One-Tube Preparation and PCR Amplification of DNA From Plant Leaf Tissue with Extract-N-Amp™

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Introduction

The use of PCR amplification to detect target DNA sequences has many applications in plant genotyping, gene mapping, diagnostics, and diversity assessment. PCR itself is simple to set up, and requires little hands-on time. However, most methods for preparing DNA from plant tissues are time consuming, tedious, and labor intensive. Virtually all require mechanical disruption, such as grinding in liquid nitrogen[1] or reciprocal shaking[2], to break the plant cell wall. Furthermore, many use multiple extraction steps with organic solvents (phenol, chloroform), detergents (CTAB, SDS)[1], salts (NaCl, ammonium acetate),[3] and/or polyvinylpyrrollidone (PVP)[3] to remove polysaccharides and polyphenolic components that can inhibit enzymatic reactions. Clearly, none of these methods is amenable to rapid analysis with more than a few test samples.

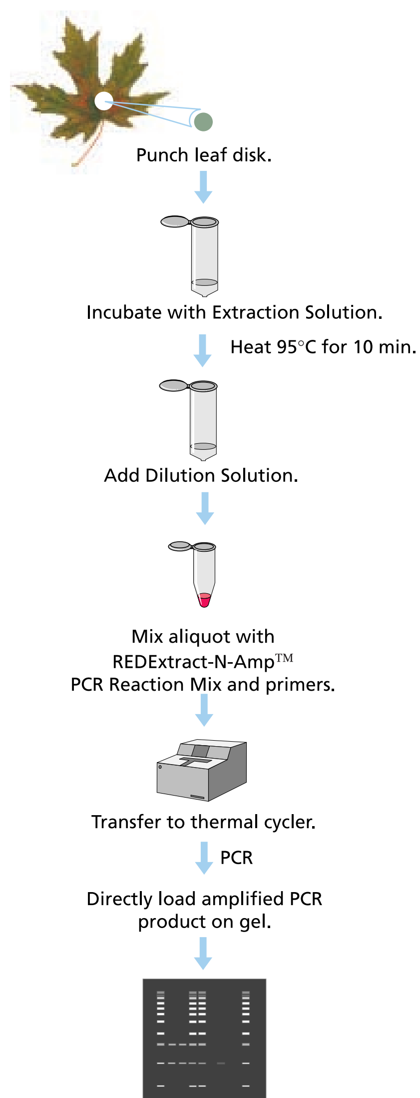

Figure 1. Overview of the REDExtract-N-Amp Plant PCR procedure

We have developed Extract-N-Amp, a one-tube extraction and PCR amplification system, for DNA from plant leaves. Briefly, a 0.5 to 0.7 cm disk of leaf tissue, cut with a standard paper punch, is incubated in Extraction Solution at 95 °C for 10 minutes (Figure 1). After an equal volume of Dilution Solution is added to the extract to neutralize inhibitory substances, the extract is ready for PCR. An aliquot of the diluted extract is then combined with Extract-N-Amp PCR Mix and user-provided PCR primers to amplify target DNA. The Extract-N-Amp PCR Mixes are optimized specifically for use with the extraction reagents, and contain JumpStart™ antibody for Hot Start PCR to enhance amplification specificity. The REDExtract-N-Amp™ PCR Mix (XNA-P) contains REDTaq™ to allow direct loading of the amplification product on an agarose gel without separate addition of loading buffers and dyes. The Extract-N-Amp™ PCR Mix in the XNA-P2 and XNA-R kits lacks the red dye for use with non-gel detection methods in which the red dye interferes.

Materials and Methods

All materials were obtained from Sigma-Aldrich Corporation (St. Louis, MO) unless otherwise noted. Leaf tissue and PCR primer sequences were graciously provided by Monsanto (St. Louis, MO). PCR primers were obtained from Sigma-Genosys (The Woodlands, TX).

DNA extraction

Leaves were frozen at -70 °C, transported on dry ice, and stored at -70 °C. For use, leaves were thawed slightly, and kept on ice. Duplicate 0.5-0.7 cm disks were cut from each leaf with a standard one-hole paper punch and transferred with forceps to a 2-ml microcentrifuge tube containing 100 µl of Extraction Solution. Paper punch and forceps were rinsed with 70% ethanol between samples. Tubes were vortexed briefly to cover the leaf disk with Extraction Solution, then incubated at 95 °C in a dry heating block for 10 minutes. After the incubation, 100 µl of Dilution Solution was added and the mixture vortexed briefly. Further dilutions of the extract (1:5 and 1:10) were made with a 50:50 mixture of the Extraction and Dilution Solutions. Four microliters of diluted extract was added to PCR, and the remainder stored at 4 °C (without removing the leaf disk).

PCR amplification

Each PCR contained 10 µl of REDExtract-N-Amp PCR mix,[4] both forward and reverse primers at 0.4 µM, and 4 µl of diluted leaf extract in a final volume of 20 µl. Reactions were assembled at room temperature (22-25 °C). PCR conditions were: 94 °C for 3 minutes, then 35 cycles of 94 °C for 30 seconds, optimum annealing temperature (45, 48, 53, or 58 °C) for 30 seconds, and 72 °C for 1.5 minutes. The final cycle was followed by incubation at 72 °C for 10 minutes. Eight microliters of each PCR product were loaded directly onto a 1.5% agarose gel for electrophoresis.

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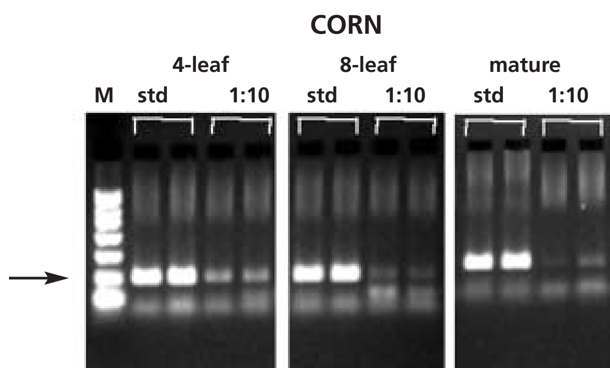

*Figure 2. **Extract-N-Amp performance with immature vs. mature leaves.** Leaves were harvested from corn seedlings having 4 or 8 leaves, or from fully mature corn plants. Duplicate samples were taken from each leaf with a single-hole paper punch, and processed as illustrated in Fig. 1 and described in Materials & Methods. The PCR product (arrow; ~143 bp) is shown on a 1.5% agarose gel stained with ethidium bromide. Std, standard procedure; 1:10, extract diluted 10-fold with a 50:50 mixture of Extraction and Dilution Solutions before PCR. Molecular weight markers(M) are 50, 150, 300, 500, 750, 1000, 1500, and 2000 bp (Product Code: P 9577).*

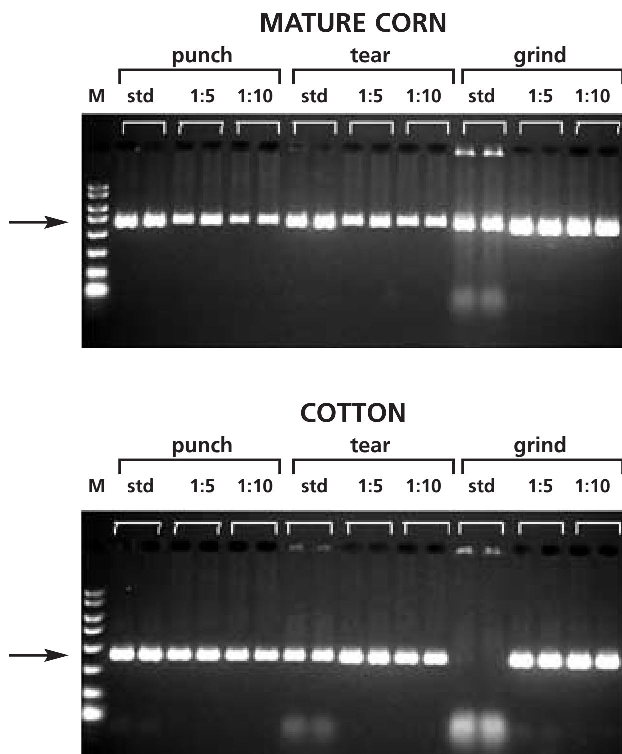

*Figure 3. **Extract-N-Amp results with leaves processed by punching, tearing, or grinding.** Samples of leaves from mature corn or cotton plants were obtained by cutting with a one-hole paper punch (punch), by tearing a piece approximately the same size as the punch (tear), or by grinding in liquid nitrogen with a mortar & pestle (grind). For the latter, a sample approximately the same weight as a punch was used (~5 mg). These samples were processed as illustrated in Fig. 1 and described in Materials & Methods. Dilutions, gel analysis, and molecular weight markers (M) are as in Fig. 2. The PCR product (arrow) is ~ 645 bp for corn and ~400 bp for cotton.*

Results and Discussion

The Extract-N-Amp kits were tested using leaves from 5 crop species: corn, cotton, soybean, potato (Figures 2-4), and wheat (no data shown). In preliminary experiments, we demonstrated that extracts from corn and wheat leaves could be added directly to PCR, but extracts from potato leaves inhibited PCR unless they were diluted at least 5-fold. Adding an equal volume of the proprietary Dilution Solution to the extracted material relieved PCR inhibition without such extensive dilution (data not shown). Therefore, addition of Dilution Solution to the extract before PCR is part of the standard procedure (Figure 1; lanes labeled "std" on Figures 2-4). Note that more than enough DNA is released from a 0.5-0.7 cm leaf punch for PCR detection with the standard procedure. In fact, PCR from a 1:10 dilution of the extract usually yields enough PCR product for detection on agarose gels (Figures 3 and 4, lanes labeled "1:10").

Extract-N-Amp performs well with both immature and mature leaves. In Figure 2, DNA was extracted from leaves of corn plants at the 4- or 8-leaf stage, and from fully mature corn plants. All gave ample PCR product with the standard procedure (std), and low, but detectable, amounts of PCR product after a 10-fold dilution of the leaf extracts (1:10).

In some situations, it may be inconvenient to prepare leaf samples by cutting disks with a non-disposable punch that must be cleaned between samples. As an alternative, pieces of tissue similar in size to the punches (0.5-0.7 cm in diameter) were torn from corn and cotton leaves (Figure 3, "tear"). For comparison, pieces of the same leaf were cut with the hole punch ("punch") or crushed by grinding in liquid nitrogen ("grind"). All were extracted and target DNA sequence was detected by PCR according to the standard procedure, or after further 5-fold or 10-fold dilutions. Both the torn and punched leaf tissue gave plenty of target DNA, detected even after 1:10 dilutions. In contrast, ground cotton leaf extracts gave no PCR product unless they were diluted, indicating that these extracts inhibit PCR. Concurrent tests with soybean leaves gave results similar to corn (data not shown). Therefore, either torn or punched pieces of leaf tissue, 0.5-0.7 cm in diameter, may be used with Extract-N-Amp.

The DNA extracted from leaves with Extract-N-Amp is quite stable in the Extraction/Dilution Solution mixture at 4 °C. For Figure 4, leaf extracts were stored at 2-8 °C for nine (cotton and soybean) or ten months (potato), and then analyzed by PCR. All contained DNA that was readily detected by PCR, even after 1:10 dilutions.

In addition to the 5 crop species used at Sigma to test the kit, other researchers have shown that Extract-N-Amp performs well with a variety of plant species. Figure 5 shows successful extraction and PCR amplification with leaves from Virginia creeper, Honeysuckle, Cottoneaster, *Ficus benjamina*, and *Cannabis sativa*.

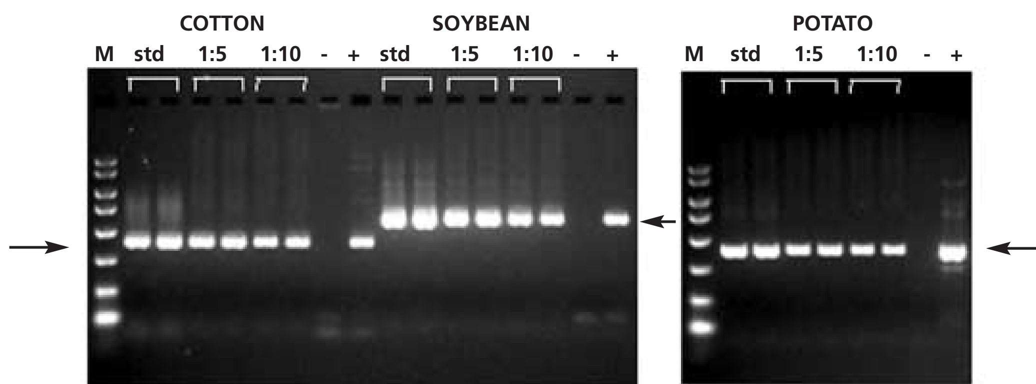

Figure 4. PCR detection of DNA in leaf extracts after storage at 4 °C for 9-months. *Leaves from cotton, soybean, and potato plants were processed as illustrated in Fig. 1 and described in Materials & Methods. The extracts (containing an equal volume of Dilution Solution, but without removing the leaf punch) were stored in a refrigerator at 2-8°C. After 9 months (cotton & soybean) or 10 months (potato), the extracts were diluted as described in Fig 2 and analyzed by PCR. Gel analysis and molecular weight markers are also as in Fig. 2. PCR products are ~ 400, 600, and 400 bp, respectively (arrows).*

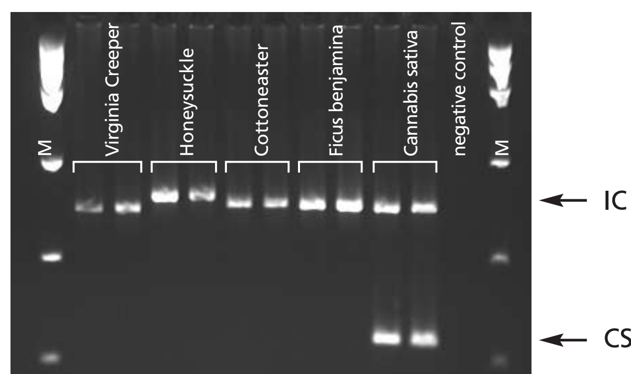

Figure 5. Extract-N-Amp performance with leaves from various plant species. *Leaves from Virginia creeper, Honeysuckle, Cottoneaster, Ficus benjamina, and Cannabis sativa were processed as illustrated in Figure 1. All samples were amplified from 1 µl of extract for 30 cycles, in duplex reactions containing primers specific for plant chloroplast DNA (IC;upper band on gel) and primers specific for Cannabis sativa (CS; lower band on gel). PCR products are shown on an ethidium bromide-stained agarose gel. Molecular weight markers(M) shown are 100, 200, & 400 bp. (Graciously provided by Andy Hopwood, Forensic Science Service, Birmingham England.)*

Conclusions

Extract-N-Amp performs well with mature as well as immature plant leaves. Torn leaf tissue may be used instead of leaf punches, as long as the size is kept at ~ 0.5-0.7 cm. The leaf extracts are stable for at least 9 months at 2-8 °C. Furthermore, leaves from a wide variety of plant species may be used with Extract-N-Amp.

Acknowledgements

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References and Note

1. Ausubel, F., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K., Short Protocols in Molecular Biology, pp. 2.9-2-11 (John Wiley & Sons, Inc., New York, NY, 1997).

2. Geuna, J., Hartings, H., and Scienza, A., Plant DNA extraction based on grinding by reciprocal shaking of dried tissue. Anal. Biochem., **278**, 228-230 (2000).

3. Kim, C.S., Lee, C.H., Shin, J.S., Chung, Y.S., and Hyung, N.I., A simple and rapid method for isolation of high quality genomic DNA from fruit trees and conifers using PVP. Nucleic Acids Res., **25**, 1085-1086 (1997).

4. The Extract-N-Amp PCR Mix is specifically formulated for use with the Extract-N-Amp Extraction Solution. Use of a PCR mixture other than the Extract-N-Amp PCR Mix will likely result in failure of the PCR.

About the Authors

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ORDERING INFORMATION

Product Code	Product Name	Unit	Price
XNA P	REDExtract-N-Amp™ Plant PCR Kit (100 Extractions & Amplifications)	1 Kit	$145.00
XNA P2	Extract-N-Amp™ Plant PCR Kit (100 Extractions & Amplifications)	1 Kit	$140.00
XNA R	Extract-N-Amp™ Reagent Kit (1,000 Extractions & Amplifications)	1 Kit	$1,300.00