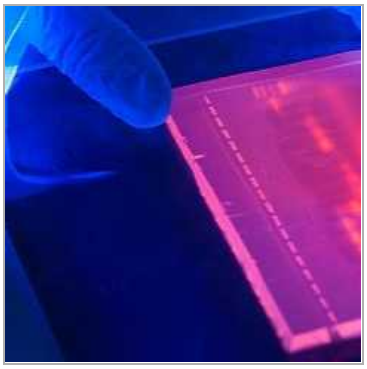

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We are still developing and optimizing this protocol

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Quantifying and Checking Genomic DNA

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ABSTRACT

Overview and Goals
Your bacterial isolate has been grown on agar plates, lysed open and genomic DNA has been isolated for sequencing. Next, we need to quantify our DNA yield and assess its integrity. For this, we will use the Nanodrop spectrophotometer and Qubit to measure our DNA quantity, We will also use an Agilent TapeStation electrophoresis system to assess the size of the DNA and quality.

After completing this lab you will gain the following lab skills:

- Lab safety and proper personal protective equipment (PPE)
- Proper use of a Nanodrop spectrophotometer and Qubit fluorometer to analyze DNA samples.
- Analysis of DNA concentration and integrity using absorbance, fluorescence, and gel electrophoresis.

MATERIALS

- One 10 µL micropipette (p10)
- Filtered tips for p10
- Chem wipes
- Tip disposal container
- Distilled water
- New England BioLabs (NEB) gDNA Elution buffer (or whatever buffer experimental DNA is stored in)
- NanoDrop spectrophotometer
- Qubit High Sensitivity (HS) 1X working solution and Qubit fluorometer
- TapeStation Genomic DNA reagents
- Your genomic DNA samples

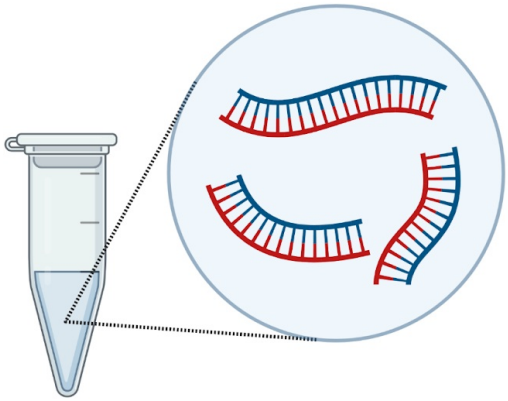
BEFORE START INSTRUCTIONS

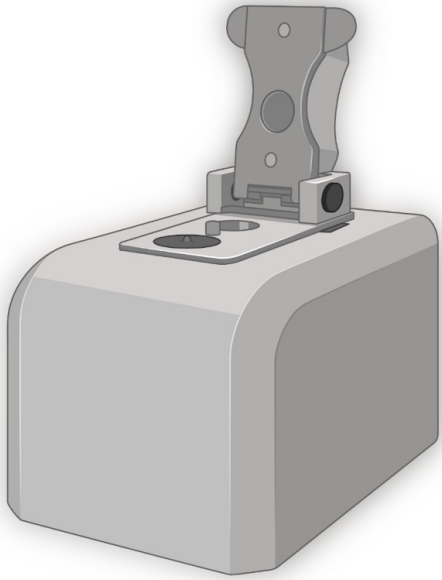
Review the protocols and figures below to learn about how the NanoDrop spectrophotometer, Qubit fluorometer, and TapeStation electrophoresis systems work.

Activity 1:Quantification of DNA with the Nanodrop Spectro..

1 Based on the NanoDrop ND-1000 Spectrophotometer Protocol.

Quantification of DNA with the Nanodrop Spectrophotometer

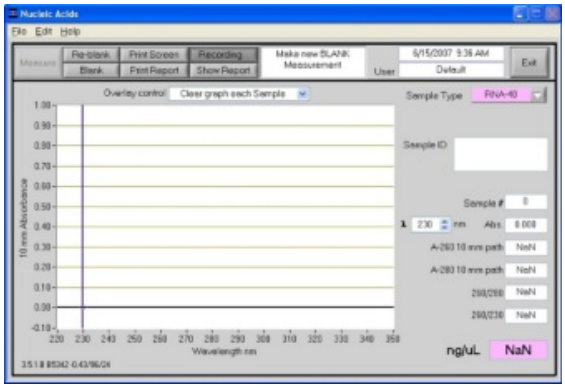




- Spectrophotometer
- DNA concentration
- Potential RNA and protein contamination.

Before starting, watch this 10-minute video: NanoDrop Microvolume Quantitation of Nucleic Acids

2 Initializing



2.1 There will be a laptop next to the NanoDrop. Log in using your NCSU ID and password.

2.2 Once you've logged in, select the ND-1000 program from the selection of apps on the left side of the screen.

2.3 In the main menu, select "Nucleic Acid" from the left column, which will take you to the screen on the right. This is where you'll see and record results.

2.4 The machine will initialize and may make some noises; do not be alarmed.

2.5 Once you have set up the NanoDrop program on the laptop, you'll be prompted to load a water sample to clean and initialize the NanoDrop.

3 Blanking

3.1 Once the machine has been initialized, lift up the bar from the pedestal and ensure the pedestal is clean by wiping each side with a KimWipe.

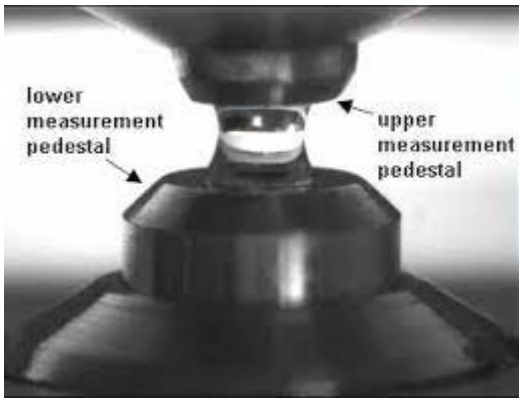
3.2 Next, you will need to load a "blank" sample of buffer. This is to calibrate the machine to the particular buffer your DNA is housed in.

3.3 Using a p10 add 1 µL - 2 µL of your DNA solvent to the lower pedestal (see image below) and gently lower the NanoDrop arm.

3.4 On the computer screen, select “Blank” at the top of the screen after loading.

3.5 Next measure your blank by selecting “Measure” at the top of the screen after loading.

The results of the blank should come back near 0 for each metric. If not, clean off the pedestal thoroughly and then load and measure another blank. Once you’ve successfully measured your blank, you may measure your sample.



4 Blanking

4.1 Using a 10 µl filtered pipette tip, add 2 µL of your sample to the center of the pedestal.

> **Note**
>
> Do not fully depress the pipette when adding the sample to the pedestal. Any air bubbles in the drop can affect the measurement.
>
> Make sure the drop is on or close to the center of the pedestal. You can use the pipette tip to slowly move the drop around if you misplace it.

4.2 Close the bar, and then look to make sure the droplet is touching both the upper and lower measurement pedestals as on the right.

4.3 After ensuring the sample is properly placed, press measure in the top left corner of the screen.

> **Note**
>
> You'll hear a click from the machine after starting the measurement - that's good.

4.4 For each read, **record the ng/µl and 260/280 values in your spreadsheet**, in addition to whatever other information your instructor requests.

4.5 Once the measurement has been taken and data recorded, open the bar and wipe off each side of the pedestal with a KimWipe.

> **Safety information**
>
> This is crucial to ensuring accurate and consistent results. Two or three wipes on each side is fine, but be sure it is dry each time before moving onto the next sample.

4.6 Only after cleaning the pedestal can you start to add another sample.

4.7 After finishing your last read, clean off the pedestal, close the NanoDrop program, and log off of the station laptop.

5 Recording you DNA quantifications

5.1 Create an Excel or Google spreadsheet to keep track of your data from NanoDrop. Title it with

the date and the name of your samples. Record the following values:

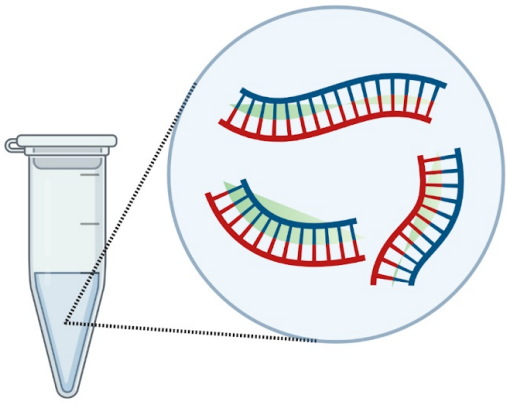
1. the concentration of your DNA in ng/µl,
2. the ratio of the absorbance at 260 and 280. DNA should have a ratio of 1.8; if RNA is present, the ratio will be higher. If protein is present, the ratio will be lower than 1.8.
3. 230/260 ratio that is used to learn about other substances that may be in your sample

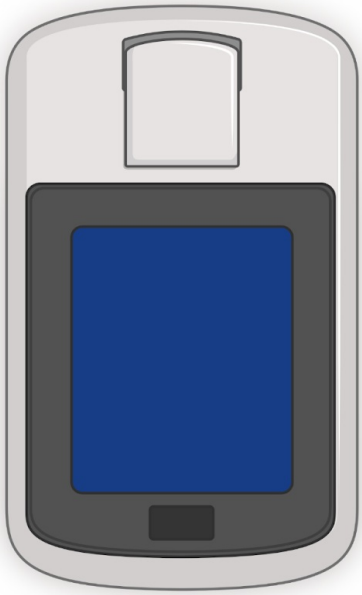
Activity 2: DNA Quantification with Qubit

2m

6

Quantification of DNA with the Qubit Fluorometer





- Fluorometer
- Accurate DNA concentration
- High sensitivity with fluorescent dye

Follow the link, scroll to the bottom of the webpage and watch this video about the Qubit fluorometer: Qubit™ 4 Fluorometer video (4:29 min)

Note

The Qubit™ 1X dsDNA HS Assay requires two standards. We use thin-wall, clear, 0.5-mL PCR tubes (Cat. No. Q32856) for the Qubit™ 4 Fluorometer.

6.1 Label the **tube lids** with a Sharpie. Label 3 tubes (2 for standards and 1 for your sample):

- "1" (standard)
- "2" (Standard)
- Bacterial species number, Ex: "105" (sample)

Safety information

Do not label the side of the tube as this could interfere with the sample read.

6.2 Prepare your standards to calibrate your sample measurement:

- Each standard tube requires 190 µL of Qubit™ working solution
- Add 10 µL of each Qubit™ standard to the appropriate tube.
- The final volume in each tube must be 200 µL

6.3 Prepare your sample:

- Each sample tube requires 195 µL of Qubit™ working solution
- Add 5 µL of each user sample to the appropriate tube using a p10.
- The final volume in each tube must be 200 µL

6.4 Mix each sample by vortexing for 00:00:05 . 5s

6.5 Allow all tubes to incubate at Room temperature for 00:02:00 . 2m

Safety information

Use a timer and keep the samples protected from light.

6.6 Calibration of the Qubit™ Fluorometer requires the standards to be inserted into the instrument in the right order. Read the prompts from the Qubit to insert the standards in the correct order. Note: we will assist you through this process. The information provided below is so you can review the entire process.

- On the Home screen of the Qubit™ Fluorometer, press the 1X dsDNA High Sensitivity (HS) assay icon. The “Read standards” screen is displayed. Press Read Standards & run samples to proceed. Note: If you have already performed a calibration for the selected assay, the instrument prompts you to choose between reading new standards and running samples using the previous calibration. If you want to use the previous calibration, press Run samples and skip to step 2.4.
- Insert the tube containing Standard #1 into the sample chamber, close the lid, then press

Run standards. When the reading is complete (~3 seconds), remove Standard #1.

- Insert the tube containing Standard #2 into the sample chamber, close the lid, and then press Read standards. When the reading is complete, remove Standard #2. The instrument displays the graphical results on the Standards complete screen.
- Press Next from the Standards complete screen to read your Samples.
- In the Sample volume screen, enter the sample volume added to the assay tube (from 1-20 µL). Enter the volume directly in the sample volume text box, use the + or – buttons or adjust the sample volume wheel to select the sample volume added to the assay tube. Note: The sample volume used (1-20 µL) changes the assay accuracy range. A different sample volume or assay may be required if the sample concentration is outside of what the assay can accurately quantify.
- Insert a sample tube into the sample chamber, close the lid, then press Run sample. When the reading is complete (~3 seconds), remove the sample tube.

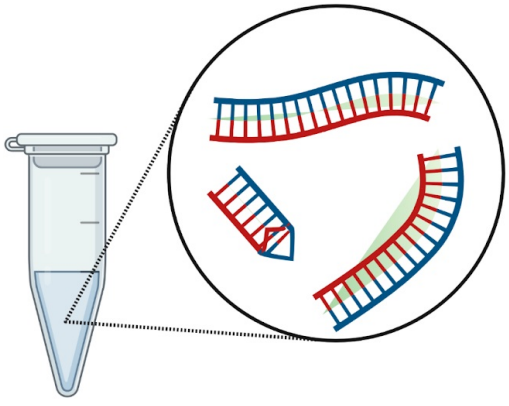
6.7 Record your concentrations in ng/µl in your notes and directly on the tubes of your genomic DNA samples.

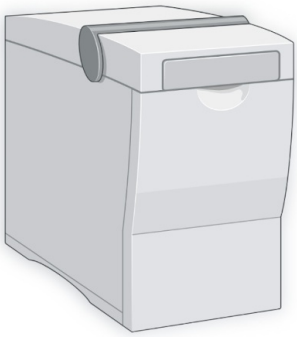
Activity 3: Genomic DNA Assessment with TapeStation

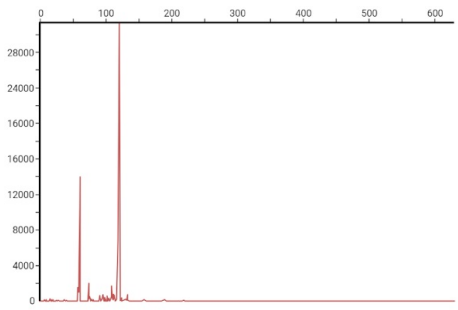
32m

7

Quantification and Size of DNA with the TapeStation Gel Electrophoresis System







- Gel electrophoresis
- Accurate DNA sizing
- High sensitivity with fluorescent dye

The Agilent TapeStation is an automated gel electrophoresis system that allows users to separate nucleic acids to obtain information about their size and distribution.

7.1 Work with your instructors to prepare samples for analysis.

7.2 Allow the Genomic DNA Reagents to equilibrate at room temperature for 00:30:00 . 30m

7.3 Flick the Genomic DNA ScreenTape device and insert it into the ScreenTape nest of the TapeStation instrument.

- Select required sample positions in the TapeStation Controller software. The required consumables (tips, further ScreenTape devices) are displayed in the TapeStation Controller software.

7.4 Vortex reagents and samples. Spin down before use.

7.5 Prepare ladder:

- Pipette 10 µL Genomic DNA Sample Buffer and 1 µL Genomic DNA Ladder at position A1 in a tube strip.

7.6 For each sample, pipette 10 µL Genomic DNA Sample Buffer and 1 µL DNA sample in a tube strip

7.7 Apply caps to tube strips .

7.8 Mix liquids using the IKA MS3 vortexer at 2000 rpm for 00:01:00 1m

7.9 Spin down samples and ladder for 00:01:00 1m

7.10 Sample Analysis

- Load samples into the TapeStation instrument.
- Place ladder in position A1 on the tube strip holder.
- Carefully remove caps of tube strips.
- Visually confirm that liquid is positioned at the bottom.
- Click Start.
- The TapeStation Analysis software opens automatically after the run and displays results.

8

Note

Critical Thinking Questions for Quantifying and Checking Genomic DNA

1. Before you measure your DNA sample using the Nanodrop, you blank it using the solvent your DNA is dissolved in.
2. What do you think the impact of using the wrong solvent could be? Ex: Using water instead of TE buffer.
3. What do you think would happen if you "blanked" the Nanodrop using your DNA sample? Would you be able to detect your DNA measurement?
4. The Nanodrop uses photospectrometry to measure your sample, while the Qubit uses fluorescence activity from chemicals that can bind DNA. What do you think are some advantages of using each method?
5. Compare your data from the Nanodrop and the Qubit: are they similar or different quantifications? If they are different, which would you trust and why?
6. From the TapeStation we will receive a DNA integrity number (DIN). What does a higher (Ex: 9) or lower value (Ex:4) tell you about your DNA?