Protocol for:

Capture and Purification of Plant Genomic DNA on a Readily-available Cellulose Matrix

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REAGENTS

Grade 238 chromatography paper (VWR International, Radnor, PA, USA)

Resealable plastic bags (available at grocery stores)

Test tube peg rack (ThermoFisher Scientific, Waltham, MA, USA)

Parchment paper (available at grocery stores)

Ceramic pestle

Self-healing rotary cutting mat (https://amazon.com)

1.5 mm micro punch (https://www.tedpella.com/histo_html/miltex-plunger-punch.htm) or razor blades

Microfuge tubes

Paper Washing Buffer (400 mM Tris-Cl, pH 8.0, 5% sodium dodecyl sulfate; 25 mM EDTA, pH 8.0)

TENT buffer (10 mM Tris-Cl, pH 8.0; 1 mM EDTA, pH 8.0; 12 mM NaCl; 2.5% Triton X-100)

$TE_{0.1}$ buffer (10 mM Tris-Cl, pH 8.0, 0.1 mM EDTA, pH 8.0)

PROCEDURE

PREPARATION OF CELLULOSE MATRIX

⇨ Wear gloves to prevent contamination by nucleases.

1. Cut chromatography paper to desired size (we use 7 cm x 13 cm).
2. Place paper pieces in a glass or plastic tray and cover completely with Paper Washing Buffer.
3. Soak paper pieces for 2 h with gentle agitation. Repeat soak if initial solution is discolored.
4. Separate paper pieces and dry overnight.

    * We dry the paper by arranging pieces vertically in a test tube peg rack in a laminar flow hood.

✋ Store dried paper in a resealable bag prior to use. Treated paper can be stored for at least 6 months.

IMPRINTING PLANT TISSUE ON TREATED CELLULOSE MATRIX

⇨ Wear gloves to prevent contamination by nucleases.

5. Place treated chromatography paper between two pieces of parchment paper slightly larger than the treated paper and staple together at one end.
6. Peel back parchment paper and place plant tissue (at least 0.25 cm^2) onto treated chromatography paper, then replace the parchment.

    ⇨ Different samples can be imprinted on the same paper but should be placed at least 1 cm apart to prevent cross-contamination of samples.

7. With moderate to firm pressure, rub the parchment with a flat, blunt object (for example: the flat end of the ceramic pestle) to disrupt the tissue and release cell contents into the chromatography paper. (There is no need to remove leaf fragments that remain on the paper as these will be removed during wash steps later.)

    * Liquid from the tissue should penetrate through the paper and be visible on the reverse side. If tissue does not contain sufficient moisture to penetrate, a second leaf can be imprinted simultaneously on the reverse side of the paper.

8. Dry paper completely (at least 1 hr at room temperature).

 Store papers in dark to prevent sample degradation. Plant tissue samples on treated chromatography paper may be stable for at least 4 months but this should be determined empirically for each species. (Samples imprinted on either untreated chromatography paper or filter paper yielded PCR products after 1 month of storage at room temperature.)

PURIFICATION OF DNA ON SINGLE PAPER DISKS

9. Peel back parchment paper and place cutting mat under chromatography paper imprinted with plant tissue.
10. Use micro punch to extract paper disks or a razor blade to cut the paper into other sizes or shapes.

    * When changing to a different tissue sample, clean micro punch by extracting paper disks from unused (untreated) chromatography paper 3-4 times; this removes trace cellulose fibers carrying DNA. Clean razor blade by running cutting edge through unused (untreated) chromatography paper. As desired, metal tools can be cleaned with full-strength bleach, rinsed with water and dried completely. Clean cutting mat by

washing with soap and water and scrubbing with a brush to remove trace cellulose fibers.

11. Dispense one disk from micro punch to a labeled 0.65-mL microfuge tube; if using razor blade, transfer disk with forceps.
12. Add 200 µL TENT buffer and incubate 5 min with gentle agitation.
13. Remove buffer by pipetting or vacuum aspiration and repeat step 12.

* Aspiration is more effective than pipetting at removing liquid and thus shortens drying time. Aspirator tip should be changed between samples. We use pipette tips on a vacuum hose.

14. Add 200 µL $TE_{0.1}$ or isopropanol and incubate 5 min with gentle agitation. If disks are to be used immediately and not stored at -20°C, isopropanol is suitable for the final two washes; however, DNA is more stable for long-term storage if the final washes are $TE_{0.1}$.
15. Remove buffer by pipetting or vacuum aspiration and repeat step 14.
16. Dry disks completely in a vacuum centrifuge at 45°C (usually10-20 min) or at room temperature (1 h).

* Incubation time can be reduced to 2 min in vacuum centrifuge when isopropanol is used for the last two washes.

Use immediately by adding PCR reagents or store dry at -20°C for up to 1 month.

BATCH PURIFICATION OF DISKS

The single disk purification can be scaled up to wash 15 disks in a 1.7-mL microfuge tube using a minimum of 100 µL of each solution per disk. Transfer individual disks to fresh tubes using clean forceps.

RECIPES

PCR Master Mix 1

50 mM KCl, 10 mM Tris-Cl (pH 9.0), 0.1% Triton X-100, 3 mM $MgCl_2$, 0.2 mM dNTPs (Sigma-Aldrich Corporation, St. Louis, MO), *Taq* DNA polymerase (homemade), 0.2 mM primer 1 (5´-GTA CAA TAG ATA GAT TAG AGG), 0.2 mM primer 2 (5´-AGT TCA TAG CCA GCA ACC)

Thermal cycling profile: Initial denaturation at 95°C for 2 min; 35 cycles of 95°C for 30 sec, 50°C for 30 sec, 72°C for 45 sec; final extension at 72°C for 6 min.

Primers amplify the A1 subunit of *Arabidopsis thaliana* protein phosphatase 2A (At1g25490)

PCR Master Mix 2

50 mM KCl, 10 mM Tris-Cl (pH 9.0), 0.1% Triton X-100, 3 mM $MgCl_2$, 0.2 mM dNTPs (Sigma-Aldrich Corporation, St. Louis, MO), *Taq* DNA polymerase (homemade), 0.2 mM Primer 1 (5´-GAB TAT GTT GTT GAR TCT TCW GG), 0.2 mM Primer 2 (5´ CAA TAA GTT GTC RTA CCA NG)

Thermal cycling profile: Initial denaturation at 95°C 2 min; 40 cycles of 95°C for 30 sec, 52°C for 30 sec, 72°C for 2 min; final extension at 72°C for 6 min.

Primers amplify glyceraldehyde 3-phosphate dehydrogenase family genes.

PCR Master Mix 3

50 mM KCl, 10 mM Tris-Cl (pH 9.0), 0.1% Triton X-100, 3 mM $MgCl_2$, 0.2 mM dNTPs (Sigma-Aldrich Corporation, St. Louis, MO), *Taq* DNA polymerase (homemade), 0.2 mM Primer 1 (5´-CT ACT GGT GTC TTC ACT GAC AA), 0.2 mM Primer 2 (5´ CAG CCT TGG CGT CAA AAA TGC T)

Thermal cycling profile: Initial denaturation at 95°C 2 min; 40 cycles of 95°C for 30 sec, 46°C for 30 sec, 72°C for 2 min; final extension at 72°C for 6 min.

Primers amplify GAPC-type glyceraldehyde 3-phosphate dehydrogenase genes.