

Lab 6 – Miniprep Plasmid Preparation and Quantification

Background: Read pages 281-284, 288-289 from *At the Bench*. Purify or “isolate” plasmid DNA from a bacterial culture.

Purpose:

To purify bacterial plasmid DNA using a miniprep kit and learn to quantitate and analyze the purity of the purified DNA.

Quantum Prep Plasmid Miniprep Quick Protocol

1. Transfer 1.5 mL of an overnight culture to a micro test tube. Centrifuge 30 sec. Remove the supernatant.
2. Add 200 μ l cell resuspension solution. Vortex until the cell pellet is completely resuspended.
3. Add 250 μ l cell lysis solution. Mix by inverting 10 times (do not vortex).
4. Add 250 μ l neutralization solution. Mix by inverting 10 times (do not vortex). Centrifuge 5 minutes.
5. Insert a spin column into a 2ml wash tube. Shake the Quantum Prep matrix until completely suspended.
6. Transfer the supernatant to a spin column. Add 200 μ l matrix. Pipet up and down to mix. Centrifuge 30 sec to pull fluid through column.
7. Remove the spin column, discard the filtrate, and replace the column. Add 500 μ l wash buffer. Centrifuge 30 sec.
8. Remove the spin column, discard the filtrate, and replace the column. Add 500 μ l wash buffer. Centrifuge 2 min followed by a 2 min spin to remove all wash buffer.
9. Remove the spin column to a clean 1.5 ml tube. Add 100 μ l H₂O. Centrifuge 1 min.

Measuring Plasmid DNA Concentration

1. Turn on the spectrophotometer and wait for it to boot up.
2. Press the “ λ ” key
3. Enter “1” for the number of wavelengths.
4. Enter 260nm for the wavelength to measure.
5. Enter “NO” when asked if you want to subtract the background. The spectrophotometer is now ready to read a sample.
6. Pipette 100 μ L of diH₂O into the cuvette and press “Read Blank”
7. Remove water from the cuvette
8. Make 100 μ L of a 1:10 dilution of the purified plasmid DNA in an autoclaved eppendorff tube.
9. Mix by inverting the tube several times.
10. Transfer the dilution to a quartz cuvette
11. Place cuvette with sample in the spectrophotometer and press “Read Sample”
12. Record the absorbance
13. Repeat steps 11 and 12 two more times.

14. Obtain a separate cuvette with water in it.
15. Reset the spectrophotometer to measure at 280nm and measure the absorbance of you sample
16. Label the remaining 100 μ L of you DNA sample and store in the class freezer box.
- 17.

Data: You will have three absorbance readings at 260 nm and one reading at 280nm. Calculate the DNA concentration for each absorbance reading.

How to Determine Plasmid Yield and Purity Using UV Absorbance Measurements

DNA yield can be determined spectrophotometrically by measuring the absorbance of a dilution of the purified plasmid at 260 nm. The normal calculation for dsDNA is $1 A_{260} = 50 \mu\text{g/ml}$, for a 1 cm path length cuvette. Assuming a plasmid yield of 1 mg resuspended in 1 ml, the expected absorbance of an undiluted sample will be approximately $A_{260} = 20$. For optimal accuracy, the target absorbance for UV spectrophotometer readings should be between 0.1–0.5. Therefore, the sample plasmid DNA should be diluted from 1:40 to 1:200 in deionized water prior to measuring UV absorbance. The recorded A_{260} should be multiplied by the dilution and the extinction coefficient ($50 \mu\text{g/ml}/1.0 A_{260}$) to obtain the concentration of plasmid DNA in $\mu\text{g/ml}$. Note that the results of A_{260} readings are accurate only in the absence of significant contamination by RNA and other UV-absorbing materials. Critical samples should also be analyzed by agarose gel analysis to confirm that the UV absorbing material is, in fact, plasmid and that the absorbance reading is consistent with the yield observed on an agarose gel. Additional information about purity can be obtained by reading the absorbance at 280 nm and determining the A_{260}/A_{280} ratio. Nucleic acids have an average absorbance maximum of approximately 260 nm, whereas proteins (assuming a normal distribution of aromatic residues) have an average absorbance maximum of 280 nm. Pure DNA and typical SpinPrep plasmid isolates have A_{260}/A_{280} ratio of 1.75–1.95. Preparations contaminated with protein have significantly lower ratios of 1.3–1.5, while higher ratios (greater than or equal to 2.0) may indicate the presence of significant levels of RNA.

Conclusion:

Calculate the average DNA concentration that you measured.

Calculate the purity of your plasmid sample.

Is your purity good? Why do you think so?

Compare the protocol for the Quantum Prep Plasmid Miniprep with the protocol from Lab 5. What could be the recipe for the lysis solution?

Which protocol, lab 5 or lab 6, do you think is more effective and why?