DNA extraction Protocol for minipreps

This protocol is modified from Edwards, K., C. Johnstone, and C. Thompson. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nuc. Acids Res. 19(6):1349.

Things to do before you begin:

1. Prepare CTAB
2. Prepare $T_1E_{0.1}$ buffer
3. Get liquid nitrogen
4. Autoclave Eppendorf tubes
5. Turn on water bath and set the temperature at 65EC
6. Autoclave 1 ml pipettor tips that have the first few cms cut off

Procedure:

1. Pinch-out 1-5 discs of leaf tissue with the lid of a sterile, 1.5 ml Eppendorf tube and place directly in ice.

2. Dip an open tube into liquid nitrogen keeping the lip (edge) of the tube above the liquid nitrogen. Hold the tube at the hinge with forceps for a better grip. Allow plant material to freeze.
   $ If the plant material doesn=t freeze well then carefully tilt the edge of the tube enough to allow a little bit of liquid nitrogen to spill into the tube. You don=t want to lose any of your material or contaminate the liquid nitrogen so be very careful with this step.

3. With a blue plastic pestle grind the frozen tissue in the tube and then put on ice until all of the samples are completed.
   $ At this point you can store the tissue in the -80E C freezer if you will not be extracting DNA in the same day.
   $ Be sure to rinse the pestle with 70% EtOH and water and to dry it between samples.

4. For one sample you need 4 µl of β-mercaptoethanol and 396 µl of CTAB. This proportion yields 400 µl of CTAB extraction buffer for each sample. Multiply these volumes by the number of samples that you need to extract that day and mix them in a sterile tube. This is your CTAB extraction buffer.
   $ This proportion is equal to 1% β-mercaptoethanol : 99% CTAB

5. Add 400 µl of the CTAB extraction buffer to a tube that contains your frozen macerated tissue.

6. Using blue pestle macerate until it becomes homogeneous.

7. Incubate at 65E C water bath for 20 minutes.
   - $ Invert tubes twice while in the water bath.
   - $ Tubes no longer need to be kept on ice.

8. For one sample you need 16 µl isoamyl alcohol and 384 µl chloroform. Add 400µl of Chloroform:isoamyl alcohol (this is at a 24:1 ratio respectively) to each tube. Mix on the orbital shaker for 15 minutes.
   - $ Be certain to tape the tubes down to the shaker well!

9. Centrifuge at 13,000 rpm for 5 minutes.
   - $ While waiting, label a new clean Eppendorf tube for every sample and put 400 µl of cold (-20 EC) isopropanol into each.

10. When the centrifuge is done transfer the top (aqueous) layer using a autoclaved blue pipett tip that has had the end cut off to the new labeled Eppendorf tube containing 400 µl. of cold isopropanol. When all of the samples have been completed invert them gently 4 times and let them stand at room temperature for 5 minutes.
    - $ At this point you should be able to see a clear or white cloud like precipitate appear in the tube. This is the DNA that has precipitated.
    - $ Instead of letting the tubes stand at room temperature you could put them in the -20E C freezer for one hour.

11. Centrifuge the tubes at 13,000 rpm for 5 minutes.

12. Discard the supernatant carefully. Leave tubes inverted to dry the pellet. This usually takes about 10-15 minutes.
    - $ The pellet may also be dried by using a ASpin-Vac@ or another sort of vacuum chamber if available.

13. Resuspend pellet in 100 µl of $T_1E_{0.1}$ buffer, then add 4 µl of RNAase (DNase free from Boehringer mannhein). Tap the tube gently to completely dissipate the RNase (Note: RNase comes in glycerol so it will sink directly to the bottom of your tube. You can see the RNase if you hold the tube up to the light). Leave at room temperature for 15 minutes.

14. Add 500 µl 100% cold EtOH (from the freezer) to each tube. Invert gently and leave tubes at room temperature for 5 to 10 minutes.
    - $ Instead of letting tubes sit at room temperature you could put them in the -20E C freezer for one hour or overnight.

15. Centrifuge the tubes at 13,000 rpm for 5 minutes.

16. Discard the supernatant carefully. Leave tubes inverted to dry the pellet (about 10 minutes).

17. Resuspend the pellet in 100 µl of $T_1E_{0.1}$.

18. Freeze samples at -20E C.

Note: When handling Chloroform use nitrile gloves.

Solutions:

CTAB Extraction Buffer:

Reagent	Amount for 300ml	Amount for 100ml	Final Concentration
CTAB	6.0g	2.0g	2.0%
NaCl (5M)	84.0ml	28.0ml	1.4M
EDTA (0.5M) pH 8.0	12.0ml	4.0ml	0.2M
TRIS-HCL (1M) pH 8.0	30.0ml	10.0ml	0.1M
Water	121.0ml	40.3ml	

Chloroform: Isoamyl alcohol (24:1):

Reagent	Amount for 250ml	Ratio
Chloroform	240ml	24
Isoamyl	10ml	1

$T_{10}E_1$ Buffer pH 8.0:

Reagent	Amount for 500ml	Final Concentration
TRIS-HCL (1M) pH 8.0	5ml	10mM
EDTA (0.5M) pH 8.0	1ml	1mM
Water	494ml	