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 gloves).

1. You will need ~2.5 gm of leaf tissue to use the mortars and pestles that we have effectively (this is ~3 leaves).
    a. Remove any large pieces of stem or veins
    b. Tare a weigh boat. Put leaf sections in the weigh boat.

2. Put the leaf into a chilled mortar (it is sitting in a Styrofoam container on wet ice).

a. Add 1gm of mixed PVP and sorbitol from the small microfuge tube (pre-measured for you).

b. Add 1ml of Grinding Buffer per gram of leaf tissue (so, if you have 2.2 gm of leaves, add 2.2 ml of grinding buffer) – it is in a 15 ml tube on your bench.

c. Add 50ul of beta-mercaptoethanol from the small microfuge tube (smells like rotten eggs but helps make clean DNA)

d. Add a few dry ice pellets (wear gloves while handling!)

3. Grind the leaf to a powder, and eventually to a smooth paste for 15 minutes.

a. Add more small pieces of dry ice at least once.

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

3. Using a wooden craft stick, scrape the paste into a 15 ml plastic tube. Estimate the volume of material present.

a. Add an equal volume (~4ml) of CTAB Extraction Buffer to the mortar and use the buffer to rinse off the mortar and pestle- then put this in your tube (you can pipette if you don't think you can pour well enough).

b. Add 200 ul of Tween20 detergent to the tube (it is very thick – make sure you pipette *slowly* up and *slowly* down and then rinse the pipette tip several times with the buffer in the tube). Cap and mix by inversion.

d. Let the solution sit for 2minutes on the bench with the lid off to make sure the dry ice is evaporated.

e. Put the cap on the tube and invert 8-10 times to mix the buffer with the leaf paste completely.

f. Label the tube and the cap so you know which one is yours.

4. Put the tube in the rack in the 60°C water bath and incubate for 30 minutes.

a. Mix every 5 minutes by inverting the tube a few times (make sure the cap is still tight first).

5. Remove the tube from the water bath. Remove the piece of Miracloth and a weigh boat from the Baggie on your bench.

a. Slowly pour the plant mixture through the Miracloth into the weigh boat – this removes large chunks of plant material. When most of the liquid is through, squeeze the Miracloth gently around the plant material that is left, but don't tear the Miracloth.

b. Discard the Miracloth in the trash

c. Carefully pour the liquid in the weigh boat into a fresh 15ml tube. Label it!

6. Take your tube to the chemical hood. Using a 10ml serological pipette and a green pipette pump, remove an equal volume (~7ml) of Chloroform from the reagent bottle and add to your sample tube. Discard the pipette in the biohazard waste bag in the hood. Cap the Chloroform.

a. Cap your sample carefully – invert slowly and check for leaks, tighten as necessary.

b. Vortex for 10-15 seconds to mix completely.

d. Balance your tube against another group's tube - they must be within 0.5gm of each other.

e. Place the equally balanced tubes directly across from each other in the centrifuge rotor.

f. Spin in the centrifuge at top speed for 60 minutes to separate the chloroform layer from the aqueous (water) layer that contains the DNA (at room temperature is fine). Note – the chloroform is denser than water so it will be on the bottom. It will also be bright green because it will extract the chlorophyll from the plant material. The DNA will be in the aqueous layer on top, but it is dissolved at this point so you won't see it. The will be a greyish mid-layer that is made up of proteins and carbohydrates.

7. Transfer the top (aqueous) layer to a new 15 ml tube, using a 10 ml (serological) pipette and a green pipette pump.

a. Label the tube and put a cap on it

b. ! Dispose of the chloroform in the bottom of your first tube by pouring it into the labeled brown Oraganic Waste bottle in the fume hood!

8. Determine the volume of your sample, multiply that number by 0.7. That is the amount of isopropyl alcohol you will add to your sample.

For example, if I have 7ml, then 7*0.7 = 4.9ml, the amount of isopropyl alcohol I will add to my solution.

a. After adding the isopropyl alcohol, put the cap on the tube and mix the solutions by inverting 8-10 times.

b. Make sure your sample tube is labeled – this will sit at room temperature until tomorrow. The DNA is not soluble in 70% alcohol, so it will slowly come out of solution - you will see it as a fluffy whitish material that has sunk to the bottom of the tube. You can compact the DNA by centrifuging the solution.

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

9. Balance tubes – add a little more isopropanol to whichever of the samples is lighter. Centrifuge your samples tubes at top speed for 60 min.

a. ! Don't forget to balance your sample tube with that of another group, they must be within 0.5 gm of each other! Make sure the balanced tubes are the ones directly across from each other in the centrifuge rotor.

b. When you take your tube out of the centrifuge, check that you can see your pellet – a white material on one side of the tube (The side away from the center of the rotor), then pour off the solution down the other side, the side opposite your pellet.

i. The solution you are pouring away can go down the sink (have the water running) and it is called the supernatant.

c. Invert your tube on a paper towel for 1-2 minutes, wipe the rim with a Kimwipe.

d. Turn the tube right side up (no cap) and air dry for 5 minutes.

11. Add 1 ml of a solution of 70% ethanol(in a 15ml tube on your bench – don't confuse this with the 100% ethanol solution..

a. Rinse your pellet and tube with this, swirling gently once or twice and then let it sit for 1 minute. You are trying to get rid of everything but the DNA pellet.

b. Pour off the ethanol.

c. Invert the tube on a paper towel for 1-2 minutes, then wipe the rim with a Kimwipe.

d. Turn the tube right side up and air dry the tube for 5 minutes

13. Add 0.5 ml of TE buffer which is in a 1.5 ml microfuge tube on your bench, put on the cap, and slosh around until your pellet is dissolved – usually 15-30 minutes.

a. Transfer the dissolved pellet to a 1.5ml microfuge tube (label it).

i. Discard the 15 ml tube in the regular waste.

14. Add 10 ul of a solution of RNAase A (in a 1.5 ml microfuge tube on your bench) to your samples. Pipette up and down 10 times to m ix it, do not vortex. This degrades the RNA that co-purified with the DNA.

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

14. Add 20ul of Proteinase K from the 1.5 ml microfuge tube on your bench. Mix by pipetting up and down 10 times, do not vortex.

a. Place the tube in a water bath set at 37C for 15 minutes.

15. Remove the tube from the water bath, dry with a KimWipe. Carry the tube to the chemical hood.

a. From the reagent bottle labeled Phenol-Chloroform, using a 1000ul micropipetter (the largest one), set ir for 500ul, seat a tip on it, remove 0.5 ml of Phenol –Chloroform and add to your sample. Discard the tip in the baihazard waste bag in the hood. Cap the reagent bottle.

a. Cap your sample tube carefully, and vortex for 10-15 sec.

b. Balance your tube against another groups tube – within 0.1 gm, or holding the tubes up, if they have the same level of solution they will be close enough. If they need adjusting add a little bit of 1X TE buffer.

c. Place in the microfuge. Centrifuge at 14,000 rpm for 30 min to separate the phenol-chloroform (it is the heavier solution) and the aqueous (mostly water- lighter) phases.

Note: This will remove the RNAase A, Proteinase K and any other proteins still around.

Note: ! Be careful with the phenol – it can cause chemical burns to your skin – make sure tubes are tightly closed and that tips used for measuring are carefully ejected into the waste boxes.

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

a. Using a 1000ul micropipette (blue tips) set at 500ul, remove the top layer to a new 1.5 ml 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).

b. The phenol is hazardous waste, put the tube containing it in the brown Organic Waste disposal bottle in the hood. Then put the tube in the hazardous waste bag in the hood.

c. Label the tube with your sample in it.

16. Measure the final volume of the aqueous solution that has your DNA.

a. Divide the sample volume by 5. Add that calculated amount of 3M sodium acetate Ithe 1.5ml microfuge tube that has 3M NaOAc on it) to the sample and mix by vortexing a few seconds. Then multiply the new total sample volume times 2 and add that much 100% ethanol to the sample.

For example, if I end up with 420ul of sample

420 /5 = 84ul of 3M sodium acetate added to the sample

(420+84)*2 = 1008ul of 100% ethanol added to the sample

Label your tube!

19. Let the sample sit in the -20 freezer for 1 hour, or overnight.

20. Balance your tube against someone else's tube

a. They should be within 0.1 gm of each other but if you hold them up together and they have the same volume you will be fine – if you need to adjust the volume use 70% ethanol to do so.

b. Put the balanced tubes directly across from each other in the rotor.

c. Centrifuge the samples at 12,000 rpm for 60 minutes at room temperature.

21. Make sure you can see your pellet (a disk of white material stuck to the side or bottom of the tube)

a. Pour off the solution on the side of the tube that is opposite your pellet.

b. Invert on a paper towel for 1 minute

c. Wipe the lip dry with a Kimwipe.

22. Add 100 ul of 70% ethanol to the tube but don't disturb the pellet, just let it sit for 5 minute.

a. Remove as much of the liquid as possible with a micropipette, but don't let the tip touch your pellet.

b. Let the pellet air dry in the open tube at room temperature for 10 minutes.

23. Add 200 ul of 1X TE buffer to the tube, cap it tightly.

a. Vortex for several 5-second bursts, until the pellet comes loose from the side of the tube.

b. Put tubes in a vortex attachment that holds a number of them and shake gently for 30 minutes or until the solution seems clear.

b. Store at 4C overnight.

24. 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.

Perform PCR to make sure there are no inhibitors.