

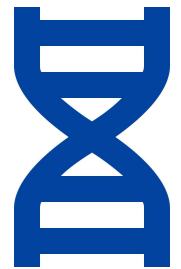
Jan 27, 2025

Version 2

Quantifying and Checking Genomic DNA V.2

DOI

<https://dx.doi.org/10.17504/protocols.io.6qpvr46epgmk/v2>



Carlos Goller¹, Carly Sjogren¹

¹[North Carolina State University]

BIT-Protocols

Tech. support phone: +91 95134-135 email: ccgoller@ncsu.edu



Nidhi Grover

North Carolina State University

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.6qpvr46epgmk/v2>

Protocol Citation: Carlos Goller, Carly Sjogren 2025. Quantifying and Checking Genomic DNA. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.6qpvr46epgmk/v2> Version created by **Carlos Carlos Goller**



License: This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: January 26, 2025

Last Modified: January 27, 2025

Protocol Integer ID: 119086

Keywords: DNA, assessment, fluorometer, nano spectrophotometer, checking genomic dna overview, bacterial isolate, dna sample, dna quality, size of the dna quality, dna concentration, dna quantity, dna yield, implen nano spectrophotometer, agilent tapestation electrophoresis system, genomic dna, lab safety, dna, gel electrophoresi, nano spectrophotometer, following lab skill, qubit fluorometer, lab, lab skill

Funders Acknowledgements:

NC State Biotechnology Program

Abstract

Overview and Goals

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

Learning Objectives

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

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

Image Attribution

Image purchased from The Noun Project and recolored by Carlos C. Goller



Materials

- One 10 μ L micropipette (p10)
- Filtered tips for p10
- Kimwipes
- Tip disposal container
- Distilled water
- New England BioLabs (NEB) gDNA Elution buffer (or whatever buffer experimental DNA is stored in)
- **IMPLEN NP80** nano spectrophotometer
- Qubit High Sensitivity (HS) 1X working solution and **Qubit fluorometer**
- TapeStation Genomic DNA reagents
- Your genomic DNA samples

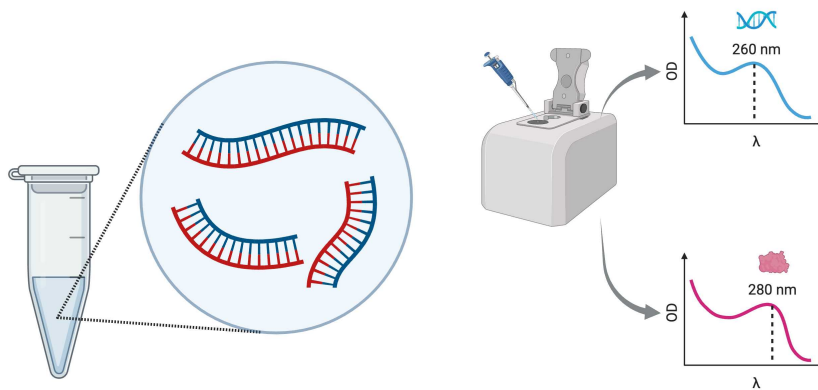
Before start

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

Activity 1: Quantification of DNA with a Nano Spectrophotometer

- 1 In the lab, we will use the **IMPLEN NP-80 nano spectrophotometer** to measure the concentration and assess the purification of your DNA.

Quantification of DNA with Nano Spectrophotometers



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

Quantification of DNA with Nano Spectrophotometers. Diagram of DNA fragments in a microcentrifuge tube and three points: spectrophotometer, DNA concentration, and potential RNA and protein contamination. On the right, a nano spectrophotometer diagram with two OD (y-axis) and λ (x-axis) graphs. The top one has a peak at 260 nm for nucleic acids; the bottom one has a peak wavelength of 280, suggesting protein contamination.

Before starting, review these two documents:

Nucleic Acid Spectrophotometer and

IMPLEN Technical Note 12 – Best Practice for Nucleic Acid Quantifications

- 2 Wipe the pedestal carefully with a damp wipe.
 - 2.1 If the device is off, initialize the instrument by pressing the power button on the back left.



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

2.3 Wait for the instrument to initialize and display the menu on the touchscreen.

2.4 Clean the pedestal one more time with a damp wipe.

3 Blank with the buffer or water used to elute your DNA.

3.1 Once the machine has been initialized, lift the bar from the pedestal and wipe each side with a Kimwipe to ensure it is clean and dry.



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

3.3 Using a p10, add  2 μ L of your DNA solvent to the lower pedestal (review image below) and gently lower the nano spectrophotometer arm.



3.4 On the computer screen, select "Blank" on the screen after loading.

3.5 Next, measure your blank by selecting "Sample" on the screen. You can enter a name such as "Blank C6 Buffer."

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 Measuring each of your samples.

4.1 Using a 10 μ l filtered pipette tip, add  2 μ L 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/ μ l 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 the pedestal for the next user. 
- 5 Recording you DNA quantifications

- 5.1 Create an Excel or Google spreadsheet to keep track of your data from the nano spectrophotometer. Title it with the date and the name of your samples. Record the following values:
1. The concentration of your DNA in ng/ μ l,
 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

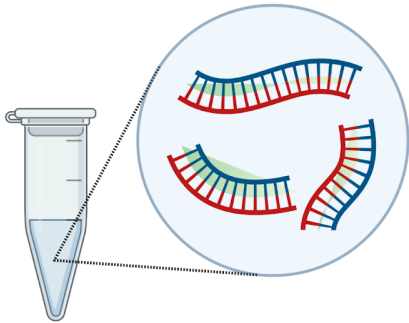


Activity 2: DNA Quantification with Qubit Fluorometer

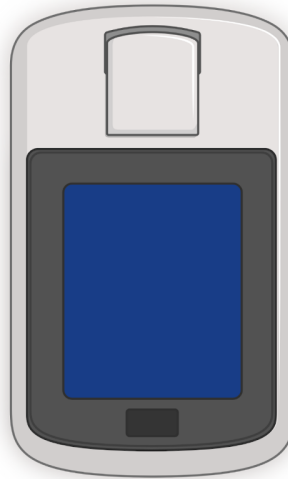
2m

6

Quantification of DNA with the Qubit Fluorometer



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



Quantification of DNA with the Qubit Fluorometer. Diagram of DNA fragments next to microfuge tube bound by green dye. Three key points are stated: fluorometer, accurate DNA concentration, and high sensitivity with fluorescent dye. A diagram of a Qubit fluorometer is on the right of the image.

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. For example: Isolate 5.

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.

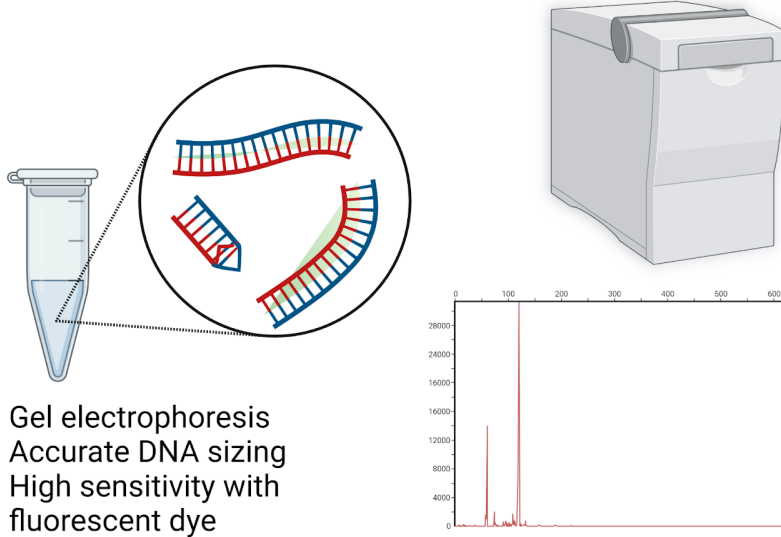
6.7 Record your concentrations in ng/µl in your notes and directly on the tubes of your genomic DNA samples.

Activity 3: Genomic DNA Assessment with TapeStation

32m

7

Quantification and Size of DNA with the TapeStation Gel Electrophoresis System



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 μ L Genomic DNA Sample Buffer and  1 μ L 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 nano spectrophotometer, you blank it using the dissolved solvent in which your DNA is dissolved.
2. What do you think the impact of using the wrong solvent could be? For example, water can be used instead of TE buffer.
3. What would happen if you "blanked" the nano spectrophotometer using your DNA sample? Would you be able to detect your DNA measurement?
4. The nano spectrophotometer 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 nano spectrophotometer 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 (for example, 9) or lower value (for example, 4) tell you about your DNA?

Acknowledgements

We appreciate the support from the NC State Biotechnology Program and students who have helped improve this and other protocols.