Plant Extraction Protocol (2 day)

This is taken from a paper by Michiels et al. “Extraction of high-quality genomic DNA from latex-containing plants” from Analytical Biochemistry, 315: 85-89 (2003).

Day 1. (wear protective gear including eye protection, lab coats, and thick gloves).

1. You will need 5 gm of leaf tissue to use the mortars and pestles that we have effectively.

2. Put the leaf into a chilled mortar (it is sitting in a Styrofoam container on dry ice). Add 500mg of polyvinyl pyrrolidone (PVP) from the small tube.

Add powdered dry ice pellets and grind the leaf to a fine powder for 5-10 minutes.

Add more dry ice as needed to keep it from melting to a paste instead of a powder, but be aware that if any chunks land they can send the powdered sample flying, so do it carefully.

! Be very careful of your hands and eyes, substances this cold can damage your skin, eyes, etc!

3. Using a spatula, scrape the powder into a 50 ml Falcon tube. Add 15ml of warm Extraction Buffer to the tube.

The buffer is in a bottle in the water bath, set at 60°C.

The Extraction Buffer contains:

0.1M Tris (pH 8.0)

1.4M NaCl

0.02M EDTA

0.2% beta-mercaptoethanol

2% CTAB.

Invert the tube repeatedly to mix the powder into the buffer.

4. Let the tube sit in the 60°C water bath and incubate for 60 minutes, with periodic (every ten minutes or so) mixing by inversion.

5. Remove the tube from the water bath. Let cool to room temperature for 15 minutes.

! The chloroform will foam over when it is warm, so you need to cool it down to room temperature before you do anything. !

Once the solution is cool, mix the contents thoroughly (by hand or with a vortex).

6. Add 15 ml of Chloroform –isoamyl alcohol (24:1 ratio) – be sure to use in a well-ventilated areal.

Make sure the sample tube cap is tightly closed. Vortex to mix completely.

Spin in a centrifuge at 2500 rpm for 5 minutes to separate the layers (room temperature is fine).

! Don't forget to balance tubes (any pair that will be across from each other in the centrifuge must have the same weight, to within a gram)!

7. Transfer the top (aqueous) layer to a new tube, using a 10 ml (serological) pipette and a green pipette pump.

! Dispose of the chloroform in the labeled brown waste bottle!

Repeat step 6 using 15 ml of a 1:1 phenol-chloroform solution.

!! Phenol burns tissues – be very careful to wear gloves, protective eyeware, and a lab coat and tell the instructors at once if you get any on yourself – it will need to be flushed with a lot of water.!!

! Dispose of the phenol-chloroform in the brown waste disposal bottle.!

8. Measure the volume of the aqueous layer with a serological pipette.

9. Add 2/3 volume isopropyl alcohol. Mix thoroughly.

Let sit at Room Temperature at least overnight.

This is a good stopping point and can be extended over the weekend.

Day 2. (wear protective gear including eye protection, lab coats, and nitrile gloves).

10. Centrifuge the tubes at 5000 rpm for 10 min.

! Don't forget to balance them!

Pour off, or pipette off, the supernatant (you want the pellet – be sure it does not float away).

The isopropanol can be disposed of in the sink, make sure the water is running.

11. Add 1 ml of a solution of Salt-EtOH solution (10mM Ammonium Acetate in 70% ethanol) .

Let sit for 5 min at room temperature.

Centrifuge at 2500 rpm for 6 min.

Remove the liquid (also called the supernatant) – it can go down the sink.

12. Repeat step 11.

13. Dissolve the pellet in 1 ml of TE buffer (10mM Tris, pH8.0, 1mM EDTA).

Add 10 ul of a solution of RNAase A that is at 1ug/ul (this degrades the RNA that co-purified with the DNA).

Place the tube into a water bath set at 37°C (note, this is called incubation) for 15 minutes.

The RNAase A is an enzyme that degrades RNA molecules to single nucleotides so that they don't contaminate your DNA.

14. Add 1 ml of Phenol (equilibrated with buffer) and vortex. Centrifuge at 2500 rpm for 5 min.

This will remove the RNAase A and any other proteins still around.

15. You have two layers of liquid. The phenol is on the bottom, the water (aqueous) phase is on the top, with your DNA in it.

Using a 1ml pipette, remove the top layer to a new tube.

Note: Get as much as you can of this layer, which has the DNA, but be willing to lose a little in order to avoid any of the bottom layer. (If you mess up, re-combine the layers, re-centrifuge and try again).

The phenol is hazardous waste, put the tube containing it in the brown waste disposal bottle.

16. Repeat step 15 with 1.0 ml of a solution that has Phenol-Chloroform-isoamyl alcohol (25:24:1 ratios).

17. Repeat step 15 with 1.0 ml of a solution that has Chloroform-isoamyl alcohol (24:1 ratio).

18. Measure the final volume.

Multiply the sample volume by 2.5 and divide by 7.5. Add the calculated amount of 7.5M ammonium acetate to the sample and mix.

19. Measure the total volume of the sample.

Multiply that number by two, and add that amount of 95% ethanol to the tube.

Mix, let sit on ice for 5 minutes ('incubate').

20. Centrifuge at 5000 rpm for 10 minutes at room temperature.

21. Remove the solution.

22. Add 100 ul of 70% ethanol, but don't disturb the pellet, just let it sit for 1 minutes

23. Remove as much of the liquid as possible, let the pellet air dry in the open tube at room temperature for 10 minutes.

24. Add 100 ul of 1X TE buffer. Finger mix to get the pellet away from the tube and into the water.

    This may have to sit overnight in order to completely dissolve. It can do this at room temperature.

25. You will need to characterize the DNA in several ways.

    Measure the concentration with a spectrophotometer

    Measure the average length of the DNA you have purified using a 1% agarose gel.