Advanced Cell Biology Lab 3.
DNA extraction

February 4, 2013

Background This lab starts a series of labs wher we will do all steps of DNA analysis, from DNA extraction to the creation phylogeny tree. The goal is to understand elucidate relationships between species of *Plantago* (plantains). Many species of this large (> 200 species) genus are not yet studied genetically. I have 28 herbarium (dry plant collection) samples of these species, and we use them as a basis of our collective research.

Warning! Since these labs are research, be ready for negative results.

Assignment We will extract DNA with MO Bio PowerPlant DNA Isolation Kit. The detailed protocol is given below.

Notes:

1. We will work in pairs, every pair will extract DNA from four samples. Since there are 8 pairs and 27 samples, some samples will be shared between pairs. If you have a shared sample (P2, P3, P4, P6, P9), do not use more than a half of it.
2. Every tube in the process (you will need 4 samples $\times$ 5 tubes = 20 tubes) should be labeled with extraction ID twice: on lid and on side. Always use black markers with stable ink.
3. Before every step, consider which pipetter and which tip to use, basing on the amount of liquid you have.
4. Use gloves at all times.
5. Use forceps to operate with herbarium samples.
6. Every DNA sample should be weighted before step 1, and extraction ID should be assigned for every extraction item. Table of samples is below. Please fill cells which you are responsible for. **This is your report** (see the next page).

Report

Extraction *Plantago* # 5

Conditions: MO Bio Power Plant DNA isolation kit

Date:

ID	Collection ID	Species	Weight, *g*	Your names
5-1	P1	*Plantago media*		
5-2	P2	*Plantago krasheninnikovii*		
5-3	P3	*Plantago krasheninnikovii*		
5-4	P4	*Plantago krasheninnikovii*		
5-5	P6	*Plantago urvillei*		
5-6	P7	*Littorella uniflora*		
5-7	P8	*Plantago arachnoidea*		
5-8	P9	*Plantago komarovii*		
5-9	P10	*Plantago minuta*		
5-10	P11	*Plantago canescens*		
5-11	P12	*Plantago griffithii*		
5-12	P13	*Plantago lagocephala*		
5-13	P14	*Plantago loeflingii*		
5-14	P15	*Plantago lessingii*		
5-15	P16	*Plantago minuta*		
5-16	P17	*Plantago polysperma*		
5-17	P18	*Plantago evacina*		
5-18	P19	*Plantago griffithii*		
5-19	P20	*Plantago squalida*		
5-20	P21	*Plantago altissima*		
5-21	P22	*Plantago argentea*		
5-22	P23	*Plantago carinata*		
5-23	P24	*Plantago monosperma*		
5-24	P25	*Plantago schwartsenbergii*		
5-25	P26	*Plantago pusilla*		
5-26	P27	*Plantago subulata*		
5-27	P28	*Plantago lagopus*		
5-28	P2	*Plantago krasheninnikovii*		
5-29	P3	*Plantago krasheninnikovii*		
5-30	P4	*Plantago krasheninnikovii*		
5-31	P9	*Plantago komarovii*		
5-32	P6	*Plantago urvillei*		

Protocol (see the next page)

Please wear gloves at all times

This protocol is written for the first time user. It is designed to be informative and describes each step in detail.

1. To the PowerPlant® Bead Tubes provided, add up to 100 mg (0.1 g) of plant tissue sample, followed by the addition of 550 µl of PowerPlant® Bead Solution.
   NOTE: THE DNA EXTRACTION EFFICIENCY WILL IMPROVE BASED ON THE CONDITION OF HOMOGENIZATION
   What's happening: After your sample has been loaded into the PowerPlant® Bead Tube, the next step is a homogenization and lysis procedure. The PowerPlant® Bead Tube contains a buffer that will (a) help wet the tissue surfaces, and (b) protect nucleic acids from degradation.

2. Gently vortex to mix.

   What's happening: Gentle vortexing mixes the components in the PowerPlant® Bead Tube.

3. **Check Solution PB1**. If Solution PB1 has precipitated, heat solution to 60°C until the precipitate has dissolved before use.

   What's happening: Solution PB1 contains SDS and other disruption agents required for complete cell lysis. In addition to aiding in cell lysis, SDS is an anionic detergent that breaks down proteins, fatty acids and lipids associated with the cell membranes. If it gets cold, it will form a white precipitate in the bottle. Heating to 60°C will dissolve the SDS and will not harm the SDS or the other disruption agents. Solution PB1 can be used while it is still warm.

4. Add 60 µl of Solution PB1 and invert several times or vortex briefly.

5. Place the PowerPlant® Bead Tubes in a water bath at 65°C

   What's happening: Heating the plant tissues help in homogenizing them in the following step.

6. Vortex at maximum speed for 20 minutes.

7. Make sure the PowerPlant® Bead Tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x *g* for 30 seconds at room temperature.
   CAUTION: Be sure not to exceed 13,000 x *g* or tubes may break.

8. Transfer the supernatant to a clean 2 ml Collection Tube (provided).

   Note: *Expect between 400 to 500 µl of supernatant at this step. The exact recovered volume depends on the absorbency of your starting material and is not critical for the procedure to be effective. The supernatant may be dark green in appearance and still contain some tissue debris. The presence of carry over tissue debris or a dark color in the mixture is expected in many plant types at this step. Subsequent steps in the protocol will remove both carry over tissue debris and coloration of the mixture.*

9. Add 250 µl of Solution PB2 and invert the tubes to mix the contents. Incubate at 4°C for 5 minutes.

   What's happening: Solution PB2 contains a reagent to precipitate non-DNA organic and inorganic material including plant polysaccharides, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

10. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g*.

11. Avoiding the pellet, transfer the entire volume of supernatant to a clean 2.2 ml Collection Tube (provided).

What's happening: The pellet at this point contains non-DNA organic and inorganic material including plant tissue debris, cell debris, and proteins. For the best DNA yields, and quality, avoid transferring any of the pellet.

12. Add 1 ml of Solution PB3 and invert the tubes at least 5 times to mix the contents. Incubate at room temperature for 10 minutes.

What's happening: Solution PB3 is 99% isopropanol and will precipitate DNA along with some organic contaminants. Most of the co-extracted impurities will be removed at this step.

13. Centrifuge the tubes at room temperature for 15 minutes at 13,000 x *g.*

14. Discard the supernatant and resuspend the pellet in 100 µl of Solution PB6. Note: The tubes do NOT have to be air dried as residual isopropanol will not affect the process.

What's happening: Isopropanol will precipitate and pellet the DNA. The pellet at this point contains relatively pure DNA along with some organic contaminants; mostly polysaccharides and phenolics depending on the plant tissues processed, leaving a majority of the contaminants in solution.

15. Shake to mix Solution PB4. Add 500 µl of Solution PB4 and vortex briefly to mix.

What's happening: Solution PB4 is a high concentration salt solution. Since DNA binds tightly to silica at high salt concentrations, this will adjust the DNA solution salt concentrations to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the Spin Filters.

16. Load the entire volume (600 µl) onto a Spin Filter and centrifuge at 10,000 x *g* for 1 minute.

What's happening: DNA is selectively bound to the silica membrane in the Spin Filter device in the high salt solution. Contaminants pass through the filter membrane, leaving only DNA bound to the membrane.

17. Remove the Spin Filter basket, discard the flow through and replace the Spin Filter basket back in the tube.

18. Add 500 µl of Solution PB5 and centrifuge at room temperature for 30 seconds at 10,000 x *g*.

What's happening: Solution PB5 is an ethanol based wash solution used to further clean the DNA that is bound to the silica filter membrane in the Spin Filter. This wash solution removes residual salts and other contaminants while allowing the DNA to stay bound to the silica membrane.

19. Discard the flow through from the 2 ml Collection Tube.

What's happening: This flow through fraction is non-DNA organic and inorganic waste removed from the silica Spin Filter membrane by the ethanol wash solution.

20. Centrifuge again at room temperature for 1 minute at 10,000 x *g*.

What's happening: This second spin removes residual Solution PB5 (ethanol wash solution). It is critical to remove all traces of wash solution because the ethanol in Solution PB5 can interfere with many downstream DNA applications such as PCR, restriction digests, and gel electrophoresis.

21. Carefully place Spin Filter in a clean 2 ml Collection Tube (provided). Avoid splashing any Solution PB5 onto the Spin Filter.

Note: *It is important to avoid any traces of the ethanol based wash solution.*

22. Add 50 µl of Solution PB6 to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica Spin Filter membrane at this step (MO BIO Catalog# 17000-10).

    Note: *Placing the Solution PB6 in the center of the small white membrane will make sure the entire membrane is wetted. This will result in a more efficient and complete release of the DNA from the silica Spin Filter membrane. As Solution PB6 (elution buffer) passes through the silica membrane, DNA that was bound in the presence of high salt is selectively released by Solution PB6 (10 mM Tris) which is a low salt solution.*

23. Centrifuge at room temperature for 30 seconds at 10,000 x *g*.

24. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application. No further steps are required.

We recommend storing DNA frozen (-20° to -80°C). Solution PB6 does not contain any EDTA. To concentrate DNA see the Hints and Troubleshooting Guide.

NOTE: Occasionally, plants such as grapes, cotton, sunflower, strawberry, pine needles, etc. will yield DNA with inhibitors, which may prevent target sequences from amplifying in PCR. Under such circumstances, it is suggested to use the clean-up protocol provided. Alternatively you can dilute the template DNA one to several fold, for successful PCR.

Thank you for choosing the PowerPlant® DNA Isolation Kit.