

Biochemistry Laboratory I CHEM 4401

DNA Extraction from Plants

In this week's lab we will be extracting DNA from dried plant tissue. While 'classic' methods of DNA extraction relied upon potentially harmful reagents such as phenol and chloroform the technique has become essential in so many fields that a variety of companies now market kits for this purpose. We will use reagents and tubes from one of these kits today. As you will discover, extraction of DNA from most tissues can be completed relatively quickly and cleanly.

All extractions begin with a disruption, where tissue is disrupted in order to release the cellular components. We will pulverize our dried tissue using a mini-pestle and disrupt the cellular membranes using a buffer solution containing detergent and NaOH. RNA, a "contaminating" nucleic acid in DNA extraction assays, will be removed through the action of RNase A, an RNA degrading enzyme. This will be followed by a precipitation step, where high molecular weight lipids, polysaccharides, and proteins are removed by centrifugation. The DNA is then bound to a filter membrane and washed to remove salts and other, low molecular weight compounds. Finally, the "clean" DNA is eluted using an alkaline (pH ~ 8.0) buffer (DNA is more stable in slightly alkaline solutions). We will then use a spectrophotometric method to estimate the concentration of our DNA.

Materials

Disposable Pestles (VWR P/N KT749521-1500)	e.Z.N.A. Plant Mini kit (P/N D5511-01)
Microcentrifuge (sterile) tubes (1.5 ml)	ice
65°C Water Bath	45°C oven

Before you start:

- Be sure plant tissue samples are dry (dry overnight at 45°C)
- Preheat Water Bath to 65°C
- Be sure appropriate buffers have had ethanol added (marked on bottles)

Procedure

1. Weigh out 30 mg of dried plant tissue.
2. Place plant tissue into a 1.5 ml microcentrifuge tube and grind to powder using the microfuge tube pestle.
3. Add **600ul of buffer SP1 Buffer** to sample.
4. Add **5ul of RNase A solution** to sample. Mix thoroughly by vortexing vigorously. Make sure to disperse all clumps.
5. Incubate for 10 min. at 65°C. Mix 2-3 times during incubation by inverting tubes.
6. Precipitate detergent, proteins and polysaccharide by adding **210ul of Buffer SP2** to sample. Vortex to mix. Incubate for 5 minutes on ice.
7. Centrifuge sample at **12,000 x g for 10 min.**
8. **Carefully transfer supernatant** to an **Omega Homogenizer column (green)** placed in a 2 ml collection tube. Avoid disturbing or transferring debris.
9. Immediately Centrifuge at 10,000 x g for 2 min.

10. Transfer cleared lysate (flow-through) to a **fresh microfuge tube** without disturbing the cell-debris pellet (if present). Measure the volume of the lysate using the pipet.
11. Add **1.5 volumes of Buffer SP3 to the lysate**. Vortex immediately to mix. Example: if you collect 500 ul of flow-through in step 10, add 750 ul of Buffer SP3.
12. Add 200 ul of Equilibration buffer to a HiBind DNA Mini column placed into a 2 ml collection tube. Centrifuge at 13,000 x g for 45 seconds. Discard flowthrough.
13. Transfer **650ul of the mixture from step 11** to the HiBind DNA Mini column placed into a 2 ml collection tube. **Centrifuge for 1 min. @ 10,000 x g** to bind the DNA. Discard flow-through and re-use collection tube for next step.
14. Repeat Step 13 with remaining sample, if necessary. Discard flow through.
15. Place **the mini column into a new 2ml collection tube, and add 650 ul of SPW Wash Buffer. Centrifuge for 1 min. @ 10,000 x g**. Discard flow-through. Re-use collection tube for following step
16. Repeat wash step by adding **an additional 650ul of SPW wash Buffer** to the mini column and **centrifuge for 1 min. @ 10,000 x g**. Discard the flowthrough and re-use the collection tube in the next step.
17. Centrifuge the empty column for 2 min at 12,000 x g to dry.
18. **Transfer the mini column** to a clean, labeled 1.5 ml microcentrifuge tube.
19. Pipet **75 ul of pre-warmed (65°C) Elution Buffer directly onto the mini column membrane. Incubate for 5 min. at room temperature. Centrifuge for 1 min. at 10,000 x g to elute.**
20. Repeat step 19 with an additional 75 ul of elution buffer. Use same collection tube.
21. Go with your instructor to quantitate your DNA.

Nanodrop Instructions

1. Turn on computer and nanodrop instrument. Select “Nucleic Acid” for measurement option.
2. Spot 2 ul of elution buffer from DNA isolation kit on nanodrop “bead”. Select “blank”.
3. Wash bead with moist tissue.
4. Spot 2 ul of DNA sample on nanodrop “bead”. Select “measure”. Instrument will provide [DNA], and purity (260/280 ratios).
5. Record values. Wash bead with moist tissue prior to next sample.

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Complete this worksheet in lieu of a laboratory report. Due next week (17 pts)

Name _____

1. What was the estimate for your DNA concentration? (1 pt)
2. What are the names of the four bases that comprise DNA. Which bases are comprised of double-ring structures and which ones are single-ring? (3 pt)
3. What are the three chemical components of a nucleotide? Draw and label these on the structures of a double ring and a single ring nucleotide. Label all atoms other than carbon or hydrogen (e.g. nitrogen, oxygen, phosphorous). Be sure to draw double bonds where present. (4 pt)
4. Draw the structure of the chemical linkage that connects nucleotides in a nucleic acid (DNA or RNA) polymer (1 pt).
5. Which bases pair with each other in a DNA double helix? How many hydrogen bonds are present in each base pair? Are these H-bonding functional groups the same contact points that are recognized by DNA-binding proteins? (2 pt)

6. What do the “5’” and “3’” terminology used to describe DNA double helices refer to? (1 pt)

7. If the length of one complete (360°) turn of B-DNA is approximately 34 angstroms, how many base pairs will this contain? (1 pt)

DNA Conformers

8. Biological DNA and DNA:RNA hybrids exists in three primary conformations, or *conformers*. In which of the DNA *conformers* (A, B or Z) does most genomic DNA naturally exist? (1 pt)

9. Which of the three conformers has a *left-handed* spiral to its helix? (1 pt)

10. Laboratory Performance (2 pt).