A high throughput DNA extraction method from chemotypically heterogeneous plant species

Parthadeb Ghosh (✉ pdggene@rediffmail.com)

Ghosh's Lab, University of Kalyani

Sanjib Kumar Chattopadhyay

Cytogenetics and Plant Breeding Section, Plant Biotechnology Research Unit, Department of Botany, University of Kalyani, Kalyani -741235, West Bengal, India

Sinchan Adhikari

Cytogenetics and Plant Breeding Section, Plant Biotechnology Research Unit, Department of Botany, University of Kalyani, Kalyani -741235, West Bengal, India

Soumen Saha

Cytogenetics and Plant Breeding Section, Plant Biotechnology Research Unit, Department of Botany, University of Kalyani, Kalyani -741235, West Bengal, India

Subhasis Mondal

Cytogenetics and Plant Breeding Section, Plant Biotechnology Research Unit, Department of Botany, University of Kalyani, Kalyani -741235, West Bengal, India

Abdul Kader

Cytogenetics and Plant Breeding Section, Plant Biotechnology Research Unit, Department of Botany, University of Kalyani, Kalyani -741235, West Bengal, India

Soura Tapas Dey

Cytogenetics and Plant Breeding Section, Plant Biotechnology Research Unit, Department of Botany, University of Kalyani, Kalyani -741235, West Bengal, India

Tapas Kumar Bandyopadhyay

Department of Molecular Biology & Biotechnology, University of Kalyani, Kalyani -741235, West Bengal, India.

Chandan Sengupta

Microbiology Laboratory, Department of Botany, University of Kalyani, Kalyani -741235, West Bengal, India

Method Article

Keywords: DNA extraction, phenol-chloroform, polyphenol, polysaccharide, SDS

Posted Date: February 7th, 2013

DOI: <https://doi.org/10.1038/protex.2013.018>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

This method is designed to recover utmost amount of DNA from heterogeneous plant tissues restraining the co-extraction of PCR inhibiting substances. It requires maceration of plant tissue of about 1.0 cm² in DNA extraction buffer [200 mM Tris-HCl (pH 8.0), 200 mM NaCl and 25 mM EDTA, and 1% PVP], followed by cell lysis with 10% SDS. The lysate was treated with Phenol: chloroform: isoamyl alcohol (25:24:1) efficiently removes all chaotropic cellular materials except water soluble polysaccharides which concomitantly excluded by DNA precipitation with ethanol and sodium chloride. It needs about one and half an hour to prepare DNA to perform thousands of PCR-based reactions and other DNA manipulation techniques. This method does not require liquid nitrogen and also bypass RNase treatment. It can be performed even in low technology laboratories.

Introduction

Chemotypic heterogeneity among species may not permit optimal DNA yields from one isolation protocol, and perhaps even closely related species may require different isolation strategies. The problem encountered due to co isolation of highly viscous polysaccharides, degradation of DNA due to endonuclease, inhibitor compounds like polyphenols and other secondary metabolites which directly or indirectly interfere with enzymatic reactions^{1, 2, 3}. Since the DNA extraction method was first reported by Marmur⁴ in 1961 using SDS and chloroform, scientists have been devoting themselves to the improvement and simplification of DNA extraction methods using either SDS or CTAB with specialized apparatus^{5, 6, 7, 8}. However, these procedures may only work for a limited number of tissue or plant species, and most are still multi step procedures. Here we present a simple, quick and low-cost method adaptable to standard laboratory conditions to isolate high-quality DNA from heterogeneous plant materials both in terms of origin and identity.

Reagents

• Sodium Chloride (Merck, Cat. No. 567441) • Tris-base (Merck, Cat. No. 648310) • Ethylene Diamine Tetraacetic Acid (EDTA) (Merck, Cat. No. 324503) • Sodium Dodecyl Sulphate (SDS) (Merck, Cat. No. 428023) • Polyvinylpyrrolidone (PVP) (Merck, Cat. No. 529504) • Phenol (SRL, Cat. No. 1624162) • Chloroform (Merck, Cat. No. 82226505001730) • Isoamyl alcohol (Merck, Cat. No. 6189699200) • Ethanol (Merck, Cat. No. 108543)

Equipment

• Centrifuge • Vortexer • Freezer (-20°C) • Spectrophotometer • Water bath • Micro-centrifuge tube (1.5 ml) • Mortar and pestle

Procedure

1. Turn on a water bath and set temperature at 65°C. 2. Grind plant material with mortar and pestle in the presence of extraction buffer [200 mM Tris-HCL (pH 8.0), 200 mM NaCl and 25 mM EDTA, and 1% PVP]. Transfer the homogenate to 1.5 ml micro-centrifuge tube and vortex for 30 seconds after adding of 10% SDS solution. 3. Incubate the tube in a water bath at 65°C for 20-40 minutes and mix the contents 2-3 times by inverting the tubes. Centrifuge at 17,000 rpm for 10 minutes, which separates the homogenate into two parts. 4. Take the upper aqueous phase into a new tube and mix with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1, v/v/v). Shake the tube well for 5 minutes and centrifuge at 12,000 rpm for 10 minutes. 5. Collect the supernatant into a fresh tube and add 1/5th volume 2 M NaCl and 2 volume pre-chilled ethanol. Keep the tube at -20°C for 10 minutes to precipitate DNA. 6. Spool the DNA and transfer to another tube. Wash the spooled DNA with 70% ethanol (2-3 times) and air dry under laminar flow chamber for 10-15 minutes. 7. Dissolve the dry pellet in nuclease free water or TE buffer (pH 8.0). Store DNA at 4°C or -20°C.

Troubleshooting

Troubleshooting section may help the researchers in solving any problems that may arise (Table 1; Figure 1).

Anticipated Results

The present protocol invariably achieve good yield of high quality of DNA from leaf tissues of plants containing high level of polysaccharides and active metabolites (Table 2; Figure 2). We have also applied the protocol in other recalcitrant plant species giving satisfactory result³. The isolated DNA is amenable to further processing in cloning experiments as well as DNA fingerprinting. Results prove the reproducibility, reliability and practicality of this protocol.

References

1. Jobes, D.V., Hurley, D.L. & Thien, L.B. Plant DNA isolation: a method to efficiently remove polyphenolics, polysaccharides, and RNA. *Taxon*. 44, 379-386 (1995).
2. Dey, T., Saha, S., Dhar T. N., Adhikary, S. & Ghosh, P. Optimization and comparison of efficiency between two DNA isolation protocols in *Cymbopogon* species. *General and Applied Plant Physiology*. 36, 232–238 (2010).
3. Adhikari, S., Chattopadhyay, S.K. & Ghosh, P.D. A simplified high yielding miniprep genomic DNA extraction protocol for three chemotypically different plant species. *Indian J. of Biotech*. 11, 337-340 (2012).
4. Marmur, J.A. procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. Mol. Biol*. 3, 208-218 (1961).
5. Doyle, J.J. & Doyle, J.L. A rapid DNA isolation procedure from small quantities of fresh leaf tissue. *Phytochem. Bull*. 19, 11-15 (1987).
6. Berthomieu, P., Berthomieu, P. & Meyer C. Direct amplification of plant genomic DNA from leaf and root pieces using PCR. *Plant Mol. Biol*. 17, 555–557 (1991).
7. Hill-Ambroz, K.L., Brown-Guedira, G.L. & Fellers, J.P. Modified rapid DNA extraction protocol for high throughput microsatellite analysis in wheat. *Crop Sci*. 42, 2088–2091 (2002).
8. Mogg, R.J. & Bond,

Acknowledgements

The authors thankfully acknowledge University Grants Commission \ (UGC), New Delhi, India for financial support and University of Kalyani for central instrumentation facilities for this research project.

Figures

Figure 1

Checkpoints in DNA extraction

Figure 2

DNA extracted from polysaccharide and polyphenol rich heterogeneous plant species resolved on 1% agarose gel.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplement0.doc](#)
- [supplement0.doc](#)