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A RAPID DNA ISOLATION PROCEDURE FOR SMALL QUANTITIES OF FRESH LEAF TISSUE

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Abstract - Of the several rapid and inexpensive DNA isolation procedures that have been described recently, one of the most popular is that of Saghai-Maroof et al. (1984), a procedure using hexadecyltrimethylammonium bromide (CTAB) and lyophilized tissue. We here describe a simple modification of this procedure that has been found to be efficient for nucleic acid isolations from fresh leaf tissue, and which we have also used for dried herbarium specimens.

Key Work Index - DNA isolation, hexadecyltrimethylammonium bromide, CTAB, molecular systematics

Introduction

Plant DNA isolation methodology has evolved rapidly in the last few years. From cumbersome, messy, and often inefficient large scale phenol procedures, DNA technology moved to more efficient methods involving ultracentrifugation in CsCl density gradients. Still, such procedures are slow and expensive, and not well suited for assaying large number of samples. More recently, a number of "miniprep" procedures have been developed that permit rapid isolation of DNA with a minimum of expense, using small amounts of tissue. There are a number of different protocols available for extraction of DNA from small samples, which is fortunate because it appears that different procedures work best for different plant groups, as might be expected considering the great diversity of plant secondary compounds that in many cases may interfere with a particular method of DNA isolation.

Here we report a method of isolation that has been found to work for a large number of different angiosperm groups, including

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both monocots (Palmae, Orchidaceae, Cyperaceae) and dicots (Rosaceae, Leguminosae, Saxifragaceae, Juglandaceae, Fagaceae, Portulacaceae, Solanaceae). We have even had success with insect larvae! It is a very simple modification of a procedure originally described for barley by Saghai-Maroof et al. (1984), differing principally in that their procedure called for using lyophilized tissue, while we use fresh leaf material, and have compensated for the increased water content by increasing the concentration of the extraction buffer.

This procedure has been found to be effective on fresh tissue, and we have recently used it to extract usable DNA (high molecular weight, digestible with restriction endonucleases) from recently-dried herbarium specimens (Doyle and Dickson, 1987). It appears to be a very versatile procedure both in our hands and elsewhere in the molecular systematics community, where versions of the protocol have been rather widely circulated.

Experimental

1. Grind 0.5-1.5 g of leaf tissue in a mortar and pestle, using 7.5 ml 2x CTAB isolation buffer (2x CTAB isolation buffer; 100mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% hexadecyltrimethylammonium bromide ["CTAB"], 0.2% 2-mercaptoethanol). Pour grindate into a 30 ml Corex tube, rinse mortar with an addition 0.5 ml 2x CTAB buffer, and add to Corex tube. In addition:

A. we preheat our mortars and 2x buffer to 60C before the extraction.

B. for tough tissues, a pinch of sterile sand can be added.

C. it is also possible to use liquid nitrogen powdered tissue.

2. Incubate at 60C for 30 to 60 min with optional gentle swirling -- somewhat longer incubations, or in some cases shorter incubations (as little as 15 min) have been found by other workers to give better yields of high molecular weight DNA. We have recently been routinely using 30 min incubations with good success.

3. Extract once with chloroform-isoamyl alcohol (24:1), mixing gently but thoroughly.

4. Spin in clinical centrifuge to concentrate layers. We use setting 7 on our IEC for 10 min. Generally the aqueous phase will be clear (though often colored) by the time we get done with our spins, but this is not always the case.

5. Pull off aqueous phase with a wide-bore pipet, transer to a clear Corex tube, add 2/3 vol of cold isopropanol, and mix gently to precipitate nucleic acids.

In the best isolations, one gets a beautiful precipitation at this stage -- long strands of nucleic acid that can then be spooled out with a glass hook. Regrettably, this is not always the case. We have seen everything from no apparent precipitation (the aqueous phase actually got clearer!) to the formation of a cloudy precipitate, and in nearly all cases have still gotten good nucleic acids.

6. If possible, spool out nucleic acids with a glass hook, and transfer to 20-25 ml of wash buffer (76% EtOH, 10 mM ammonium acetate) for approximately 20 min.

If this is not possible, several approaches can be followed:

A. (best alternative) Spin in clinical centrifuge at low speed (e.g. setting 3 on IEC) for 1-2 min. Gently pour off as much of the supernatant as possible without losing nucleic acids, which will generally be a diffuse and very loose pellet at the bottom. Add wash buffer to the pellet and gently swirl to resuspend the pellet.

B. If you just don't see much of any precipitation, harder, more prolonged spins are unavoidable -- do whatever it takes, but the less the better as far as purity is concerned. After more than about 3 min at setting 3 on the IEC, the pellet is hard and very difficult to resuspend in wash buffer -- we generally resort to "tearing" it with a glass hook to try to wash it as much as possible. In other cases the precipitate at this point may look flaky, and that, too, can be disconcerting.

Generally, nucleic acids will become much more white when rinsed in the wash buffer.

7. Spin down (or spool out) nucleic acids after around 20 min of leaving in wash buffer (the time of "washing" is not apparently critical).

8. Allow to air dry briefly, then add resuspension buffer (usually 1 ml, but this will depend on the amount of precipitate seen). (Resuspension buffer: 10 mM ammonium acetate, 0.25 mM EDTA; we have also been known to use TE [10 mM Tris-Cl pH 7.4, 1 mM EDTA, pH 8.0]).

A. If you check this preparation on a minigel, in many cases there is not only good quality DNA, but also visible bands of ribosomal RNAs.

B. Saghai-Maroof et al. (1984), from whom this protocol was modified, use DNA at this stage for restriction digests. We have found some instances where DNA at this stage was resistant to endonuclease digestion, so we do some further cleaning.

9. Add RNAse A to a final concentration of 10 ug/ml, incubate 30 min at 37C.

10. Ethanol precipitation of the DNA: dilute sample with 2 volumes of distilled water, add ammonium acetate (7.5 M stock) to a final concentration of 2.5 M, mix, add 2.5 volumes of cold ethanol, and gently mix to precipitate DNA. Spin down DNA at high speed (10,000 xg for 10 min in refrigerated centrifuge, or setting 7 in clinical for 10 min).

A. By this precipitation, the DNA usually looks great, even in preps that didn't look so good initially.

B. In the past, we precipitated directly from the 1 ml volume, and found that the DNA formed a gelatinous complex with the alcohol. Even a hard spin leaves a lot of alcohol in prep, and if you resuspend too soon you still get a great deal of alcohol. Increasing the volume by diluting with water helps a great deal, but it is still necessary to dry the pellet, either under vacuum or, as we often do, by air drying on the benchtop overnight.

11. Air dry and resuspend in TE (or spool out, dab dry on a kimwipe, and resuspend directly).

Results and Discussion

DNA isolated by this method has been of uniformly good quality in terms of size, with the majority in excess of 30 kilobases in length. A260/A280 ratios are a bit low, however--generally anywhere from 1.4-1.7, so there obviously is some impurity. Yields are generally quite high -- apparently up to nearly 1 mg/g fresh wt, which is a bit deceptive because whenever possible we use young, unexpanded leaves that are packed with DNA. The DNA is digestible with virtually all restriction enzymes (we have not found any examples of an enzyme that consistently will not digest DNA prepared this way, though for any particular plant taxon there may be some enzymes that will not work, just as is true for DNA prepared in other ways). It is, of course, possible to clean the DNA further -- additional precipitations, for example or, even phenol extractions or passing the DNA over CsCl gradients. It is also possible to add reagents to the extraction buffer; for example, we have added 1% polyvinylpyrollidine (PVP 40) in one or two cases where we anticipated large amounts of phenolic compounds to be present.

We routinely use DNA prepared in this way for all studies in our laboratory as it is a total DNA preparation that includes both nuclear and organellar components. Using specific recombinant probes, it is therefore possible to detect chloroplast DNA restriction fragments as well as sequences from any nuclear gene by using conventional transfer and hybridization procedures.

Literature Cited

Doyle, J. J. and E. L. Dickson (1987) Taxon (in press).

Saghai-Maroof, M. A., K. M. Soliman, R. A. Jorgensen and R. W. Allard (1984) Proc. Natl. Acad. Sci. USA 81: 8014-8019.

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ANNOUNCEMENTS: BIOLOGICALLY ACTIVE NATURAL PRODUCTS FOR POTENTIAL USE IN AGRICULTURE

The American Chemical Society, Division of Agrochemicals, will be holding a symposium titled "Biologically Active Natural Products for Potential Use in Agriculture", from 4-7 September 1987, in New Orleans, Louisiana. The Symposium starts at 1:55 p.m. on Tuesday and ends at 11:50 a.m. on Friday. The content is tripartite with the first section devoted to "Biologically Active Natural Products from Microorganisms", the second, "Biologically Active Natural Products from Higher Plants", and third, "Biologically Active Natural Products from Insects, and Natural Products that Affect Insects". Some thirty six papers will be presented and it is expected that they will be incorporated into an ACS Symposium Series Book with some findings of Japanese scientists who will be presenting their research at the Natural Products Symposium, sponsored by the Plant Growth Regulator Society of America Meetings, to be held at Waikiki Beach, Hawaii, (2-6 August 1987). The reason for holding the ACS Symposium is because of the renewed interest in natural products by industry, academic and government concerns who believe that secondary metabolites may be of significant use in agriculture for the following reasons. Secondary metabolites generally have high specific activity, a narrow spectrum of activity against target organisms, are biodegradable, and offer novel templates for chemical (synthetic) manipulation to produce agrochemicals. Presentations at the meetings in New Orleans range from discussions about the avermectins, a very successful series of industrial metabolites from Streptomyces avermitilis, to newly isolated secondary metabolites and their preliminary activities in biological systems.

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