II. Isolate DNA from Plant or Animal Tissue

REAGENTS, SUPPLIES, & EQUIPMENT

For each group

Container with cracked or crushed ice
DNA rehydration solution (250 µL)
70% Ethanol (1.5 mL)
Isopropanol (1.5 mL)
4 microcentrifuge tubes (1.5 mL)
Micropipettes and tips (100-1000 µL)
*Nuclei Lysis solution (1.5 mL)**
Permanent marker
Protein Precipitation solution (0.5 mL)
RNAse solution (10 µL)
2 Plastic pestles
2 Specimen tissue samples (from Part I)

To share

Microcentrifuge
Water bath or heating block at 65°C
Vortexer (optional)
Microcentrifuge tube rack

*Store on ice

This universal DNA extraction method uses a commercial kit. Although it is more expensive than the alternate method for plants using Edward's buffer (see part IIa), it has the advantage of working reproducibly with almost any kind of plant or animal specimen.

The large end of a 1000 µL pipette tip will punch leaf disks of this size. Animal tissue should be about the size of a pencil eraser. Using more than the recommended amount can inhibit the DNA extraction or amplification.

1. Obtain plant or animal tissue ~10-20 mg or ¼ inch diameter from your sample. If you are working with more than one sample, be careful not to cross contaminate specimens. (If you only have one specimen, make a balance tube with the appropriate volume of water for centrifuge steps.)
2. Place sample in a clean 1.5 mL tube labeled with an identification number.
3. Add 100 µL of nuclei lysis solution to tube.

Lysis solution dissolves membrane bound organelles including the nucleus, mitochondria, and chloroplast.

4. Twist a clean plastic pestle against the inner surface of 1.5 mL tube to *forcefully* grind the tissue for 1 minute. Use a clean pestle for each tube if you are doing more than one sample.

Grinding the plant tissue breaks up the cell walls. When fully ground, the sample should be a green liquid. There may be some particulate matter remaining.

5. Add 500 µL more nuclei lysis solution to tube.
6. Incubate the tube in a water bath or heat block at 65°C for 15 minutes.
7. Add 3 µL of RNAse solution to tube. Close cap, and mix by rapidly inverting tube several times.

Step 7 degrades RNA that could interfere with PCR.

8. Incubate the tube in a water bath or heat block at 37°C for 15 minutes. Then stand tube at room temperature for 5 minutes.

Step 9 causes many proteins to precipitate out of the solution, leaving DNA in the supernatant.

9. Add 200 µL of protein precipitation solution to each tube. Vortex tubes for 5 seconds: Securely grasp the upper part of tube, and vigorously hit the bottom end with the index finger of the opposite hand. Use a vortexer if available.
10. Stand tube on ice for 5 minutes.

Centrifugation pellets the nucleic acids. The pellet may appear as a tiny teardrop-shaped smear or particles on the bottom side of the tube underneath the hinge. Do not be concerned if you cannot see a pellet. A large or greenish pellet results when cellular debris carried over from the first centrifugation.

11. Place your tube and those of other groups in a balanced configuration in a microcentrifuge, with cap hinges pointing outward. Centrifuge for 4 minutes at maximum speed to pellet protein and cell debris.
12. Label a clean 1.5 mL tube with your sample number. Use a fresh tip to transfer 600 µl of supernatant to the clean tube. Be careful not to disturb the pelleted debris when transferring the supernatant. Discard old tube containing the precipitate.
13. Add 600 µL of isopropanol to the supernatant in tube. Close cap, and mix by rapidly inverting tubes several times.
14. Place your tube and those of other groups in a balanced configuration in a microcentrifuge, with cap hinges pointing outward. Centrifuge for 1 minute at maximum speed to pellet the DNA.
15. Carefully pour off the supernatant from tube, and add 600 µL of 70% ethanol. Close cap, and flick the bottom of each tube several times to "wash" the pellet.

Dry the pellets quickly with a hair dryer. To prevent blowing the pellet away, direct the air across the tube mouth, not into the tube.

16. Centrifuge the tube for 1 minute at maximum speed.
17. Carefully pour off the supernatant. Use a micropipette with fresh tip to remove any remaining ethanol, being careful not to disturb the pellet.
18. Air dry the pellet for 10-15 minutes to evaporate remaining ethanol.

In Part III, you will use 2.5 µL of DNA for each PCR. This is a crude DNA extract and contains nucleases that will eventually fragment the DNA at room temperature. Keep the sample cold to limit this activity.

19. Add 100 µL of the DNA rehydration solution to each tube, and dissolve the DNA pellet by pipetting in and out several times.
20. Incubate the DNA at 65°C for 45-60 minutes, or overnight at 4°C.
21. Store your sample on ice or at -20°C until you are ready to begin Part III.

IIa. Isolate DNA from Plant Tissue (Alternate)

REAGENTS, SUPPLIES, & EQUIPMENT

For each group
Container with cracked or crushed ice
Edward's buffer (2.2 mL)
70% Ethanol
Isopropanol
4 microcentrifuge tubes (1.5 mL)
Micropipettes and tips (100-1000 µL)
Permanent marker
Plant specimens
2 Plastic pestles
3M Sodium Acetate (150 µL)
Tris/EDTA (TE) buffer with RNase A (250 µL)
dH2O (1.5 mL)

To share
Microcentrifuge
Vortexer (optional)
Water bath or heating block

This method is optimized for plants. Although it takes about 20 minutes longer than the previous method, it uses readily available reagents.

The large end of a 1000-µl pipette tip will punch disks of this size.

1. Obtain plant tissue ~¼ inch diameter from a specimen. If you are working with more than one sample, be careful not to cross-contaminate the specimens. If you only have one specimen, make a duplicate prep to provide a balance for centrifuge steps.

Centrifugation pellets the nucleic acids. The pellet may appear as a tiny teardrop-shaped smear or particles on the bottom side of the tube underneath the hinge. Do not be concerned if you cannot see a pellet. A large or greenish pellet results when cellular debris carried over from the first centrifugation.

11. Place your tube and those of other groups in a balanced configuration in a microcentrifuge, with cap hinges pointing outward. Centrifuge for 4 minutes at maximum speed to pellet protein and cell debris.
12. Label a clean 1.5 mL tube with your sample number. Use a fresh tip to transfer 600 µl of supernatant to the clean tube. Be careful not to disturb the pelleted debris when transferring the supernatant. Discard old tube containing the precipitate.
13. Add 600 µL of isopropanol to the supernatant in tube. Close cap, and mix by rapidly inverting tubes several times.
14. Place your tube and those of other groups in a balanced configuration in a microcentrifuge, with cap hinges pointing outward. Centrifuge for 1 minute at maximum speed to pellet the DNA.
15. Carefully pour off the supernatant from tube, and add 600 µL of 70% ethanol. Close cap, and flick the bottom of each tube several times to "wash" the pellet.

Dry the pellets quickly with a hair dryer. To prevent blowing the pellet away, direct the air across the tube mouth, not into the tube.

16. Centrifuge the tube for 1 minute at maximum speed.
17. Carefully pour off the supernatant. Use a micropipette with fresh tip to remove any remaining ethanol, being careful not to disturb the pellet.
18. Air dry the pellet for 10-15 minutes to evaporate remaining ethanol.

In Part III, you will use 2.5 µL of DNA for each PCR. This is a crude DNA extract and contains nucleases that will eventually fragment the DNA at room temperature. Keep the sample cold to limit this activity.

19. Add 100 µL of the DNA rehydration solution to each tube, and dissolve the DNA pellet by pipetting in and out several times.
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The large end of a 1000-µl pipette tip will punch disks of this size.

1. Obtain plant tissue ~¼ inch diameter from a specimen. If you are working with more than one sample, be careful not to cross-contaminate the specimens. If you only have one specimen, make a duplicate prep to provide a balance for centrifuge steps.

2. Place sample in a clean 1.5 mL tube labeled with an identification number.
3. Add 100 µL of Edward's buffer to tube.

Detergent in the Edward's buffer, sodium dodecyl sulfate (SDS), dissolves lipids of the cell membranes.

4. Grind the tissue for 1 minute by forcefully twisting a clean plastic pestle against the inner surface of the 1.5 mL tube. If you are doing more than one sample, use a clean pestle for each sample.
5. Add 900 µL more Edward's buffer to tube, and grind briefly to remove tissue from the pestle.

Step 7 denatures proteins, including enzymes that digest DNA.

6. Vortex tube for 5 seconds, by hand or machine (if available).
7. Boil sample at 100°C for 5 minutes in a water bath or heating block.

Step 8 pellets insoluble material at the bottom of the tube.

8. Place tube, along with those from other groups, in a balanced configuration in a microcentrifuge, and centrifuge for 2 minutes to pellet any remaining cell debris. Centrifuge longer if there is still unpelleted debris.

Step 9 precipitates nucleic acids, including DNA.

9. Label a clean 1.5 mL tube with your sample number. If doing more than one sample, use fresh tips to transfer 350 µL of supernatant for each sample to the appropriate fresh tubes. Be careful not to disturb the pelleted debris when transferring the supernatant. Discard old tube containing the precipitate.
10. Add 400 µl of isopropanol to the supernatant in tube. Close cap and mix by rapidly inverting several times.

Centrifugation pellets the nucleic acids. The pellet may appear as a tiny teardrop-shaped smear or particles on the bottom side of the tube underneath the hinge. Do not be concerned if you can't see a pellet. A large or greenish pellet results when cellular debris carried over from the first centrifugation.)

11. Stand tube at room temperature for 3 minutes.
12. Place your tube and those of other groups in a balanced configuration in a microcentrifuge, with cap hinges pointing outward. Centrifuge for 5 minutes at maximum speed to pellet the DNA.
13. Carefully pour off the supernatant from tube, and add 500 µL of 70% ethanol. Close cap, and flick the bottom of tube several times to "wash" the pellet.

Nucleic acid pellets are not soluble in ethanol and will not dissolve during washing.

14. Place the tube in a balanced configuration in a microcentrifuge, and spin for 1 minute. Align tubes in the rotor with the cap hinges pointing outward.
15. Carefully pour off the supernatant from tube. Centrifuge the tube again for 30 seconds to force any remaining ethanol to the bottom.

Dry the pellets quickly with a hair dryer. To prevent blowing the pellet away, direct the air across the tube mouth, not into the tube.

16. Use a micropipette to carefully remove the remaining ethanol from tube. Be careful not to disturb the pellet.
17. Air dry the pellet for 10 minutes to evaporate remaining ethanol.

If needed, you may store DNA in TE/RNase solution at –20°C until ready to continue.

18. Add 100 µL of TE/RNaseA buffer to tube. Dissolve the nucleic acid pellet by pipetting in and out. Take care to wash down the side of the tube underneath the hinge, where the pellet formed during centrifugation. Use a fresh tip for each tube if you are doing more than one sample.
19. Incubate TE/RNaseA solution at room temperature for 5 minutes.
20. Add 400 µL of dH_2O to tube.
21. Add 50 µL of sodium acetate to tube. Close cap, and mix by rapidly inverting tube several times.
22. Add 550 µL of isopropanol to tube to precipitate the DNA. Close cap, and mix by rapidly inverting tube several times.

23. Stand tube at room temperature for 3 minutes.
24. Place the tube in a balanced configuration in a microcentrifuge, and spin for 5 minutes. Align tubes in the rotor with the cap hinges pointing outward.

Nucleic acid pellets are not soluble in ethanol and will not dissolve during washing.

25. Carefully pour off the supernatant from tube, and add 500 µL of 70% ethanol. Close cap, and flick the bottom of tube several times to "wash" the pellet.
26. Place the tube in a balanced configuration in a microcentrifuge, and spin for 1 minute. Align tubes in the rotor with the cap hinges pointing outward.

Dry pellet quickly with a hair dryer. To prevent blowing the pellet away, direct the air across the tube mouth, not into the tube.

27. Carefully pour off the supernatant from tube. Centrifuge the tube again for 30 seconds to force any remaining ethanol to the bottom.
28. Use a micropipette to carefully remove the remaining ethanol from tube. Be careful not to disturb the pellet.
29. Air dry the pellet for 10 minutes to evaporate remaining ethanol.

In Part III, you will use 2.5 µL of DNA for each PCR. This is a crude DNA extract and contains nucleases that will eventually fragment the DNA at room temperature. Keep the sample cold to limit this activity.

30. Add 100µl of dH_2O to tube, and dissolve the DNA pellet by pipetting in and out several times.
31. Store your sample(s) on ice or at -20°C until you are ready to begin Part III.