



QUANTIFICATION

Using the NanoDrop to Quantify DNA or Phage

OBJECTIVE

To quantify the amount of DNA in a phage or genomic DNA sample.

BACKGROUND

Nucleic acids absorb light at a wavelength of **260 nm**. If a 260 nm light source shines on a sample, the amount of light that passes through the sample can be measured, and the amount of light absorbed by the sample can be inferred. For double-stranded DNA, an **Optical Density (OD)** of 1 at 260 nm correlates to a DNA concentration of 50 ng/μl, so DNA concentration can be easily calculated from OD measurements.

These measurements were traditionally taken with standard spectrophotometers, but we now use a tabletop spec called a NanoDrop that requires only 2 μl of a sample for quantification. The principle of action is the same, but the practical usage is much easier. That said, there's no reason phage samples can't be quantified by traditional spectrophotometers.

APPROXIMATE TIME NEEDED

~**10-15 minutes** for one sample, add ~**2 minutes** for each additional sample

MATERIALS NEEDED

Sample to be measured
NanoDrop
P2 or P10 micropipettor with tips
Lint-free lab wipes
Purified water
Blanking solution (H₂O, TE, EB, Tris, or other depending on your sample)

HELPFUL TIPS

- Significant absorbance at **270 nm** generally indicates **phenol contamination**. An OD₂₆₀:OD₂₇₀ ratio of 1.2 indicates a clean DNA sample. If your ratio is lower, bear in mind that there may be some phenol contamination and (as a result) your DNA measurement may be too high.
- Absorbance at **230 nm** is due to the presence of organic compounds and is generally not a concern. Often DNA prepped with a kit shows a significant peak at 230 nm.
- In our experience, phage genomic DNA samples (and in particular concentrated ones) are very difficult to accurately quantify because the DNA tends to aggregate. We generally heat these samples for **15 minutes at ~55°C** before measuring to help break up clumps. If you're having trouble getting consistent readings from the same tube, we recommend diluting your sample down to the 100-200 ng/μl range. **DO NOT VORTEX**, however, since this may fragment DNA.



- ✿ Because genomic DNA readings tend to be inconsistent, **MEASURE YOUR GENOMIC DNA SAMPLE MORE THAN ONCE**. If you measure 3 times and readings are fairly consistent, you can calculate the average and use that as your concentration. If you can't seem to get consistent readings, you can give the sample another day or two to resuspend OR try heating it for a bit longer and re-measuring OR try diluting it.
- ✿ Generally, extracted DNA samples are measured. You can, however, measure the amount of DNA in a **phage sample**. Just bear in mind that some of the absorbance at 260 will be due to the capsid and tail proteins, so we generally **subtract about 20%** from readings taken with complete phage particles present. They're not totally accurate, but these measurements can give a good enough ballpark measurement to decide, for example, how much sample to begin a phenol extraction with.
- ✿ The NanoDrop requires 2 μ l of sample to make a measurement. If your sample is very concentrated, this could represent a substantial loss of material. **You can, however, recover your sample after making a measurement**. Simply make sure the pedestal is clean before using it, then pipet your sample up off the pedestal after measuring and back into your tube.

PROCEDURE

1. Open and turn on the computer attached to the NanoDrop.
2. Wash the NanoDrop pedestal.
 - a. There should be a lab wipe in the pedestal from the previous user. Lift the upper arm of the NanoDrop and remove the wipe.
 - b. Add 4-5 μ l of purified water to the lower pedestal, then lower the arm.
 - c. Wait 30-60 seconds.
 - d. Lift the upper arm and use the wipe to vigorously scrub both the upper and lower pedestals.
3. Open the NanoDrop software on the computer by double-clicking the "ND-1000" icon that looks a bit like an hourglass.
4. Initialize the NanoDrop.
 - a. Click on the "Nucleic Acid" button in the NanoDrop software. This will bring up a dialog box. **DO NOT** click "Okay" until you've added water.
 - b. Add 2 μ l of purified water to the lower pedestal, then lower the upper arm.
 - c. Click "Okay" on the computer and wait ~20 seconds while the NanoDrop initializes.
 - d. When it's done, lift the upper arm and dry the pedestal with a wipe.
5. Blank the NanoDrop.
 - a. Add 2 μ l of the buffer your sample is in. If you resuspended a DNA pellet using TE, for example, blank now with TE.
 - b. Lower the upper arm of the NanoDrop and click the "Blank" button on the software.
 - c. Wait ~20 seconds for the blank measurement to be made.
 - d. When it's done, lift the upper arm and dry the pedestal with a wipe.



6. Measure your sample.
 - a. Add 2 μ l of your sample to the lower pedestal, then lower the upper arm.
 - b. In the "Sample ID" box, type in the name of your sample.
 - c. Click the "Measure" button on the software and wait ~20 seconds for measurement.
 - d. When it's done, lift the upper arm and dry the pedestal **OR** lift the upper arm and carefully pipet up as much of the sample as you can to retain it for further use (see final Helpful Tip, above).
7. Collect your data.
 - a. Write down any measurements you're interested in. You can move the cursor to check the absorbance number at various wavelengths.
 - b. Click the "Print Screen" button to print the complete spectrum, if desired.
 - c. When finished making all measurements, click "Print Report" to get a table of all data.
8. Clean the pedestal.
 - a. Add 4-5 μ l of purified water to the lower pedestal, then lower the arm.
 - b. Wait 30-60 seconds.
 - c. Lift the upper arm and use a wipe to vigorously scrub both the upper and lower pedestals.
 - d. Place a new folded lab wipe on the lower pedestal and close the upper arm.
9. Shut down and close the computer.