample ID field.
   10. Load 1.5 – 2 ul of sample, lower cover, and press the ‘measure’ icon or press F1.
   11. Wipe off the sample from the loading pedestal. Be sure to clean between each loaded sample.
   12. Re-blank the instrument after every 5-6 samples.
   13. If you need to blank using a different diluent, clean the pedestals of the nanodrop with water, wipe dry, apply the new diluent, and click on ‘blank’.
   14. The Nanodrop 2000 calculates the concentration, 260 nm and 280 nm readings, and 260/280 and 260/230 ratios.
   15. **Export Excel File from Nanodrop 2000**
       1. When all samples have been measured, select the **Reports** button in the menu on the left side of the screen.
          1. If all Nanodrop readings should be exported to an Excel worksheet:
             1. Select **Export**.
             2. Name the file using the format: [YYYYMMDD\_Nanodrop\_readings]

Dilution: [YYYYMMDD\_Nanodrop\_readings\_DIL]

PM Extraction: [YYYYMMDD\_Nanodrop\_readings\_PM]

Organic Extraction: [YYYYMMDD\_Nanodrop\_readings\_ORG]

RNA: [YYYYMMDD\_Nanodrop\_readings\_RNA]

MGMT: [YYYYMMDD\_Nanodrop\_readings\_MGMT]

* + - * 1. Save the document in the MGP\_Nanodrop Import files folder in the RICMBLAB$ shared drive.
      1. If only select Nanodrop readings should be exported:
         1. Use the **Ctrl** button on the keyboard to select the Nanodrop readings to be exported.
         2. Select **Export**.
         3. Name the file using the format: [YYYYMMDD\_Nanodrop\_readings]

Dilution: [YYYYMMDD\_Nanodrop\_readings\_DIL]

PM Extraction: [YYYYMMDD\_Nanodrop\_readings\_PM]

Organic Extraction: [YYYYMMDD\_Nanodrop\_readings\_ORG]

RNA: [YYYYMMDD\_Nanodrop\_readings\_RNA]

MGMT: [YYYYMMDD\_Nanodrop\_readings\_MGMT]

* + - * 1. Save the document in the MGP\_Nanodrop Import files folder in the RICMBLAB$ shared drive.
    1. Navigate to and open the exported file.
    2. Select **Save As**.
    3. Change the file type from **XML Spreadsheet 2003** (\*.xml) to **Excel Workbook** (\*.xlsx).
       1. **Note**: When the file is saved, two documents with the same name will appear in the folder. The document with Type: Microsoft Excel Worksheet will appear when selecting a file to import into Soft Molecular.
    4. Select **Save**.

1. **DILUTION CALCULATIONS:**
   1. If a given sample has a DNA concentration of less than 100 ng/ul, proceed with the appropriate clinical assay.
   2. **Soft Molecular will automatically calculate dilutions to 50ng/ul.**
      1. This should be done on all samples with a concentration >100ng/ul and <300ng/ul.
   3. **The Nanodrop is not linear with concentrations greater than 300ng/ul.**
      1. When the original reading is greater than 300ng/ul, dilute to 100ng/ul and utilize an internal note in Soft Molecular to state original nanodrop reading and how much buffer was added.
   4. **Dilution Formula :**
      1. To dilute DNA to a final concentration of 50ng/ul or 100 ng/ul:
         1. Use formula: (C1)(V1)=(C2)(V2)
         2. C1 = concentration read from spectrophotometer
         3. V1 = volume in tube from extraction (typically 35ul per tube on Maxwell, 100ul organic extraction)
         4. C2 = 50ng/ul or 100ng/ul
         5. Solve for V2, which is the total volume.
         6. To get final desired concentration, volume to add = V2 -V1.
   5. To dilute RNA for *BCR-ABL1*:
      1. The required volume of RNA is 15ul.
      2. RNA samples with a concentration greater than 100ng/ul must be diluted using the same formula listed above, where:
         1. C2 = 100ng/µl
         2. V2 = 15ul
         3. C1 = concentration read on the spectrophotometer
         4. Solve for V1, which is the volume of sample to add to the reaction
         5. After solving for V1, calculate Volume dH2O to add = V2 – V1 = 15 – V1
         6. Remember that the reaction requires 15ul, so subtract V1 from 15 to determine the volume of distilled water to add to make up to 15ul
2. **MAINTENANCE:**
   1. The Nanodrop 2000 may need to be cleaned of buildup periodically using bleach or mild 0.2N Hydrochloric Acid.
   2. Twice a year the linearity and concentration are checked for accuracy.
      1. Thaw HindIII fragments, vortex, and flash spin.
      2. In a 2ml tube, dilute the HindIII fragments to 25ng/ul by adding 75ul of HindIII fragments and 1.425**ml** of dH2O.
         1. Dilute further according to Table 1.

|  |  |  |
| --- | --- | --- |
| Volume of Diluted HindIII DNA ul | Volume of dH2O ul | Expected DNA conc ng/ul |
| 2 | 0 | 25 |
| 225 | 25 | 22.5 |
| 200 | 50 | 20 |
| 175 | 75 | 17.5 |
| 150 | 100 | 15 |
| 125 | 125 | 12.5 |
| 100 | 150 | 10 |
| 75 | 175 | 7.5 |
| 50 | 200 | 5 |
| 25 | 225 | 2.5 |

* + 1. From the *undiluted* HindIII tube, dilute further according to Table 2.

|  |  |  |
| --- | --- | --- |
| Volume of HindIII DNA ul | Volume of dH2O ul | Expected DNA concentration ng/ul |
| 15 | 135 | 50 |
| 30 | 120 | 100 |
| 60 | 90 | 200 |
| 75 | 75 | 250 |
| 90 | 60 | 300 |

* + 1. Vortex all dilutions and flash spin.
    2. Blank the Nanodrop with dH2O.
    3. Measure each dilution and record the A260, A280, 260/280 ratio, and 260/230 ratio.
    4. In the QC folder on the CMB drive, enter the readings into the Spectrophotometer excel sheet.

1. **TROUBLESHOOTING:**
   1. See Nanodrop 2000 Procedure Appendix A for expected Nanodrop curves and for curves that indicate interfering substances are present. Any unusual curves should be brought to the attention of the Laboratory Director or Pathologist.
2. **REFERENCES:**
   1. Davis, L.G., Dibner, M.D, & Battey, J.F. ‘Basic Methods in Molecular Biology’, Elsevier, New York, Amsterdam, London, 1986.
   2. Handbook of Molecular Biology, CRC Press, 1990.
   3. Freifelder, D., Physical Biochemistry, W.H. Freeman and Co. San Francisco, 1976.
3. **ATTACHMENTS:**
   1. NanoDrop 2000 Instrument Procedure Appendix A: Curve Analysis
4. **REVISIONS:**
   1. 3/16/2018: Addition of Appendix A, elimination of Eppendorf Biophotometer instructions and reformatting.
   2. 1/6/2020: Updated with new procedure name, added Dilution Calculations section, steps for Soft Molecular and updated footer with new lab name.
   3. 10/6/2020: Added Appendix A for expected curve analysis.
   4. 1/16/2024: Clarifications on when to dilute samples.