DNA Extraction from Dried Plant Tissues Using 96-well Format (CTAB Method)

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[Abstract] This high throughput DNA isolation protocol is used to extract DNA of high quality from plant tissues for various genetics studies, like genotyping, and mapping etc. This protocol uses the well-established CTAB extraction procedure, and has been adapted to be used with 96-well plates.

Materials and Reagents

1. Hexadecyltrimethyl Ammonium Bromide (CTAB) (Thermo Fisher Scientific)
2. Sodium bisulfite
3. Tungsten carbide beads
4. Sodium chloride
5. EDTA
6. Tris-HCl (pH 8.0)
7. β-mercapto-ethanol (BME)
8. Chloroform
9. Octanol
10. Isopropanol
11. Ethanol
12. Sodium-acetate
13. Ammonium-acetate
14. TE (pH 8.0)
15. 100 ml of CTAB (see Recipes)

Equipment

1. Centrifuges (Eppendorf)
2. Mixer mill
3. Glass beaker
4. Water bath
5. 96-square well blocks

Procedure

A. Tissue grounding:

Before this step, the plant tissue should have been dried (either air-dried or vacuum dried).

1. Add tungsten carbide beads to freeze-dried samples. These will grind the sample.
2. Grind leaf samples four times in the mixer mill, reversing orientations of the trays and switching shaker arms between grinds. Make sure the sample is ground into a fine powder (this influences yield).
3. Add 350 µl of CTAB (don't forget the β-mercaptoethanol). Grind the sample again in the mixer mill.
4. Wrap the boxes with tape and incubate in the 60 °C water bath for 30 min, shaking them gently every 10 min (be sure the caps on the tubes are secure before shaking-the pressure from heating the tubes can pop them off).
5. Sit the tubes on the bench for 10 min to allow them to return to room temperature.
6. Spin the samples in the tabletop centrifuge for a few seconds to get the leaf tissue off the lid.

B. Phase separation:

7. Add 350 µl of chloroform: octanol (24:1) to the tubes (use a 1 L glass beaker for the chloroform: octanol). Shake the tubes continuously for 5 min under the fume hood.
   * Use new caps during this step.
8. Spin the samples in the tabletop centrifuge for twenty minutes at 3,250 rpm.
9. Add 200 µl of chloroform: octanol (24:1) to a new set of tubes and label the tubes.
10. Remove the upper (aqueous) phase to the new tubes. Try to get about 200 µl of fluid, but less is okay.
11. Shake the tubes continuously for 5 min under the fume hood.
12. Spin in the tabletop centrifuge for 20 min at 3,250 rpm. The upper (aqueous) phase will be used in the following steps.

C. DNA precipitation:

1. Add 150 µl of -20 °C isopropanol to a set of 96-square well blocks (deep well, V-bottom).
2. Collect the upper (aqueous) phase from tubes after step 6 in the phase separation section to the square blocks. Try to get 12-150 µl of fluid, but less is okay. Gently mix the solution by swirling the trays.
3. To increase the yield, let the DNA precipitate overnight at -20 °C.
4. Set the tabletop centrifuge to 4 °C. Once it has cooled down, spin the DNA samples in the tabletop centrifuge for 15 min at 3,250 rpm.

5. Pour out isopropanol into sink and very gently tap out trays over a paper towel.

D. DNA wash:

1. Add 500 µl of 76% ethanol/0.2 M sodium-acetate. Let the samples sit in this solution for 20 min.
2. Spin in the tabletop centrifuge for ten minutes at 3,250 rpm.
3. Pour out the 76% ethanol/0.2 M sodium-acetate and very gently tap out the trays over a paper towel.
4. Add 250 µl of 76% ethanol/10 mM ammonium-acetate. Let the samples sit for 2 min.
5. Spin in the tabletop centrifuge for 10 min at 3.250 rpm.
6. Pour out the 76% ethanol/10 mM ammonium-acetate and very gently tap out the trays over a paper towel.
7. Air dry on the bench for 10-15 min or until dry.
8. Add TE (pH 8.0) to the trays. Place in the cold room at 4 °C overnight to let DNA re-suspend.
   a. 50 µl TE for corn (40x concentration)
   b. 200 µl TE for *Arabidopsis* (working concentration)
9. Store the trays at -20 °C for future use.

Recipes

1. 100 ml of CTAB
   2 g CTAB
   1 g sodium bisulfite
   28 ml 5 M sodium chloride
   4 ml 0.5 M EDTA
   10 ml 1.0 M Tris-HCl (pH 8.0)
   1.0 ml BME right at time of use

References

1. Saghai-Maroof, M. A., Soliman, K. M., Jorgensen, R. A. and Allard, R. W. (1984). Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. *Proc Natl Acad Sci U S A* 81(24): 8014-8018.

