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UTILITY OF FTA CARDS FOR THE PRESERVATION OF MARINE GREEN AND RED ALGAL DNA

D. WILSON FRESHWATER and SAMANTHA L. SCHMITT

Center for Marine Science, University of North Carolina Wilmington, Wilmington, NC 28409

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Fast Technology for Analysis of nucleic acids (FTA) cards are a paper-based matrix impregnated with chelators, denaturants and free-radical traps that inhibit enzymes, chemicals and microbes that might degrade DNA or RNA. Originally developed for Phenylketonuria screening in newborns (Guthrie and Susi 1963), they were subsequently used in human medical and forensic science for detecting DNA by the Polymerase Chain Reaction (PCR) (e.g., Devost and Choy 2000; Vanek et al. 2001). Further research has expanded the use of FTA cards to both DNA and RNA based studies of many organisms including plants (e.g., Roy and Nassuth 2005; Tsukaya et al. 2005), but their utility with marine algae has not been tested.

Many different methods for the preservation of marine algal samples and the subsequent extraction of DNA have been published. Whereas DNA extractions from live material work best, it is often not practical to transport living algae back to the lab. Frozen samples are known to work well, however difficulty with transporting frozen material from remote collecting locations prompted the use of silica gel desiccant to quick dry samples in the field (Freshwater and Rueness 1994). Other researchers have found that ethanol preservation of some algal groups works better than quick drying (e.g., Koistra et al. 2002; Lin and Fredericq 2003). Similar to differences in preservation success, the best method for extracting DNA from preserved samples varies in the different algal groups, and consequently a large number of methods and commercial kits have been used (e.g., Mayes et al. 1992; Goff and Moon 1993; Hughey et al. 2001; Curtis et al. 2008). The FTA card system would seem to be an ideal method for both the preservation and later extraction of marine algal DNA, and therefore a series of tests were carried out to determine its utility.

METHODS AND OBSERVATIONS

A variety of marine green and red algae were included in FTA card tests (Table 1). All specimens were collected in New Hanover County, NC, USA from November 2008 to January 2009 or Quintana Roo State, Mexico during December 2008. Live specimens were cleaned of any macrophytic epiphytes and pressed onto FTA PlantSaver Cards (Whatman Inc., Florham Park, NJ, USA) following manufacturer's instructions. The cards were stored at room temperature (22–24°C) in resealable plastic bags containing ca. 5 cm^3 silica gel desiccant to maintain dryness. Small 1.2 mm disks were punched from the sample area of the FTA cards and cleaned prior to their use in PCR. The manufacturer's instructions call for cleaning each disk in a separate tube and then using them as PCR reaction templates within 3 h, or storing at 4°C or −20°C. Whereas the disk cleaning procedure is relatively easy and fast (we have found that at a methodical pace 12 tubes can be processed in approximately 1 h), the number of tubes to be handled can multiply quickly when using this method for large studies or when multiple loci need to be amplified from the same specimen. The disk cleaning step also adds a significant amount of extra preparation time when setting up PCR reactions.

Four disks per sample were simultaneously cleaned in a single tube to test a more practical application of FTA cards. The disk cleaning method was as follows:

1) Four 1.2 mm disks were punched from each sample card and placed in a single 1.5 ml tube.
2) 200 µl of FTA purification reagent was added, and the tubes incubated for 5 min at room temperature with periodic inverting (either on a rocking table or by hand).
3) As much of the FTA purification reagent as possible was removed from the tube and discarded, leaving the disk in the tube.
4) Steps 2 thru 3 were repeated twice for a total of three FTA purification reagent washes.
5) 200 µl of either $TE_{0.1}$ (10 mM Tris, 0.1 mM EDTA) or isopropanol was added and the tubes incubated for 5 mins at room temperature with periodic inverting.
6) As much of the $TE_{0.1}$ or isopropanol as possible was removed from the tube and discarded, leaving the disk in the tube.
7) Steps 5 thru 6 were repeated once for a total of two $TE_{0.1}$ or isopropanol washes.
8) Disks were dried completely by placing tubes in a vacuum centrifuge for 10 min.
9) Using forceps the disks were transferred to individual PCR tubes and stored at −20°C until use.

Table 1. Classification and amplification success of red and green algae used in FTA card trials. Classification follows Wynne (1998).

Species	Division	Order	Family	Amplification
Ulva fasciata	Chlorophyta	Ulvales	Ulvaceae	Yes
Cladophora albida	Chlorophyta	Cladophorales	Cladophoraceae	No
Penicillus sp.	Chlorophyta	Bryopsidales	Udoteaceae	No
Bryopsis sp.	Chlorophyta	Bryopsidales	Bryopsidaceae	Yes
Hypnea musciformis	Rhodophyta	Gigartinales	Hypneaceae	Yes
Gracilaria tikvahiae	Rhodophyta	Gracilariales	Gracilariaceae	Yes
Rhodymenia pseudopalmata	Rhodophyta	Rhodymeniales	Rhodymeniaceae	No[a]
Rhodymenia pseudopalmata 2	Rhodophyta	Rhodymeniales	Rhodymeniaceae	No
Antithamnion cruciatum	Rhodophyta	Ceramiales	Ceramiaceae	Yes
Antithamnionella elegans	Rhodophyta	Ceramiales	Ceramiaceae	Yes
Neosiphonia harveyi	Rhodophyta	Ceramiales	Rhodomelaceae	Yes

[a] One of six amplification trials amplified weakly.

PCR amplification reactions for portions of the plastid-encoded *rbc*L were set up as described in Freshwater et al. (2005), but using GoTaq DNA polymerase (Promega Corporation, Madison, WI, USA) and the thermocycling protocol outlined in Freshwater et al. (2000). The amplification primers for green algae were ''rbc1'' and ''rbc5'' of Hanyuda et al. (2000), and for red algae ''F753'' and ''RrbcSstart'' of Freshwater and Rueness (1994). These primer pairs amplify 682 bp and 715+ bp fragments of *rbc*L respectively.

The partial *rbc*L fragments were successfully amplified from most but not all tested species (Table 1). Five species representing three red algal orders amplified consistently, but *Rhodymenia pseudopalmata* (Rhodymeniales) did not. The original FTA card preserved *R. pseudopalmata* sample only amplified once in 6 trials, and a second FTA card preserved sample also did not amplify successfully. The partial *rbc*L fragment was amplified using DNA extracted by the Freshwater and Rueness (1994) and Hughey et al. (2001) methods from all six red algal species included in these trials. Only two of the four green algal species successfully amplified. The failure of the *Cladophora albida* sample to amplify was not an absolute test of the FTA card system because DNA extracted with another method was not available as a positive control. Our *rbc*L primers may also have been the reason for the unsuccessful *C. albida* amplifications, as a recent study found that amplifying plastid DNA from Cladophorales species is impossible using standard primer combinations (Verbruggen et al. 2009). FTA card preserved *Penicillus* samples also did not amplify, but DNA extracted from the tested *Penicillus* sp. using the Curtis et al. (2008) method did amplify successfully.

An often-cited advantage of FTA cards is the ability to preserve samples at room temperature for extended periods. Amplifications with disks punched and cleaned up to 102 days after the sample was pressed onto FTA cards worked as well, or better, than those punched and cleaned on the same day that the sample was initially preserved (Table 2, Fig. 1). This suggests that the reported ability to preserve samples at room temperature for extended periods will also be applicable to the marine algal species for which the FTA card system works. It is also important for the practical application

Table 2. Results for time of preservation on FTA paper before cleaning of punches trials. Yes = good amplification, Yes* = weak amplification, No = no amplification, – = not tested for that sample.

Species	Date of FTA card preservation	Days before cleaning of punches						
		0	14	42	48	60	89	102
Ulva fasciata	20 Nov 2008	Yes	–	–	–	Yes	–	Yes
Bryopsis sp.	20 Nov 2008	Yes	–	–	–	Yes	–	Yes
Neosiphonia harveyi	20 Nov 2008	Yes	–	–	–	Yes	–	Yes
Cladophora albida	02 Dec 2008	–	No	–	No	–	No	–
Hypnea musciformis	02 Dec 2008	–	Yes	–	Yes	–	Yes	–
Gracilaria tikvahiae	02 Dec 2008	–	Yes	–	Yes	–	Yes	–
Rhodymenia pseudopalmata	02 Dec 2008	–	No	–	Yes*	–	No	–
Antithamnion cruciatum	02 Dec 2008	–	Yes	–	Yes	–	Yes	–
Rhodymenia Pseudopalmata 2	17 Jan 2009	–	–	No	–	–	–	–
Antithamnionella elegans	17 Jan 2009	–	–	Yes	–	–	–	–

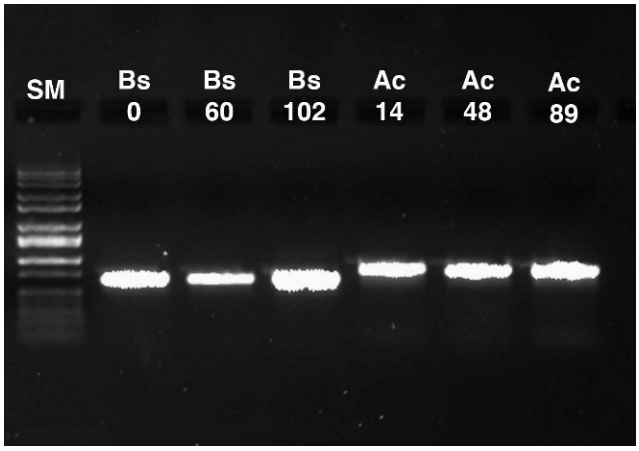

FIG. 1. Example PCR amplification products for FTA card preserved sample disks prepared for PCR up to 102 days after the samples were preserved. Sample designations are Bs = *Bryposis* sp. and Ac = *Antithamnion cruciatum* and the number of days after sample preparation are shown below the sample designations. SM = DNA size marker.

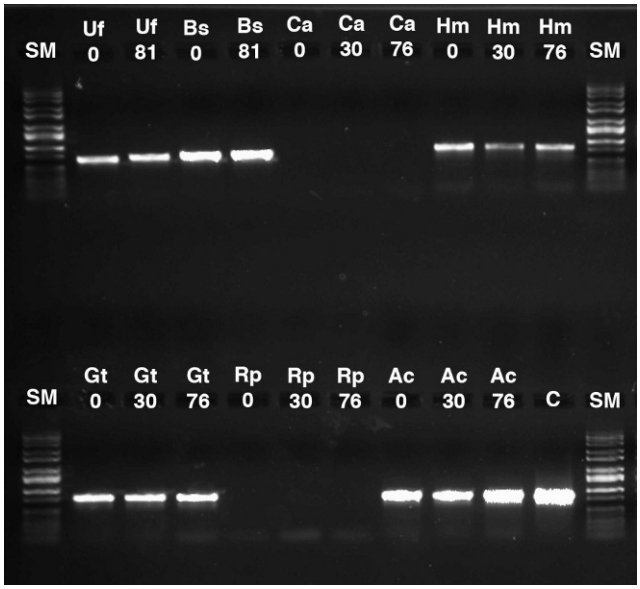

FIG. 2. Example PCR amplification products for FTA card preserved sample disks cleaned and stored at −20°C for up to 81 days. Sample designations are Uf = *Ulva fasciata*, Bs = *Bryopsis* sp., Ca = *Cladophora albida*, Hm = *Hypnea musciformis*, Gt = *Gracilaria tikvahiae*, Rp = *Rhodymenia pseudopalmata* and Ac = *Antithamnion cruciatum*. The number of days disks were stored is shown below the sample designations. C = positive control DNA (*Gelidium americanum* extracted following the method of Freshwater and Rueness [1994]) and SM = DNA size marker.

of the FTA card system that cleaned disks can be stored for extended periods before use in PCR reactions. Amplifications using disks cleaned up to 81 days prior to the reactions and stored at −20°C were as successful as those using disks cleaned as part of the PCR set up (Table 3, Fig. 2).

The FTA card system for preserving PCR quality DNA worked very well for a majority of the marine green and red algae tested. However, FTA preserved samples of some species would not amplify, indicating that preliminary assessments of the FTA card system should be made for specific algal groups before extensive sample preservation with this method. Simultaneous cleaning of multiple FTA paper disks from the same sample, and their long-term storage at −20°C, was found to have no adverse effect on subsequent PCR. This ability greatly increases the utility of the FTA card preservation method in large studies requiring the amplification of multiple loci from individual samples.

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Table 3. Results for time between cleaning of FTA paper punches and PCR amplification trials. Yes = good amplification, No = no amplification, – = not tested for that sample.

Species	Date FTA card punches cleaned	Days before PCR amplification reaction			
		0	30	76	81
Ulva fasciata	11 Dec 2008	Yes	–	–	Yes
Bryopsis sp.	11 Dec 2008	Yes	–	–	Yes
Cladophora albida	15 Dec 2008	No	No	No	–
Hypnea musciformis	15 Dec 2008	Yes	Yes	Yes	–
Gracilaria tikvahiae	15 Dec 2008	Yes	Yes	Yes	–
Rhodymenia pseudopalmata	15 Dec 2008	No	No	No	–
Antithamnion cruciatum	15 Dec 2008	Yes	Yes	Yes	–

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