Total Plant Genomic DNA Isolation
(Nuclear, chloroplast and mitochondria)
Modified 04-16-06

Grow plants under ideal conditions.

Harvest the tissue into a convenient container. Some people collect it first into a beaker of cold water until the entire sample is harvested and then transfer it to a moist paper tower and then bring that into the lab. Others prefer to collect the tissue into a prelabeled Aluminum foil package that is sitting in liquid N_2. Ideally only the very young leaves; i.e., the shoot tips, should be harvested. (There are more cells and hence more nuclei; i.e., DNA per gram fresh weight in young expanding leaves than there is in old mature leaves.) Definitely don't use old leaves or leaves that look like they are senescing. I routinely collect tissue in the greenhouse (from about 10 different plant types), wrap each into a moistened paper towel, bring that into the lab and then transfer the tissue into prelabeled Aluminum foil packages and then put this into the -80°C. If you are uncomfortable with this practice or have an excessive number of samples, then harvest the tissue directly into liquid nitrogen. Be sure you label the tissue container appropriately: Plant type, Date, Your initials, Total weight, Tare weight (i.e. pre weigh the container before you put tissue in it so that you can determine your actual tissue weight), etc. Then either use the tissue immediately or freeze/store it at –80°C.

Determine approximately how much tissue you have:

Total weight: _____
Tare weight: _____
Net weight: _____

You can also freeze dry the tissue. (If you freeze dry it then the isolation of nuclei is not possible.) The following DNA extraction protocol is written for freeze-dried tissue. To freeze dry tissue it must first be frozen. You can do this either by putting it into the –80°C or by having it in liquid N_2. Then put the frozen tissue into a brown paper bag or foil pack or coin envelope into the Freezemobile lyophilizer in Dr Streeter's lab. Follow the direction for operation of the Freezemobile on the machine itself and let the tissue dry down. A gram will dry down overnight; 10 grams might take a day or two. Once the tissue has been freeze-dried it should be quite stable as long as it is kept dry. For long-term storage put it into a seal a meal type bag, heat seal the bag and store it at –20°C.

DNA Extraction:

Grind the tissue in a mortar and pestle. Continue to grind until it is a very fine powder. This takes a fair amount of effort but it is worth it because the finer the grind, the greater the DNA yields.

Transfer the tissue to an appropriate size tube:

Appropriate Tube	Beginning Fresh Weight	Estimated Dry Weight	Buffer Vol
250ml bottle	~20g	~2g	~100ml
50ml Oak Ridge tube	~5g	~0.5g	~25ml
1.7ml microfuge tube	~1g	~0.1g	

If multiple samples are to be processed, place the container with ground tissue into the –20°C freezer until all samples have been ground up.

Add a volume of EEB about 5X to that of the fresh weight (fw) of the tissue; i.e., if you started with 20g of fresh tissue (~2g dw), add 100ml EEB to the powder. You can probably add less EEB initially and then add more buffer if the tissue does not disperse well (next step).

Gently mix the tissue and EEB until all the tissue is evenly dispersed. Add more EEB if the tissue starts to feel like concrete. Realize that once EEB is added to the ground tissue and from this point on, the DNA is susceptible to shear forces; i.e., the rougher you treat the DNA the more sheared and smaller it will become, therefore be gentle.

Incubate at 60°C (up to 70°C works fine too) for 20 to 30 minutes. (A little longer is okay too.)

Add a volume of chloroform approximately equal to ½ of the volume of EEB.

Very gently mix the chloroform and the aqueous phases by gently rocking or inverting the tube until a complete emulsion is achieved.

Spin for 10 minutes at max speed in a microfuge. If using either the 250ml bottles and the Sorval SLA1500 rotor or the Oak Ridge Tubes and the SS34 rotor, spin for 10 minutes at 9,000RPM (the 250ml centrifuge bottles will collapse at higher speed although if left they will eventually return to their original shape).

Remove most of the lower organic layer (using a P1000 or 25ml pipette depending on the container and volume) and dispose of it into the organic liquid waste container. Recentrifuge the bottle/tube for another 5 minutes to pellet any debris that mixed with the aqueous phase during removal of the organic phase.

Transfer the resulting upper aqueous layer to another container by either decanting it or by pipetting it using a P1000 for mini preps or a 25ml glass pipette for large volumes. Most likely there is a small volume of chloroform remaining in the one tube, avoid transferring this over to the new fresh container.

Add two volumes of 1% CTAB approximately equal to the volume of the aqueous phase removed. Mix by gently inverting the tube several times.
Note: The two volumes of 1% CTAB was a modification made subsequent to the 07-30-02 protocol and is critical for obtaining a CTAB-DNA pellet.

Incubate at RT for about 30 minutes. (Note however that you should see a white precipitate form immediately after adding the CTAB to the DNA solution. Thus it is probably safe to immediately spin rather than waiting 30min.)

Spin at 9000RPM in the Sorval for 5 minutes. The recommended g-force is 2000-5000 x g for 5 minutes. Note that this 9000RPM is much higher than the recommended 2000g.

Look for the DNA-CTAB pellet at the bottom of the tube. It should be opaque and real viscous looking. Very gently tip the tube and decant the supernatant off into the waste. The DNA-CTAB pellet should stay attached to the side of the tube; however it does occasionally come loose so you need to pay attention. If you are afraid of loosing the DNA-CTAB pellet, decant it into another tube so that you can later recover it.

To the CTAB DNA pellet, add a volume of $T_{50}N_{700}E_2$ that will still leave room for ~2 volumes of ethanol; i.e., if you are doing mini preps in microfuge tubes, resuspend in 400µL; for 250ml bottles resuspend in ~50ml; for Oak Ridge Tubes resuspend in about 10ml.

Resuspend the DNA by incubating the sample at 65°C. Periodically invert the tube and gently mix until the DNA is completely in solution. This will take about 1 to 2 hours. (It is also okay to leave the tissue to resuspend overnight at 4°C although it may not entirely go into solution until incubated at 65°C.) If the DNA seems difficult to redissolve, add about 1/10 volume of 5M NaCl (the excess salt helps to dissolve the DNA) and/or some additional $T_{50}N_{700}E_2$.

Add two volumes of RT Ethanol. Invert gently to mix.

Spin for 5 minutes at max speed in the microfuge or 9,000 RPM in the Sorvall Superspeed. Room temperature spins are fine; 4°C is okay too.

Decant off the supernatant. Pay attention to the pellet on the bottom of the tube. If it comes loose and slides off of the side of the tube, don't pour it off. (Similarly as with the DNA-CTAB precipitation, decant the ethanol into another tube so that you can later recover it if you are afraid of loosing the DNA pellet.) However even if the DNA comes loose, if you take care when decanting off the supernatant usually you can still keep the pellet in the tube.

Add 70% ethanol (~1ml for a microfuge tube, ~25ml for an Oak Ridge tube and ~200ml for a 250ml bottle). Invert gently to mix. Try to rinse the entire DNA pellet. Don't worry about the pellet coming loose now, because you will spin the tube a second time.

Spin for 2-5 minutes at RT at max speed in the microfuge or 15,000 RPM in the Sorvall Superspeed to insure the DNA pellet is stuck to the side of the tube/bottle.

Decant off the supernatant as before.

Give the tube one additional quick spin to pull all the liquid down to the bottom of the tube. Pipette this excess liquid off.

Allow the DNA pellet to air dry. If most of the Ethanol has been removed with a pipette then 1h to 90 minutes should be more than enough time to air-dry the sample. Leaving it overnight on your bench with the cap off is also fine. Just place something over the tubes(s) so that dust and other crap do not make their way in to your DNA.

Resuspend the DNA in $T_{10}E_{0.1}$. What volume depends on the amount of starting material. A rough estimate would be ~200µl and ~3ml for 1g and 25g beginning fresh weight of plant material, respectively. The easiest way is to start with a small volume, heat to aid resuspension and then add more $T_{10}E_{0.1}$. For example if the beginning fresh weight was 25g: (1) add 2ml of $T_{10}E_{0.1}$, (2) incubate at 65^{o}C for one to two hours with intermittent gentle swirling until the DNA appears completely resuspended, (3) quick spin the bottle/tube to bring all liquid down to the bottom of the bottle/tube, (4) transfer these 2 ml to a sterile 15ml conical tube, (5) add a second 1ml of $T_{10}E_{0.1}$ to the original bottle to remove as much of the remaining DNA as possible, (6) add this to the 2ml already collected in the sterile conical tube, (7) mix these by gentle swirling, (8) dispense this DNA into small convenient volumes; e.g., 1-1.5ml in a microfuge tube, and (9) store at –20^{o}C.

Check the quality and quantity of the DNA by running a small volume 1-10µl on a low percentage (~0.4%) agarose TEA gel overnight at very low volts (~20-30).

Sol'ns and stuff:

1. EEB

	Stock Sol'n	100ml	Final [ ]
(a)	1.0M Tris (~8.5- 9.5)	20.0ml	200mM
(b)	0.5M EDTA	0.1ml	0.5mM
(c)	5.0M NaCl	14.0ml	700mM
(d)	5% CTAB	25.0ml	1.25%
(e)	1M Sorbitol	12.5ml	125mM
(f)	2-Mercaptoethanol*	2.0ml	2.0%
(g)	dH_2O		

2. Chloroform
3. 1% CTAB
   Filtering the CTAB is probably not necessary but if you choose to do be sure to heat it first otherwise the CTAB will not pass through the filter membrane.)
4. $T_{50(pH\ 8)}N_{700}E_2$
5. EtOH
6. $T_{10(pH\ 7.4)}E_{0.1}$

Notes:

*Add the mercaptoethanol (2-ME) to the EEB immediately before using it. Once 2-ME has been added, the EEB should be stored at $+4^oC$ (a few days to a week) or -20^oC (for longer periods).

This protocol works well at quite different volumes. When using ~1g tissue (fw) (about that much which can be jammed into a microfuge tube, this procedure is basically like a mini prep in that can be completely carried out in a microfuge tube.

Young tissue gives the best yields.

Lyophilization is not required but simplifies things and increases the yield.

Grinding can be done right in the microfuge tube. Simply prepare pestles by melting the end of a blue pipette tip in a flame, then sealing the end in a microfuge tube. Alternatively use the drill setup in Esther van der Knaap's lab.

The DNA forms a salt with CTAB and precipitates when the sodium concentration is less than 0.7M. Often times some people have problems getting a pellet at this step (decant the s/n into another m/f tube to be sure the DNA does not get lost here). When the NaCl concentration is > 0.7M the DNA will form a salt with Na^+.

Original Protocol Ref:

Murray and Thompson (1980) Rapid isolation of high molecular weight plant DNA. Nuc. Acids Res. 8:4321-4325.

My modifications:

Stockinger et al (1996) A linkage map of sweet cherry based on RAPD analysis of a microspore-derived callus culture population. J. Heredity 87:214-218.