

A simple and effective method for RNA isolation and cDNA library construction from the brown seaweed *Sargassum polycystum* (Fucales, Phaeophyceae)

Mei-Chea Sim · Chai-Ling Ho · Siew-Moi Phang

Received: 3 October 2012 / Revised and accepted: 7 January 2013 / Published online: 23 January 2013
© Springer Science+Business Media Dordrecht 2013

Abstract The isolation of high-quality RNA from brown seaweeds has always been problematic due to a high content of polyphenolic compounds and polysaccharides in their tissues. This study presents a simple and effective method for isolating high-quality RNA from the brown seaweeds *Sargassum* (*Sargassum polycystum*, *Sargassum siliquosum*, *Sargassum baccularia*, and *Sargassum binderi*), *Padina tetrastromatica*, *Turbinaria conoides*, *Dictyota* sp., and *Hormophysa cuneiformis* using cetyltrimethylammonium bromide and selective lithium chloride precipitation. Approximately 25–43 $\mu\text{g g}^{-1}$ fresh weight of total RNA was obtained from these brown algae. The A_{260}/A_{280} absorbance ratio of these RNA samples are between 1.995 ± 0.027 and 2.122 ± 0.013 , with distinct 28S and 18S ribosomal RNA bands visible on a formaldehyde agarose gel. The RNA obtained from *S. polycystum* was found to be suitable for many downstream applications such as reverse transcription polymerase chain reaction, cloning, synthesis of cDNA, and construction of cDNA library for the generation of expressed sequence tags.

Keywords cDNA library construction · CTAB · RNA isolation · *Sargassum polycystum* · Phaeophyta · Biotechnology

M.-C. Sim · S.-M. Phang
Institute of Biological Sciences, Faculty of Science,
University of Malaya, 50603, Kuala Lumpur, Malaysia

C.-L. Ho
Department of Cell and Molecular Biology,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia

S.-M. Phang (✉)
Institute of Ocean and Earth Sciences (IOES),
University of Malaya, 50603, Kuala Lumpur, Malaysia
e-mail: phang@um.edu.my

Introduction

Sargassum is an economically and ecologically important brown seaweed, being one of the main raw materials for the production of alginate in Asian countries (Kaladharan and Kaliaperumal 1999) with many applications in textile, in food industries, and as feed (Mahmood and Siddique 2010). Its nutritional and medicinal properties (Blunt et al. 2007; Murakami et al. 2011) make *Sargassum* an interesting model for studying molecular ecology (Cheang et al. 2010; Hu et al. 2011) and functional genomics (Wong et al. 2007; Kim and Itabashi 2012) in the tropical region.

A fast and cost-effective method for the extraction of high-quality nucleic acids, especially RNA, is a prerequisite for successful functional genomics studies. However, the high content of phenolic compounds and alginate–cellulose cell walls (Chkhikvishvili and Ramazanov 2000; Rioux et al. 2007) in brown algae have always been a problem in RNA extraction. In most cases, the quality and yield of the extracted RNA are affected by algal polysaccharides, which often coprecipitate with the RNA (Wang et al. 2008). The task is even more difficult due to the enzymatic degradation of RNA molecules by endogenous RNase (Jun et al. 2008). Furthermore, the isolation of intact and high-quality RNA without DNA contamination is always important and necessary for gene expression studies such as reverse transcription polymerase chain reaction (RT-PCR), northern blot analysis, microarray hybridization, and cDNA library construction (Yao et al. 2009). This is often a challenge for the nonmodel organisms, especially in the development of these molecular studies.

There are numerous methods for the isolation of total RNA from recalcitrant plants and algae such as the acidic guanidinium thiocyanate-based method (AGTI) (Chomczynski and Sacchi 1987; Hong et al. 1997), sodium dodecyl sulfate (SDS)–phenol method (Wilkins and Smart 1996), lithium chloride (LiCl)–SDS method (Kim et al.