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RESEARCH ARTICLE

A rapid and cost effective protocol for plant genomic DNA isolation using regenerated silica columns in combination with CTAB extraction

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Abstract
Isolation of high quality DNA from multiple samples can be both time-consuming and expensive. We have developed a combined protocol to reduce the time component of the hexadecyltrimethylammonium bromide (CTAB) extraction method and reduced costs by regenerating the silica columns used to purify genomic DNA. We present data that shows, by increasing the temperature used during the CTAB method, the time required to extract crude genomic DNA can be reduced. We show that silica columns can be regenerated using HCl and still maintain their DNA-binding capacity. Furthermore, we show both spectrophotometrically, and by restriction enzyme cutting, that the quality of the eluted DNA is high. Critically, using both genomic DNA from pea and perennial ryegrass we demonstrate, using species-specific PCR primers, that there is no carry-over of DNA from repeated use of a single column. The main advantages of the method are high yield, high quality, cost effectiveness and time-saving. This method could satisfy demand when large numbers of plant genomic DNA samples are required, for example from targeting induced local lesions in genomes (TILLING) populations.

Keywords: CTAB, DNA isolation, silica columns

1. Introduction

Isolation of high quality genomic DNA is necessary for many molecular biology applications. For plant genomic DNA isolation the popular method using hexadecyltrimethylammonium bromide (CTAB) is cost-effective with high DNA yield and acceptable DNA quality, but this protocol is time-consuming (Doyle and Doyle 1987; Allen *et al*. 2006). On the other hand, high quality DNA can be isolated rapidly using commercial DNA extraction kits with easy protocols and then the used columns are discarded (Deavours and Dixon 2005; Tesniere *et al*. 2006). However, the application of either the CTAB method or the use of commercial kits can be limited both in terms of time and expense when large numbers of DNA samples are required. As silica matrices are extraordinarily stable over extended time periods under mild acid conditions, it has been suggested that silica columns may be reused after acid treatment to remove any DNA carried-over on the binding matrix (Siddappa *et al*. 2007). The authors reported on the successful use of regenerated columns to purify plasmids. However, there are few attempts to isolate plant genomic DNA using regenerated silica columns. Lemke *et al*. (2011) reported on the regeneration and reuse of Qiagen 'DNA Easy 96 column plates' and columns, but these were regenerated

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using a commercial regeneration kit.

We are interested in detecting cytokinin oxidase/dehydrogenase mutants in perennial ryegrass for yield enhancement (Jameson and Song 2016), but this requires analysing leaf material from multiple single seed lines from numerous cultivars using EcoTILLING (eco-targeting induced local lesions in genomes) (Comai *et al*. 2004; Till *et al*. 2006; Song *et al*. 2015). In this paper, we report a rapid and cost-effective plant genomic DNA isolation protocol to meet the requirements for preparation of large numbers of genomic DNA samples for such applications as genotyping and large-scale mutation screening. In this method, regeneration of commercial silica columns (Qiagen DNeasy Plant Mini Kit, Cat. No. 69104; Qiagen, Hilden, Germany) was assessed. To test their reliability, silica columns regenerated over 10 cycles were used for plant genomic DNA isolation in combination with a modified CTAB method. We assessed retention capacity and quality of eluted DNA, as well as using PCR to test for cross contamination when isolating genomic DNA from two different species.

2. Materials and methods

2.1. Column regeneration

New Qiagen DNA extraction columns (Qiagen DNeasy Plant Mini Kit, Cat. No. 69104) were used to isolate genomic DNA from *Lolium perenne* (perennial ryegrass) following the manufacturer's protocol. The used columns were then washed thoroughly with water to remove any cell debris, and soaked in either 0.5 or 1 mol L^{-1} HCl for either 1, 4, 24, or 48 h. The columns were then rinsed thoroughly with sterile distilled water 3–5 times, and 500 µL equilibration buffer (QBT buffer) were added. The QBT buffer was prepared in-house based on the recipe supplied in the Qiagen Plasmid Purification Handbook and comprised 750 mmol L^{-1} NaCl, 50 mmol L^{-1} MOPS (3-(N-morpholino) propanesulfonic acid) (pH 7.0), 15% (v/v) isopropanol, and 0.15% (v/v) Triton X-100. The columns were spun at 13 000×g for 1 min. The columns were ready for a fresh application and then used for up to 11 rounds of regeneration following the above steps. Two sets of controls were used. These were new columns used to isolate perennial ryegrass genomic DNA, but the columns were either not soaked or soaked in H_2O for 1, 4, 24, or 48 h. Elution buffer was applied to the columns and the eluate collected and treated as if it contained DNA. Eluted DNA was assessed using a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and by gel electrophoresis on 1% (w/v) agarose gel prepared in 35 mL 1×TAE buffer (40 mmol L^{-1} Tris-acetate, 1 mmol L^{-1} EDTA (ethylenediaminetetraacetic acid)) and 2 µL SYBR™ Safe DNA Gel Stain (Invitrogen, USA).

2.2. DNA extraction procedure

Lysis buffer (2% CTAB buffer with 2% PVP40 (polyvinylpyrrolidone-40) (w/v); 2% β-mercaptoethanol (v/v) was added just before use) was pre-heated to 85°C. Up to 100 mg fresh weight (FW) of plant material was ground to a fine powder under liquid nitrogen using a pre-chilled mortar and pestle. Alternatively, when an Omni International Bead Ruptor 24 (Omni International, Kennesaw, Georgia, USA) was used, 100 mg tissue and 3 ceramic beads (2.8 mm Ceramic Bead Media 19-646-3; Omni International) were placed into a 2-mL freestanding microtube and stored in liquid nitrogen. Samples were disrupted using the bead ruptor with speed set at 3.9 m s^{-1} for 20 s. The powdered tissue was then scraped into a 1.7-mL dry Axygen microtube and 400 µL preheated lysis buffer was added, and the mixture was incubated at 85°C for 10 min (this is a modification of the standard CTAB procedure). After 5 min incubation, the tubes were gently shaken for 5 s to disperse the material, and the incubation continued. The sample was spun at 20 000×g for 5 min at room temperature; 300 µL of the supernatant was transferred into a new 1.5-mL tube and mixed with 500 µL of binding buffer (2 mol L^{-1}) guanidine hydrochloride, 75% (v/v) ethanol) and then 700 µL of this mixture was transferred onto new or regenerated columns. The columns were spun at 13 000×g for 1 min at room temperature. The flow-through was discarded. 700 µL of washing buffer I (10 mmol L^{-1} NaCl, 10 mmol L^{-1} Tris-HCl, pH 6.5, 80% (v/v) ethanol) was applied to each column, and the column centrifuged again at 13 000×g for 1 min. This step was repeated once. The columns were then washed with 700 µL of washing buffer II (96% v/v ethanol) and spun at 13 000×g for 1 min. The flow-through was discarded and the column centrifuged again at 13 000×g for 2 min. 50 µL elution buffer (comprising 10 mmol L^{-1} Tris-HCl, pH 8.5, with RNase A (Qiagen Cat. No. 145012547) was added to a final concentration of 10 µg mL^{-1}), was preheated to 65°C, and applied to the column. The columns were spun at 13 000×g for 1 min to elute DNA. This step was repeated once using the elution buffer from the collection tube to obtain a greater DNA concentration. Alternatively, addition of a further 50 µL of elution buffer could be added and the column centrifuged again which would potentially increase yield, but reduce the DNA concentration. The eluted DNA was incubated at 37°C for 1 h to remove any remnants of RNA, and the DNA stored at –20°C.

2.3. Yield and quality determination

In order to determine the yield and quality of DNA obtained

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