Phycological Research

Phycological Research 2017 doi: 10.1111/pre.12165

Optimization of high quality total RNA isolation from the microalga, *Chlorella* sp. (Trebouxiophyceae, Chlorophyta) for next-generation sequencing

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SUMMARY

The advent of high-throughput next-generation sequencing (NGS) has enabled more comprehensive transcriptome analyses to obtain gene expression data and understand metabolic pathways essential for functional analysis and systems biology. The isolation of high purity and intact RNA of sufficient quality and yield is crucial to the success of NGS sequencing. In green microalgae, extraction of nucleic acids is hindered by high concentration of lipids and polysaccharides that co-precipitate with nucleic acids thus resulting in reduced yield and poor quality extracts. The present study compared the performance of standard and modified total RNA isolation protocols using different cell disruption techniques combined with TRIzol reagent or a commercially available kit, in meeting the stringent requirements for downstream NGS application of a green freshwater microalga, *Chlorella* sp. The protocols were evaluated for (i) the yield of RNA, (ii) integrity of RNA as determined by the RNA integrity number and (iii) the purity of RNA as determined by the A_{260}/A_{280} and A_{260}/A_{230} ratios. In general, higher yields were obtained by cell disruption via flash freezing and homogenizing with liquid nitrogen followed by lysis with TRIzol reagent. We recommend the incorporation of a salt precipitation step to improve the purity and integrity of RNA isolated. Results of this study served as a simple and low-cost practical guide for researchers working on isolation of high quality total RNA from microalgae, considering that most relevant publications do not provide a detailed methodology for total RNA isolation or use more expensive methods (e.g. bead-beater).

Key words: cost-effective, PureLink RNA mini kit, RNA integrity number, RNA-Seq, TRIzol.

INTRODUCTION

Green microalgae are an ecologically and economically important group. The chlorophytes contribute approximately 20% (~7 $PgC{\cdot}y^{-1}$) of the total phytoplankton production (Rousseaux & Gregg 2014). The genus *Chlorella* is commercially important as a health supplement, aquatic feed and other products. Species of *Chlorella* are often used for various studies including bioremediation (Lim *et al.* 2010), potential use in biophotovoltaic platforms (Ng *et al.* 2014), lipid productivity for biodiesel production (Vello *et al.* 2014), and physiological and biochemical responses to abiotic stress (Teoh *et al.* 2013; Wong *et al.* 2015).

For the past 5 years, characterization and quantification of microalgal transcriptome data by high-throughput sequencing have been actively conducted to obtain gene expression profiles and elucidate metabolic pathways essential for functional analysis and systems biology (Guarnieri *et al.* 2011; Rismani-Yazdi *et al.* 2011; Lv *et al.* 2013; Ouyang *et al.* 2013; Carrier *et al.* 2014; Li *et al.* 2014; Zheng *et al.* 2014). This approach is also used to investigate the molecular mechanisms underlying the adaptation of algae from diverse habitats to various biotic and abiotic stress factors (Sun *et al.* 2014). As summed up by Hong and Lee (2015), transcriptomic analyses are essential for systems biology because *de novo* and comparative transcriptomic analyses can provide a framework for metabolic reconstruction.

Isolation of RNA of sufficient quality (i.e., non-degraded, free of impurities) and yield is crucial for many applications (Santiago-Vázquez *et al.* 2006; Johnson *et al.* 2012). Often, harsh conditions are required to disrupt the highly resistant cell wall of microalgae (Kim *et al.* 2012). In addition, extraction of nucleic acids from green microalgae is hindered by the high concentration of lipids and polysaccharides that tend to co-precipitate with nucleic acids in low ionic strength buffers (Gehrig *et al.* 2000; Carra *et al.* 2007) thus resulting in reduced yield and poor quality extracts. Isolation of intact RNA is challenging due to the ubiquitous presence of RNases which rapidly degrades RNA.

Various cell disruption methods have been used for RNA isolation from green microalgae. Bead-beating (Kim *et al.* 2012; Mansfeldt *et al.* 2016) and repeated freeze-thawing and vortexing (Singh *et al.* 2010) are some of the techniques used to break rigid cell walls. The lack of standardized unit of measurement for RNA yields (e.g. ng, Kim *et al.* 2006; μg/μL, Pinto *et al.* 2009; mg/g of fresh cell weight, Thanh *et al.* 2009; and μg/mg dry cell weight, Kim *et al.* 2012), and the lack of information supplied to standardize the units hinders comparison of yield obtained from different protocols. In our opinion, expressing RNA yield as weight of RNA per fresh cell weight is simple and useful.

Here we compare standard and modified total RNA extraction protocols using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and/or PureLink RNA mini kit (Ambion, Life

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Email: phaikeem@um.edu.my
Communicating Editor: Giuseppe Zuccarello
Received 21 June 2016; accepted 24 October 2016.