



VERSION 1

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[https://docs.google.com/document/d/1ast6JkatnReXsEVp\\_z5QDU1yDQLXtaSnX04tFLcKZwY/edit](https://docs.google.com/document/d/1ast6JkatnReXsEVp_z5QDU1yDQLXtaSnX04tFLcKZwY/edit)

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**Protocol status:** In development  
We are still developing and optimizing this protocol

## Quantifying Checking Genomic DNA V.1

 Forked from [Quantifying and Checking Genomic DNA](#)

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BIT Metagenomics

Portable Genome Sequencing



Carlos Goller

### 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 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 and quality the different bands.

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

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

### IMAGE ATTRIBUTION

Photo taken by Francesca Balestrieri, NC State Biotechnology Program

### GUIDELINES

Follow the steps carefully and pay attention to your instructor's guidance. Ask questions when necessary.

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**Keywords:**

nanospectrophotometer,  
IMPLEN, Qubit, quantification,  
TapeStation

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State

## MATERIALS

- One 10  $\mu\text{L}$  micropipette (p10)
- Filtered tips for p10
- Wipes
- Tip disposal container
- Distilled water
- QIAGEN PowerSoil Pro Buffer C6 (Elution), 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/or Qubit Broad Range (BR) 1X Working Solution and [Qubit fluorometer](#)
- Agilent TapeStation Genomic DNA reagents (buffer and ladder)
- Your genomic DNA samples

## SAFETY WARNINGS



Be careful with the instrument and take care to wear gloves at all times.

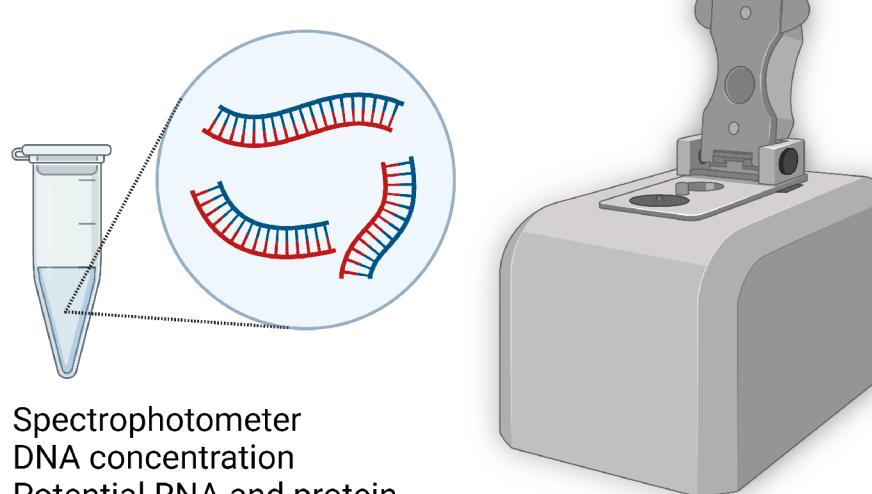
## BEFORE START INSTRUCTIONS

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

## Activity 1: Quantification of DNA with the IMPLEN Nano Spectrophotometer

- 1 Based on the NanoDrop ND-1000 Spectrophotometer Protocol.

## Quantification of DNA with the Nanospectrophotometer



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

Use of nano spectrophotometer for DNA quantification.

Before starting, watch this 3-minute video: [The NanoPhotometer Introduction Video](#) (3:43 min video).

2 Turn on the Implen and select "Nucleic Acids."

Ensure that the arm is **down** when you turn on the Implen to ensure that it can properly perform its initialization step. Additionally, check to ensure the device is set to measure "dsDNA."



IMPLEN NP80 NanoPhotometer initializing screen.



Implen NP80 menu.

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

**Note:** If you are the first to use the Implen, perform steps 1 and 2. If someone else has already used the Implen, skip to [go to step #2.3](#).

- 2.2** Pipet  2  $\mu\text{L}$  of “blank” sample buffer onto the sample window. Lower the arm and click “Blank.”

- 2.3** Wipe off the sample window and top sensor with a Kimwipe.



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.

- 3** Pipet  2  $\mu\text{L}$  of your  Sample onto the sample window. Lower the arm and click “Sample.”



- 3.1** Record the concentration (given in ng/ $\mu\text{L}$ ) and the A260/A280 ratio.



- 4** Wipe off your sample from the sample window and top sensor with a Kimwipe. You may use distilled water sprayed on a Kimwipe and dried with another one to clean the pedestal and mirror.



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

- 4.1** Clean the sample window and top sensor by pipetting 2  $\mu\text{L}$  of water onto the sample window, lowering the arm, and gently tapping the arm to make sure that the water contacts the top sensor. Wipe off the pedestal and top sensor with a Kimwipe.



- 4.2** **Lower the arm** to prevent dust from accumulating on the sample window.

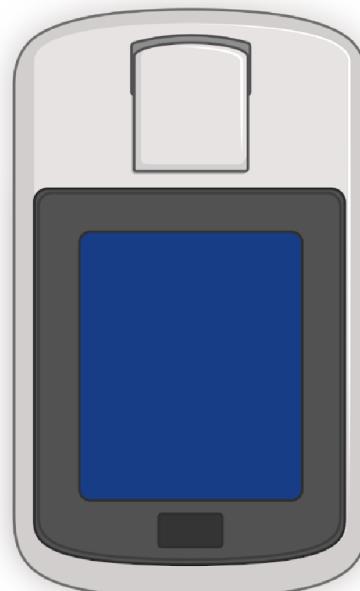
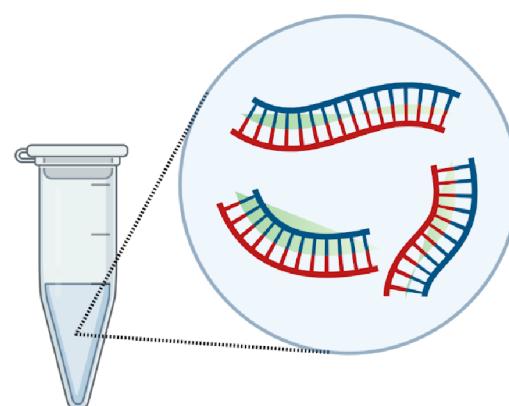
- 5 Create an Excel or Google spreadsheet to keep track of your data from Implen. Title it with the date and the name of your samples. Record the following values:
- the concentration of your DNA in ng/ $\mu$ L,
  - the ratio of the absorbance at 260 and 280. DNA should have a ratio of 1.8; if RNA is present, the ratio will be higher. If protein is present, the ratio will be lower than 1.8.
  - 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.

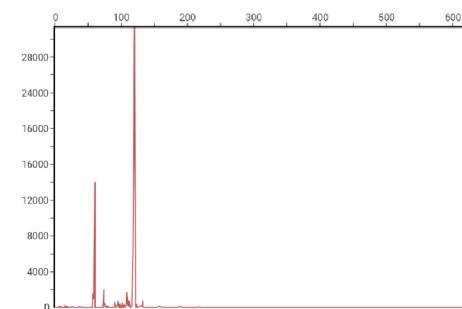
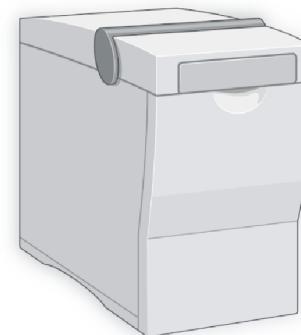
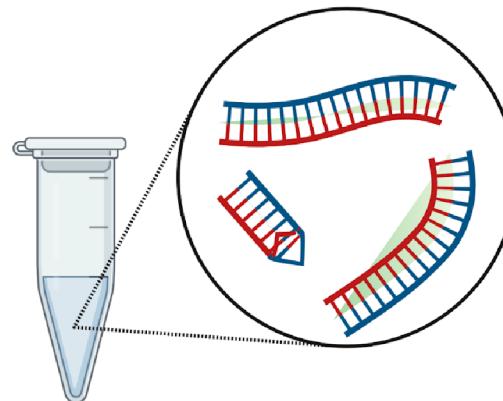
- On the Home screen of the Qubit™ Fluorometer, press the 1X dsDNA High Sensitivity (HS) assay icon. The “Read standards” screen is displayed. Press Read Standards & run samples to proceed. Note: If you have already performed a calibration for the selected assay, the instrument prompts you to choose 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

## 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 Genomic DNA Sample Buffer and Genomic DNA Ladder at position A1 in a tube strip.

**7.6** For each sample, pipette Genomic DNA Sample Buffer and DNA sample in a tube strip**7.7** Apply caps to tube strips .**7.8** Mix liquids using the IKA MS3 vortexer at for 

1m

**7.9** Spin down samples and ladder for 

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.

## 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?