TOOLBOX

Measuring DNA Concentration by NanoDrop

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 a tabletop spec called a NanoDrop that requires only 2 µL of a sample for quantification is now commonly used. The principle of action is the same, but the practical usage is much easier. That said, there's no reason why 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

Equipment

- NanoDrop
- micropipettor

Consumables/Reagents (See online media preparation guides)

- Lint-free lab wipes
- Sample to be measured
- Purified water
- Appropriate micropipette tips
- Blanking solution (dH_2O, TE, EB, Tris, or other depending on the 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 the ratio is lower, there may be some phenol contamination and (as a result) the 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.

- Phage genomic DNA samples (and in particular concentrated ones) are very difficult to accurately quantify because the DNA tends to aggregate. These samples are generally heated for **15 minutes at ~55°C** before measuring to help break up clumps.

- If there are difficulties in obtaining consistent readings from the same tube, diluting the sample down to the 100 – 200 ng/µL range is recommended. **DO NOT VORTEX**, however, since this may fragment DNA.

- Because genomic DNA readings tend to be inconsistent, **MEASURE THE GENOMIC DNA SAMPLE MORE THAN ONCE**. If readings from 3 measurements are fairly consistent, average the numbers as use that as the concentration. If there are difficulties in obtaining consistent readings, 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. However, the amount of DNA in a **phage sample** can be measured as well. Just bear in mind that some of the absorbance at 260 will be due to the capsid and tail proteins, so it is generally a good idea to **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 µL of sample to make a measurement. If the sample is very concentrated, this could represent a substantial loss of material. **However, the sample can be recovered after a measurement has been made.** Simply make sure the pedestal is clean before using it, then pipet the sample up off the pedestal after measuring and back into the tube.

PROCEDURES

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. Life the upper arm of the NanoDrop and remove the wipe.

    b. Add 4 – 5 µL 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 thoroughly clean both the upper and lower pedestal.

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 water has been added in step #4b.

    b. Add 2 µL of purified water to the lower pedestal, and 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 µL of the buffer the sample is in. For example, if the DNA pellet was re-suspended using TE, 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 the DNA sample.

    a. Add 2 µL of the sample to the lower pedestal, and then lower the upper arm.

    b. In the “Sample ID” box, type in the name of the sample to be measured.

    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 possible can to retain it for further use (see final Helpful Tip, above).

7. Collect data.

    a. Write down any measurements of interest. 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 µL 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 thoroughly clean 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.