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Simple and rapid RNA extraction from freeze-dried tissue of brown algae and seagrasses

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Simple and rapid RNA extraction from freeze-dried tissue of brown algae and seagrasses

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An inexpensive and rapid RNA extraction protocol for brown algae and seagrasses is presented, based on homogenization in a simple CTAB buffer and selective precipitation of RNA with lithium chloride. The protocol avoids the use of toxic chaotropic agents and phenol; high concentrations of dithiothreitol are used to inhibit RNase activity and to prevent oxidative cross-linking of nucleic acids by phenolics. A relatively high throughput of about 100 samples in 24 h can be achieved for, for example, Northern analysis. Yields of 50–200 $\mu g\, g^{-1}$ fresh weight are comparable with those obtained for higher plants using commercially available kits. Tests of the extraction procedure demonstrate that high quality, intact RNA can be obtained from a variety of lyophilized brown algal tissues, even after prolonged storage at room temperature. Lyophilization is therefore suggested as an alternative to freezing tissue at −70°C to −80°C. The RNA obtained was used directly in several downstream applications to detect *Fucus* plastid-encoded transcripts by RNA-labelling, RT-PCR and Northern analysis.

Key words: brown algae, *Fucus*, lyophilization, plastid gene expression, *rbcL*, RNA extraction, seagrass

Introduction

Gene expression studies require the isolation of high-quality RNA for applications such as Northern blotting, macroarray hybridization, RT-PCR analysis, and cDNA library construction. This is often a challenge for the development of molecular ecology studies in non-model organisms. The broad geographical range of fucoids (Lüning, 1990), and the sympatric occurrence of several species with different vertical distributions and physiological characteristics, makes *Fucus* an interesting model for molecular ecology and for studying the functional and evolutionary aspects of abiotic stress tolerance (Pearson *et al.*, 2001; see MGE link at: http://www.marine-genomics-europe.org/index.php). In addition, there is a long history of research using fucoid zygotes as a model in developmental biology (see reviews by Kropf, 1992; Brownlee *et al.*, 2001). However, the realization of these goals will require the development of high-throughput and cost-effective methods of sample processing and extraction.

The alginate–cellulose cell walls and high content of phenolic compounds in brown algae initially make them appear unpromising candidates for high-throughput approaches. Most procedures developed for RNA extraction from 'difficult' plant and similar tissues rely on the chaotropic action of phenol/SDS used to directly extract ground tissue (Harris & Dure, 1978). Also commonly applied, the hot-borate method (Wan & Wilkins, 1984) uses high concentrations of borate to combat phenolics (which oxidatively cross-link nucleic acids) and proteinase K to inhibit ribonucleases (RNase). Other procedures use chaotropic salts of guanidinium (e.g., Chomczynski & Sacchi, 1987) that effectively inhibit RNase, but may be unsuitable for tissues high in polysaccharides or phenolics (Wilkins & Smart, 1996). Commercially available kits using guanidinium salts (e.g., the RNeasy Mini Kit; Qiagen, Portugal) have been tried without success for brown algae in our laboratory. The subsequent isolation of RNA after extraction is best achieved by selective precipitation with LiCl (Apt & Grossman, 1993; Apt *et al.*, 1995; further references in Wilkins & Smart, 1996; see also La Claire & Herrin, 1997), which is preferable to CsCl ultracentrifugation (Roell & Morse, 1991). The latter is not amenable to high-throughput sample processing, and the RNA is susceptible to degradation during the overnight centrifugation at room temperature.

In this paper we describe a simple and cost-effective RNA-extraction procedure based on a CTAB (cetyl trimethyl ammonium bromide) extraction buffer and selective LiCl precipitation,

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modified from that previously developed by Apt and co-workers for red and brown algae (Apt & Grossman, 1993; Apt *et al*., 1995), that requires neither phenol nor other chaotropic agents, but rather relies on high concentrations of dithiothreitol (DTT) to inhibit RNase activity and prevent oxidative cross-linking by phenolics. Importantly, with mechanical grinding, this method is applicable to high-throughput sample preparation – e.g. more than 100 samples per day for Northern analysis can be achieved. We present results showing that the RNA prepared using this protocol can be used without further treatment for Northern analysis, RT-PCR, cDNA library construction, and RNA-labelling techniques. Furthermore, a test of the same procedure with marine angiosperms (the seagrass *Zostera noltii* Hornemann) gave equally good results.

Another constraint for studies of gene expression analysis, particularly for multiple samples, is that of sample storage and/or transport. This is a particular problem in, for example, collaborative studies between different laboratories, where it is often desirable to do experimental treatments or collections at one site, but to extract and analyse RNA at another. The most common method of sample storage is to use a −80°C freezer, although space can be a limiting factor when many samples are required for a particular study. However, the transport of samples by air or surface mail between laboratories for extraction at a second location is even more fraught with danger of RNA degradation during transport. We assess the usefulness of lyophilization as a method for preserving RNA integrity and show that this allows for transport and longer-term storage of tissue, even at room temperature.

Materials and methods

Sample storage and RNA extraction

Fresh apical tips of *Fucus vesiculosus* L. were flash-frozen in liquid nitrogen (LN_2) and stored at −80°C before extraction without prior lyophilization. Lyophilized tissue (from various sources) was first flash-frozen in LN_2 and after lyophilization was either mailed from the laboratory of origin, stored at −80°C, or stored on the bench top at room temperature in the presence of silica gel to prevent re-hydration.

Fresh frozen tissue (approx. 250 mg fresh weight per sample) was ground to a fine powder in 1.5-ml Eppendorf tubes in LN_2 using a Kontes pestle. Lyophilized tissue (approx. 50 mg dry weight per sample) was ground for 5 min at room temperature using a mixer-mill (Retsch MM 301) in 2-ml Eppendorf tubes with the tungsten beads supplied. Extraction buffer consisted of Tris-EDTA (100 mM Tris, 50 mM EDTA, pH 7.5), with 2 M NaCl and 2% CTAB. The buffer was stored in 50-ml aliquots at −20°C. Immediately prior to use, 50 mM DTT as antioxidant was added from 2 M stocks dissolved in water. At this concentration, DTT effectively prevents oxidation of nucleic acids by phenolic compounds, and inhibits RNase activity by disrupting disulphide bond formation. Extraction buffer was added to the tissue (1 ml per 250 mg fresh or 50 mg dry weight) and the suspension was mixed vigorously by vortexing. After 10–15 min at room temperature, the mixture was extracted with 1 ml of chloroform:isoamyl alcohol (24:1 v/v), vortexed vigorously, and centrifuged at 12,000*g* for 20 min at 20°C. The upper aqueous phase was transferred to a new tube and 0.3 vol absolute EtOH was added gently and mixed by rocking the tube. EtOH addition results in the precipitation of polysaccharides (Fang *et al*., 1992). There followed a second chloroform extraction, under the same conditions as the first, and the aqueous phase was carefully removed. RNA was precipitated with 0.25 vol of 12 M LiCl in the presence of 1% (v/v) β-mercaptoethanol as antioxidant. Precipitation is complete in 20–30 min at −80°C. Alternatively, the RNA can be precipitated overnight at 4°C in the refrigerator – extended periods at lower temperatures tend to cause co-precipitation of impurities. The RNA was collected by centrifugation at 14,000*g* for 30 min at 4°C, and the pellet was washed with 70–80% EtOH and centrifuged at 14,000*g* for 10 min at room temperature. After drying (10–20 min at room temperature), the RNA was resuspended in RNase-free water, TE (10 mM Tris-HCl, 1 mM EDTA) or 10 mM Tris-HCl and stored at −80°C. After determining the purity by spectrophotometry ($A_{260/280}$), RNA integrity was assessed by agarose–formaldehyde gel electrophoresis. The RNA can be used directly for applications such as Northern blotting, RT-PCR, and cDNA synthesis for library construction. Hence the use of phenol and other chaotropic agents is avoided completely in this protocol.

Downstream applications: RT-PCR

Total RNA extracted from lyophilized *F. vesiculosus* collected in the Tagus Estuary, Lisbon, Portugal was used to test RT-PCR conditions. Several primer pairs for plastid genes sequenced in our lab were tested (see Table 1 for a list of primers, genes, and GenBank accession numbers). A multiplex reverse transcription (RT) reaction was performed containing: 1 µg total RNA, 200 U Superscript™ II (Invitrogen), reverse primer mix (10 µM each) for 16S rRNA, *atpB*, *ccsA*, and *tsf*, 5 × first-strand buffer, dNTPs (0.5 mM each), 0.1 mM DTT, and 1 µl (40 U) RNaseOUT (Invitrogen), in a total volume of 20 µl with RNase-free water (Sigma). The reaction mix was denatured at 65°C for 5 min, extension was at 42°C for 50 min, and the RT was inactivated by heating at 75°C for 15 min. Each target gene fragment was amplified in a separate 50 µl PCR reaction containing 5 µl of the first-strand reaction mix, 10 × PCR buffer with 2 mM $MgCl_2$, dNTPs (0.2 mM each), forward and reverse gene-specific primers (0.2 µM each), 1 µl Taq polymerase (5 U, EuroClone, Biocat, Germany) and PCR-grade H_2O.

Table 1. Forward and reverse primers used to generate plastid DNA probes and for RT-PCR amplification in this study, the corresponding plastid genes from *Fucus vesiculosus,* and GenBank accession numbers.

Gene	Forward primer	Reverse primer	Product size (bp)	Accession number (NCBI GenBank)
16S rRNA (1507 bp)	5′-TACCAGGGTTTGACATTTT-3′	5′-ACGGCTACCTTGTTACGA-3′	528	DQ307678
psbA (360 AA)	5′-CATCGGTTGGTTTGGTTG-3′	5′-TTCCAAGCTGAGCACAAC-3′	487	DQ307679
rbcL (488 AA)	5′-GGATTCCTTACGCTTACC-3′	5′-CCAACATAATCACGACCT-3′	898	DQ307680
atpB (476 AA)	5′-TAGAAGTAGAAGGTAAAGATGG-3′	5′-CACCTAAAATCATATTAAAACC-3′	1236	DQ307681
ccsA (343 AA)	5′-AATCTAAAGCTCAAAGTCC-3′	5′-CACCAGCAATAATACCAA-3′	525	DQ307682
tsf (200 AA)	5′-CTTTACAAGAAACAAATGGGAAT-3′	5′-GTTGGTCTATCAAACTTAACCGT-3′	411	DQ307683
ycf4 (186 AA)	5′-TATTATTATAGGGTCAAGGCGTT-3′	5′-ATAGCAATAACTCCATAAAAGGT-3′	189	DQ307684

The cycling program for PCR was as follows: 5 min denaturation at 95°C, followed by 16, 17, 18, 19, and 20 cycles of 94°C for 30 s, 55°C for 45 s, and 72°C for 1 min. Between 16 and 20 cycles, 5 µl of PCR product was successively removed and electrophoresed on an agarose gel. Relative amounts of PCR products were estimated from the pixel density of a digital image of the gel using the GelEval software (FrogDance software, Dundee, UK).

Downstream applications: Northern Analysis

We used RNA extracted from lyophilized *F. serratus* as described above for RNA gel-blot analysis, and probed with an 898-bp fragment of the RuBisCO large subunit gene (*rbcL;* Table 1).

Samples were rapidly frozen in LN_2 before lyophilization and extraction as described. Sample RNA was run on denaturing formaldehyde-agarose gels and transferred to Nylon membranes (Hybond-XL, Amersham Biosciences, UK) following standard protocols (Sambrook & Russell, 2001). For Northern analysis, blots were hybridized (PerfectHyb™ Plus, Sigma) with a ^{32}P-labelled *rbcL* probe from the closely related species *F. vesiculosus*. After hybridization, the blots were washed as recommended by the manufacturer, and exposed for 24 h at −80°C on X-ray film (BioMax MS, Kodak) for autoradiography.

Downstream applications: RNA-labelling techniques

Two methods of RNA-labelling were tested to assess their utility for detecting plastid gene expression on nylon membrane-based macroarrays. In the first, 15 µg total RNA was 5′-end-labelled with 10 µCi of γ-^{32}ATP in the presence of 10 U of T4 polynucleotide kinase (PNK; USB Corp., Cleveland, OH, USA). After incubation at 37°C for 30 min, the reaction was terminated by adding 2 µl of 0.5 M EDTA (pH 8.0). Labelled RNA was separated from precursor ATP by chromatography on ProbeQuant G-25 sephadex spin-columns (Amersham Biosciences, UK).

In a second approach, cDNA was labelled in a RT reaction using 20 µg total RNA from *F. vesiculosus* in the presence of 5 µl (50 µCi) α-^{32}ATP using gene-specific reverse primers for the following genes: *psbA*, *rbcL*, *atpB*, *ccsA*, and 16S rRNA (see Table 1). The cDNA probes were purified with size-exclusion chromatography (G-25 spin columns) to remove unincorporated radionucleotides. Both RNA (5′-end-labelled) and cDNA probes were hybridized to gene-specific targets spotted on Hybond© XL nylon membranes (Amersham Biosciences, UK). After washing, membranes were exposed to X-ray film (BioMax MS, Kodak) with intensifying screens at −80°C for 1 (5′-end-labelling) or 6 days (labelled cDNA).

Results

Sample storage and RNA extraction

Extraction of fresh-frozen and lyophilized frozen tissues of *F. vesiculosus* gave essentially equivalent results, both in terms of the estimated yield and integrity of the total RNA (Fig. 1). In this case, both types of tissue were stored at −80°C prior to extraction in order to test only the effects of lyophilization on the extraction procedure. Thus, lyophilization appears to have no detrimental effects on RNA integrity. In addition, we have found that the yields of RNA from lyophilized tissue, in comparison to the laborious grinding of fresh-frozen tissue in LN_2, results in more consistent extraction-to-extraction yields (data not shown), probably because of the more efficient and consistent disruption of dry tissue in the mixer mill. The average yields obtained from *F. vesiculosus* used in this study were $135.3 \pm 47.3\ \mu g\ g^{-1}$ fresh weight (FW) with a $A_{260/280}$ of 2.014 ± 0.035 (values are means ± SD, $n = 6$).

We next wished to determine if lyophilization could be an effective method of transporting and

storing experimental tissue, as a convenient alternative to storage at −80°C and/or transport on dry ice. We obtained a variety of samples, including adult *F. vesiculosus* from the Baltic and North Sea coasts of Sweden (Tjärö Marine Laboratory, University of Göteborg, Sweden), 16-h embryos of *Fucus serratus* L. from the UK (Marine Biological Association, Plymouth, UK), and cultured *Laminaria digitata* (Hudson) Lamouroux and *Ectocarpus siliculosus* (Dillwyn) Lyngbye (Roscoff Marine Laboratory, France). In each case, tissues were lyophilized on site and mailed to the University of the Algarve on silica gel to prevent rehydration. On arrival, the samples were stored at room temperature in the laboratory until extraction. In the case of the adult *F. vesiculosus* tissues from Sweden, the storage period was 3 months from the arrival date. Figure 2 shows that intact RNA could be extracted from these samples even after this period of storage at room temperature, and yield and quality were comparable to those obtained from lyophilized tissue stored at −80°C (c.f. lanes 1–4 with 5 and 6 in Fig. 2). Both the storage and extraction methods are suitable for brown algae in general, since high quality intact RNA was obtained from early *Fucus* stages (16-h embryos), as well as from representatives of the Laminariales and Ectocarpales (Fig. 3). As a more general test, freshly collected and cleaned whole plants (roots, rhizomes and leaves) of the intertidal seagrass, *Zostera noltii*, from the Ria Formosa, Portugal, were lyophilized and extracted in the same way. An average yield of 363 $\mu g\,g^{-1}$ dry weight (DW) of high purity RNA ($A_{260}/A_{280} = 1.953$) was obtained, which was shown to be intact by electrophoresis (Fig. 3; lane 6).

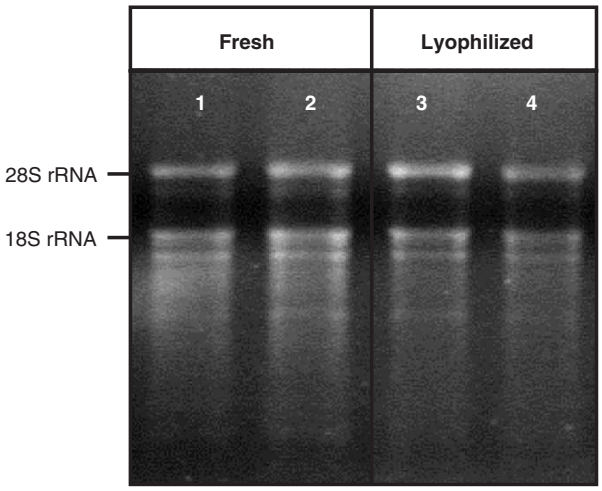

Fig. 1. Total RNA extracted from fresh (lanes 1 and 2) and lyophilized (lanes 3 and 4) tissue of *Fucus vesiculosus*. RNA was extracted from *c*. 300 mg FW or FW-equivalent. The final pellet was resuspended in 100 µl TE, and 5 µl was run on a formaldehyde–agarose gel.

RT-PCR

We optimized a multiplex RT with four plastid primers for *tsf*, *atpB*, *ycf4* and the 16S rRNA. All the predicted size fragments amplified in the subsequent gene-specific PCR reactions with both gene specific primers, with no evidence of non-specific amplification products (Fig. 4*a*). Amplification was semi-quantitative, as shown in Fig. 4*b* by the increased amount of product with successive cycle number between 16 and 19 cycles. The maximum product size (1.2 kb for *atpB*) indicated that the RT successfully transcribed cDNA of at least this size from the starting template RNA.

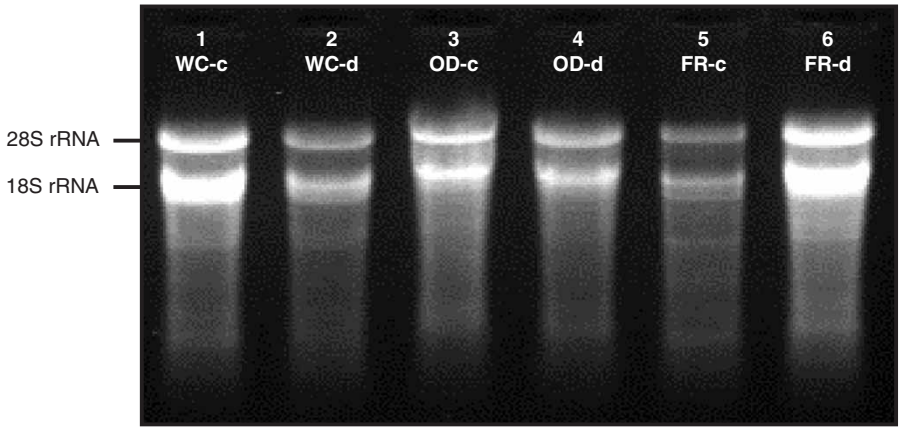

Fig. 2. Effects of long-term, room-temperature storage of lyophilized tissue on RNA quality. In lanes 1–4, treated (c: control; d: desiccated), lyophilized tissue was air-mailed to Portugal from Sweden and stored at room temperature for 3 months in Falcon tubes in plastic bags in the presence of silica gel to prevent rehydration from the air. In lanes 5 and 6, algae from Roscoff, France were maintained in culture in Faro for 2 weeks before treatment (c: control; d: desiccated), lyophilization and storage at −80°C. Total RNA was extracted from 50-mg samples of powdered, lyophilized tissue. WC-c and WC-d: Tjärnö, Swedish west coast; OD-c and OD-d: Öregrund dwarf morph, central Baltic Sea; FR-c and FR-d: Roscoff, France.

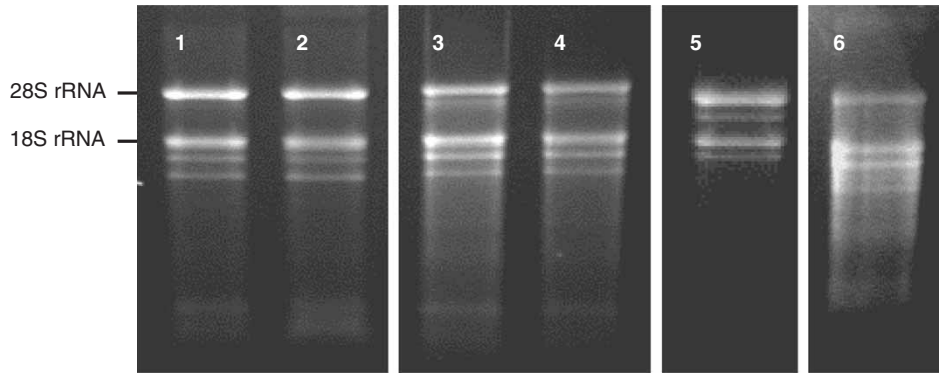

Fig. 3. Total RNA extracted from lyophilized tissue of *Laminaria digitata* (lanes 1 and 2), *Ectocarpus siliculosus* (lanes 3 and 4), 16-h embryos of *F. serratus* (lane 5), and the seagrass, *Zostera noltii* (lane 6).

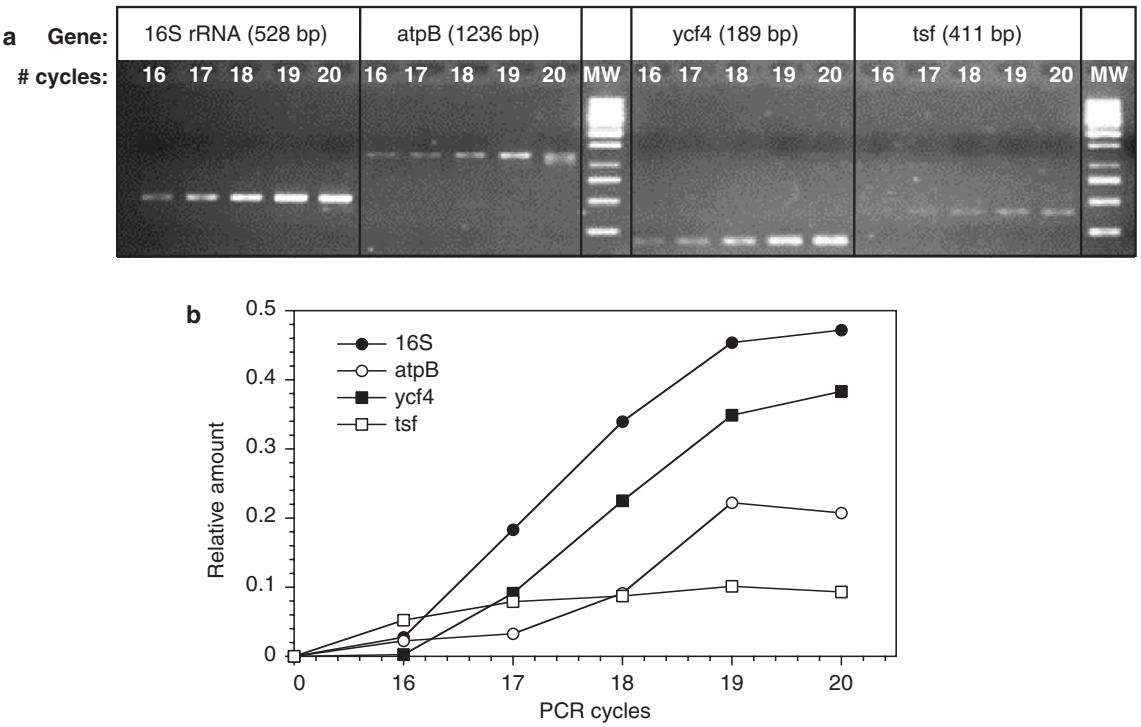

Fig. 4. RT-PCR of the plastid gene products 16S rRNA, *atpB*, *ycf4*, and *tsf* from *F. vesiculosus*. (*a*) Agarose gel showing 5 µl of PCR product (total reaction volume 50 µl) run after 16, 17, 18, 19 and 20 cycles as indicated; MW: molecular weight markers. (*b*) The relative amount of each gene product calculated from the gel bands shown in (*a*).

Northern blotting and RNA-labelling techniques

Total RNA (5 µg) obtained from *F. serratus* was sufficiently pure to use directly in RNA gel-blotting experiments, as illustrated using a radiolabelled probe for *rbcL* (Fig. 5*a*). Thus a single extraction in a 2-ml Eppendorf tube (equivalent to *c.* 250 mg FW tissue) typically yields enough total RNA for Northern analysis of high-abundance mRNAs.

Hybridization of 5′-end-labelled RNA with plastid gene probes for *psbA*, *rbcL*, *atpB*, *ccsA*, and 16S rRNA on nylon membranes is shown in Fig. 5*b*. Strong signals were obtained with *psbA* and 16S rRNA, encoding the photosystem II (PSII) D1 protein subunit and the 16S plastid ribosomal RNA subunit, respectively, while *rbcL* (encoding the large subunit of RUBISO) hybridized less strongly. Weaker signals were obtained with *atpB* (encoding the plastid ATP synthase CF_1 β subunit) and *ccsA* (encoding the CcsA protein, and involved in catalysis of haem attachment to plastid cytochrome c). Essentially similar hybridization signals were obtained with radiolabelled, reverse-transcribed, first-strand cDNA (Fig. 5*c*), with the exception of 16S rRNA, which gave a signal weaker than *psbA* and *rbcL*. Since both *psbA* and *rbcL* are less abundant than 16S rRNA, the lower signal for the latter appears likely to be an artefact of the cDNA-labelling technique. This method requires successful and efficient

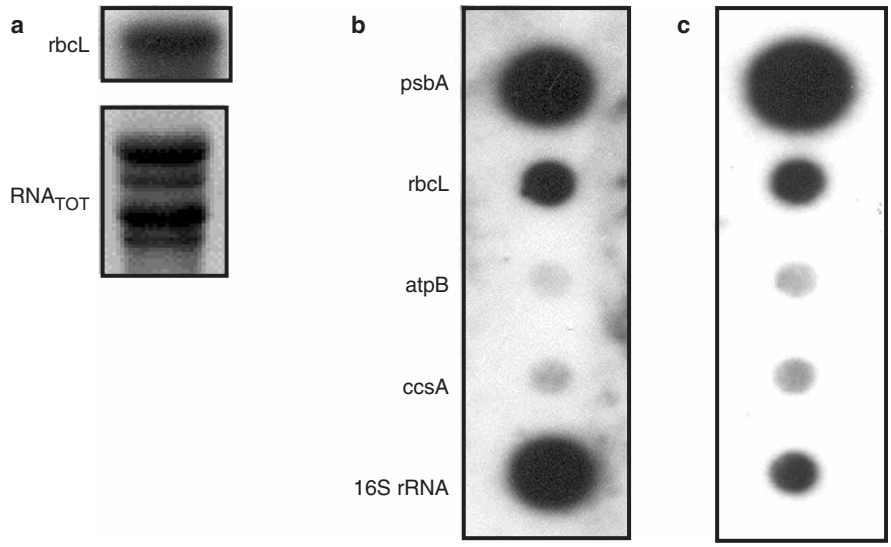

Fig. 5. Detection of plastid genes using (a) Northern analysis of *rbc*L expression, and detection of plastid target probes using (b) 5′-end-labelled RNA, and (c) RT-labelled first-strand cDNA. (a) *rbc*L expression in *F. serratus*. Transferred RNA was hybridized with a specific ^{32}P-labelled probe for *rbc*L (upper panel). RNA integrity was assessed with ethidium bromide staining (lower panel, RNA$_{TOT}$). (b) Labelling of 15 µg total RNA from *F. vesiculosus* with T7 polynucleotide kinase (PNK) in the presence of 1 µl (10 µCi) of γ-^{32}ATP. (c) cDNA was labelled in a multiplex RT reaction using 20 µg total RNA from *F. vesiculosus* in the presence of 5 µl (50 µCi) α-^{32}ATP using gene-specific primers for the genes indicated. RNA or cDNA probes were purified with size-exclusion chromatography (G25 spin columns) to exclude unincorporated radioactivity, and hybridized to gene-probes spotted on Hybond© nylon membranes for 1 (b) or 6 days(c).

transcription of first-strand cDNA in order to incorporate radionucleotides into the probe. We hypothesize that secondary structure in the 16S rRNA may have reduced the efficiency of transcription. In contrast, 5′-end-labelling requires no transcription and is, in principle, unaffected by secondary structure. RT-PCR and the two RNA-labelling methods gave qualitatively similar results for the relative expression of *atpB* and 16S rRNA (cf. Figs 4, 5).

Discussion

In this paper we demonstrate that lyophilization is a convenient method of preserving RNA yield and integrity in tissue samples, and present a rapid, high-throughput and cost-effective RNA-extraction protocol. The suitability of RNA obtained using these procedures was tested successfully in several downstream applications: RT-PCR, RNA-labelling, and Northern analysis. Although we have applied these techniques to studies involving brown algae, we present data showing that both lyophilization and the extraction protocol described are effective methods for higher plants such as seagrasses, suggesting that they may be of general use for a variety of algal and plant groups.

Visual inspection of total RNA on denaturing (formaldehyde) agarose gels indicated no differences in quality between lyophilized and freshly ground tissue (Fig. 1). Given this, it is surprising that there are few reports of freeze-drying as a sample preservation method in the literature, although extraction of double-stranded RNA (dsRNA) from dried and lyophilized plant tissues has been reported previously (Karan *et al.*, 1991). Provided that samples are maintained in a dry atmosphere, lyophilized tissue can be stored for a period of at least 3 months on the bench-top at room temperature (Fig. 2). As a method of sample preparation and preservation, lyophilization offers several advantages over low temperature storage: (i) The need for −80°C freezer space is reduced, (ii) transport of samples (e.g. between laboratories) is made far easier, (iii) the initial grinding step of RNA extraction is simplified, since the need for maintaining low temperatures with LN_2 is removed. A corollary of this in our experience is that yields of RNA are more repeatable, probably due to more uniform grinding of dry tissue in the mixer mill, when compared with hand-grinding of frozen tissue in LN_2.

The second main aim of this study was to present a rapid, relatively non-toxic, and inexpensive RNA-extraction protocol for brown algae, which can accommodate high-sample throughput. In addition to the presence of a cell wall, the metabolic activity of plants, algae and fungi presents further obstacles to the extraction and purification of high quality RNA (e.g. a high abundance of polysaccharides that can co-purify with RNA), and secondary metabolites such as phenolic compounds that can cross-link

RNA under oxidizing conditions. The extraction protocol was modified from that developed for the brown alga *Macrocystis* by Apt *et al*. (1995). By using powdered lyophilized tissue, and increasing the antioxidant concentration (DTT), we were able to increase the tissue: buffer ratio from 1:10 (w/v) to 1:4 (FW equivalent), and scaled the protocol down so that it could be performed in microfuge tubes. To circumvent the problem of polysaccharide contamination, a high-salt CTAB buffer was used, in addition to selective precipitation with ethanol. Oxidative cross-linking of RNA by phenolic compounds was avoided by the presence of DTT at 50 mM, which has the additional advantage of inactivating RNases during the homogenization and extraction steps. Finally, LiCl was used to precipitate the RNA selectively (Wilkins & Smart, 1996).

Using the methods described, total RNA was obtained from several morphologically dissimilar brown algal species (*Fucus*, *Laminaria*, *Ectocarpus*). We also decided to test a seagrass, *Zostera noltii*, in order to assess the general usefulness of lyophilization and the extraction procedure. Intact, high quality RNA was obtained, judged by denaturing agarose gel electrophoresis (Fig. 3), suggesting that commonly used extraction procedures using toxic chaotropic agents, such as phenol or guanidinium salts, can be avoided for seagrasses and possibly other higher plant species.

The total RNA obtained from *F. vesiculosus* was pure enough for direct use in applications such as RT-PCR, RT and 5′-end-labelling for macroarray hybridization, and Northern blotting. Using specific primers designed for plastid sequences from *F. vesiculosus*, semi-quantitative RT-PCR was performed with four plastid genes, with PCR products ranging in size between 189 bp (*ycf4*) and 1.2 kb (*atpB*) (Fig. 4). Enzymes such as reverse-transcriptase and *taq*-polymerase are sensitive to the presence of various impurites, and the successful amplification in particular of the 1.2 kb *atpB* gene fragment indicates that the template was pure. It should be noted, however, that quantitative gene expression protocols using RT-PCR (e.g. real-time PCR), should include an additional step to remove any DNA contamination from the RNA samples, by digestion using RNase-free DNase (Sambrook & Russell, 2001).

One of the research goals of our laboratory is to develop tools for the study of genome-wide expression of plastid-encoded genes in the genus *Fucus*. To achieve this, we are testing membrane-based macroarrays of plastid gene probes and labelling methods to monitor expression. We show here that the RNA extracted using the methods described is suitable for either direct 5′-end-labelling, or for radionucleotide incorporation into reverse-transcribed cDNA. It has been suggested in the literature that 5′-end-labelling produces probes that most accurately reflect levels of mature transcripts in plastid gene expression studies (Legen *et al*., 2002). The reasons for this include: (i) only processed (mature) transcripts having a free 5′-OH group can be labelled by PNK – this is not the case with RT-labelling, and (ii) secondary structure in the RNA template may interfere with RT-labelling, but not with end-labelling methods. This may have been the reason why the signal for 16S rRNA was stronger when end-labelled probes rather than cDNA probes were used.

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