COMPARISON OF RNA EXTRACTION METHODS FROM SPONGES

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ABSTRACT

Many sponges produce a variety of chemical compounds that inhibit the downstream applications of both DNA and RNA. Therefore, robust extraction methods need to be developed.

Three extraction methods were compared; TRIzol® reagent, TRIzol® plus columns from the FastDNA® Spin kit for soil and lastly the FastRNA® Green kit. We investigated these methods in terms of yield of RNA recovered and quality of RNA. Presence of inhibitory compounds was ascertained by the ability to produce double stranded cDNA.

Results indicate that all methods were able to extract stable RNA that could be converted to cDNA. However, the amount of RNA and 260/280 ratios varied between the methods, while the size of RNA was similar for all three methods. Yields of cDNA varied from 900ng to 500ng.

In conclusion the FastRNA® Green kit produced not only largest amount of RNA per gram of sponge but also the highest quality based on conversion to cDNA.

KEY WORDS

Sponge, molecular biology, RNA extraction, method.

INTRODUCTION

Sponges are known to produce a variety of chemical compounds that inhibit downstream applications of both DNA and RNA, such as PCR and RT-PCR reactions. RNA by nature is unstable and easily degraded by environmental RNAses (FARRELL, 1998). Previously RNA was extracted using guanidine thiocyanate, mercaptoethanol, guanidine hydrochloride, guanidine-caesium chloride and other hazardous chemicals (CHIRGWIN *et al.,* 1979; WRIGHT & HETZEL, 1985; PFEIFER *et al.,* 1993; FARRELL, 1998; FERNANDEZ-BUSQUETS *et al.,* 1998). However, more recently this has been replaced by less hazardous chemicals in the single - step RNA extraction method and by the commercially available reagents such as TRIzol® reagent (Invitrogen) and TRI reagent (Sigma) (CHOMCZYNSKI & SACCHI, 1987; CHOMCZYNSKI, 1993; CHOMCZYNSKI & MACKEY, 1995; BROWER *et al.,* 1997; MANUEL & LE PARCO, 2000; WIENS *et al.,* 2000). These reagents are able to extract high quality RNA from small sample sizes (CHOMCZYNSKI, 1993; CHOMCZYNSKI & MACKEY, 1995). TRIzol® reagent has been effectively used to isolate RNA and mRNA from sponges for cDNA library construction (MANUEL & LE PARCO, 2000). The quality of RNA extracted is important for downstream application such as cDNA library construction, gene expression and micro-array analysis (FARRELL, 1998). If the RNA quality is poor no or few full-length mRNAs will be extracted and

subsequently converted to cDNA, which may lead to incomplete data sets. Contamination of RNA by protein and/or DNA may inhibit cDNA synthesis. One easy way to evaluate the quality of RNA is by measuring absorbance at 260 and 280nm and performing an absorbance scan between 240 and 320 nm (FARRELL, 1998). Pure RNA should give a ratio of absorbance 260 / 280 of 2.0 ± 0.15 and a characteristic skewed bell curve, which exhibits a maximum close to 260 nm (FARRELL, 1998). If these parameters are not met, the RNA maybe be of poor quality and may contain contaminants and it may be necessary to subject the RNA to clean up procedures (FARRELL, 1998).

We investigated two commercial RNA extraction methods, TRIzol® reagent (Invitrogen) and the FastRNA® Green kit (Q-BIOgene) and one method which combined two commercial methods, TRIzol® reagent and the extraction columns of the FastDNA® Spin kit for soil (Q-BIOgene), to determine which produced the highest quality of RNA for downstream applications.

MATERIALS AND METHODS

Mycale (Carmia) hentscheli Bergquist & Fromont, 1988 samples were collected by scuba diving. Wet weight was determined before RNA extraction and between 0.1 - 0.2 g of sponge was used for each extraction. Extractions were performed in duplicate and all methods were performed on the same day and with sub-samples of one sponge to reduce variability between samples and possible effects of storage on the samples. Three extraction methods were compared including TRIzol® reagent (Invitrogen), TRIzol® reagent plus the extraction columns of the FastDNA® Spin kit for soil (Q-BIOgene) and the FastRNA® Green kit (Q-BIOgene) (Fig. 1).

TRIzol® and FastRNA® Green kit were used according to the manufactures instructions. The third method combined the extraction columns of the FastDNA® Spin kit for soil and TRIzol® into one method (Fig. 1). We investigated the extraction methods in terms of yield of RNA recovered and quality of RNA as measured spectrophotometerically and by gel electrophoresis (FARRELL, 1998).

Absence of inhibitory compounds was ascertained by the ability to produce double stranded cDNA using SuperScript II reverse transcriptase (Invitrogen). The SuperScript™ Choice System for cDNA Synthesis was used to convert 4μg of the total RNA extracted from the duplicate extractions to cDNA using Oligo(dT) according to the manufacturers instructions. The amount of cDNA converted was measured using a DyNA Quant 200 fluorometer (Amersham).

RESULTS AND DISCUSSION

Results indicated that all methods were able to extract stable RNA that could be converted to cDNA. The amount of RNA per gram wet weight sponge extracted ranged from 698 to 181 μg. The FastRNA® Green kit extracted 698 μg per gram sponge (wet weight) compared to 181 and 558 μg per gram sponge (wet weight) for TRIzol® with extraction column and TRIzol® only respectively (Tab. I). The size of RNA as analysed by gel electrophoresis was similar for all three extraction methods (Fig. 2).

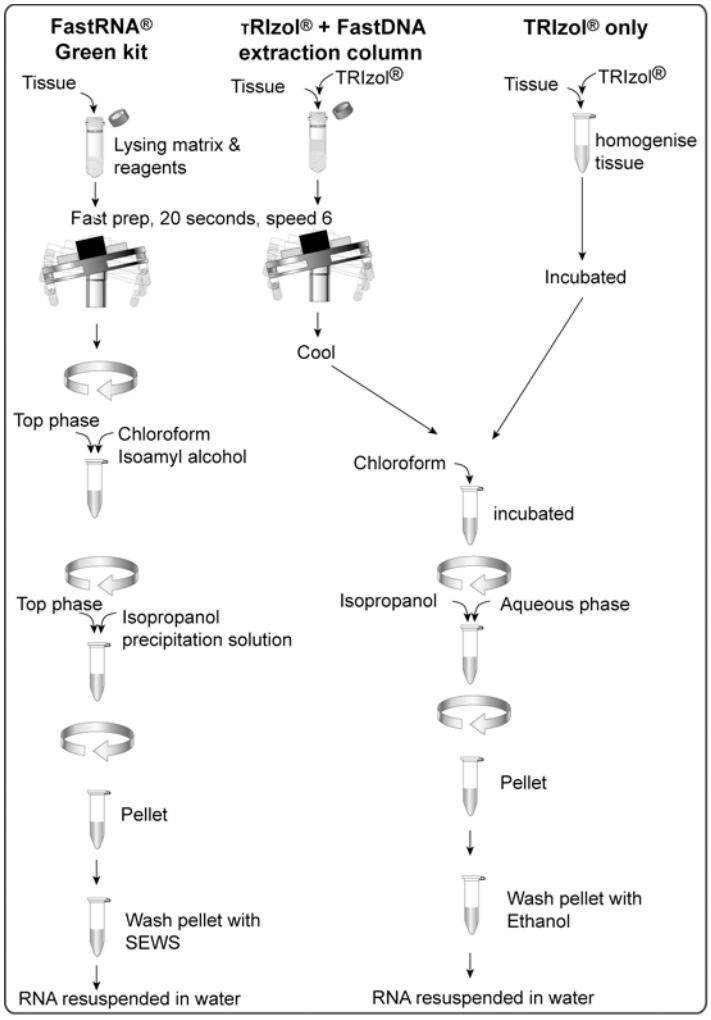

Fig. 1. Flow diagram showing the major steps in each method, FastRNA® Green kit (Q-BIOgene), TRIzol® plus the extraction columns of the FastDNA® Spin kit for soil (Q-BIOgene) and TRIzol® only.

UV absorbance spectra were obtained for each sample (Fig. 3) and show the typical profile for nucleic acids (FARRELL, 1998). Ratios were obtained for each sample and are shown in Tab. I. A ratio of 2.0 ± 0.15 is expected for pure RNA (FARRELL, 1998). The FastRNA® Green kit produced an average ratio of 2.00 indicating a pure sample of RNA, the other methods deviated from 2.0 by 0.08 and 0.05.

Tab. I. The amount of total RNA recovered, the A260 / 280 ratio and the amount of cDNA synthesised from 4 μg total RNA for each method.

Method	Absorbance at 260 nm	280 nm	Ratio 260/280	μg total RNA/g sponge*	Average μg total RNA/g sponge*	Average Ratio	Total cDNA (ng)
FastRNA®	1.0245	0.5176	1.98	756.55			
FastRNA®	0.9991	0.4967	2.01	639.42	697.99	2.00	900
TRIzol® plus extraction column	0.3626	0.1889	1.92	217.56			
TRIzol® plus extraction column	0.2259	0.1145	1.97	144.58	181.07	1.95	600
TRIzol® only	0.3967	0.2118	1.87	544.05			
TRIzol® only	0.6551	0.3332	1.97	571.72	557.88	1.92	828

* = wet weight

The SuperScript™ Choice System for cDNA synthesis (Invitrogen) was used to convert 4μg of each of the total RNA to cDNA using Oligo(dT) (Fig. 4). Yields of total cDNA varied from 900 ng to 500 ng with the FastRNA® Green kit again showing the best conversion rate (Tab. I).

The FastRNA® Green kit produced not only the greatest amount of RNA per gram of sponge sample but also the highest quality based on conversion to cDNA.

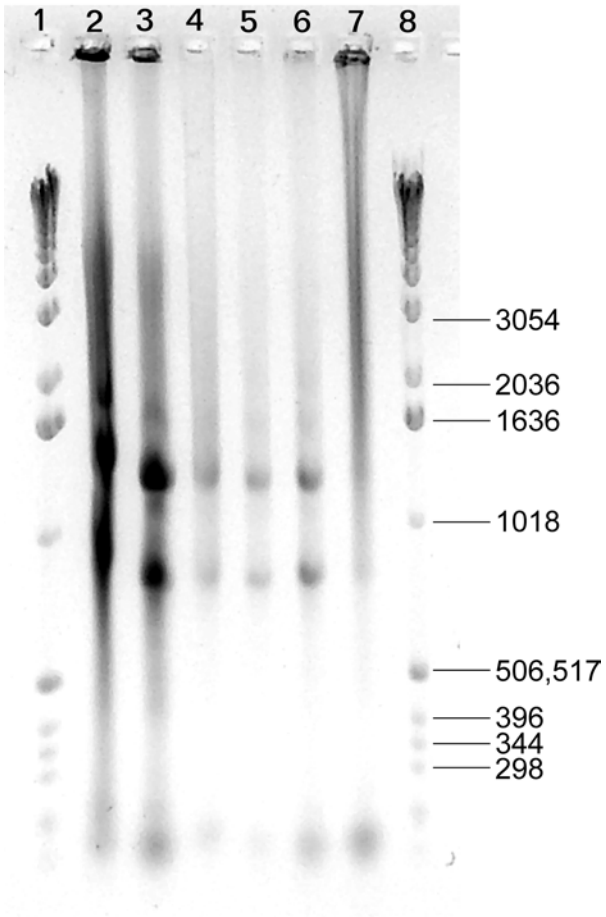

Fig. 2. Gel electrophoresis (1% agarose) showing 5µl of total RNA extracted. Lanes 1 & 8 1Kb ladder (Invitrogen), Lanes 2 & 3 total RNA extracted using the FastRNA® Green kit, Lanes 4 & 5 total RNA extracted using TRIzol® plus the extraction columns of the FastDNA® Spin kit for soil and Lanes 6 & 7 total RNA extracted using TRIzol® only.

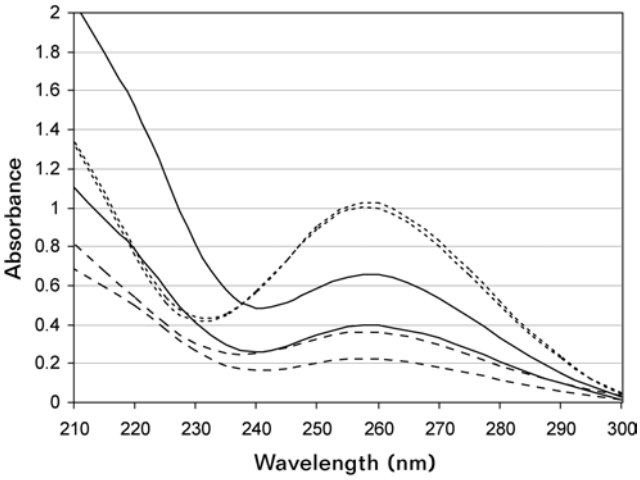

Fig. 3. Graph showing the UV absorbance spectrum obtained for each sample between 210 - 300 nm. FastRNA® Green kit (·····), TRIzol® (- - -) and the extraction columns of the FastDNA® Spin kit for soil, TRIzol® only (——).

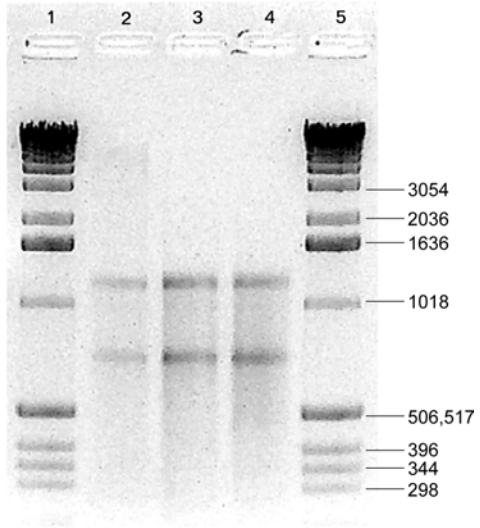

Fig. 4. Gel electrophoresis (1 % agarose) showing 2 µl cDNA synthesised from each method using the SuperScript™ choice system for cDNA synthesis. Lanes 1 & 5 1Kb ladder, Lane 2 cDNA synthesised from the RNA extracted by the FastRNA® Green kit, Lane 3 cDNA synthesised from the RNA extracted by TRIzol® and the extraction columns of the FastDNA® Spin kit for soil and Lane 4 cDNA synthesised from the RNA extracted by TRIzol® only method.

ACKNOWLEDGEMENTS

We thank Dr Michelle Kelly-Shanks for identification of the sponge samples. This work was partially supported by the Foundation for Science, Research and Technology Grant, New Zealand, FRST C01X0001.

REFERENCES

BROWER D.L., BROWER S.M., HAYWARD D.C., BALL E.E., 1997 - Molecular evolution of integrins: Genes encoding integrin beta subunits from a coral and a sponge. *Proc. Natl. Acad. Sci. USA,* **94** (17): 9182-9187.

CHIRGWIN J.M., PRZYBYLA A.E., MACDONALD R.J., RUTTER W.J., 1979 - Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry,* **18** (24): 5294-5299.

CHOMCZYNSKI P., SACCHI N., 1987 - Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.,* **162** (1): 156-159.

CHOMCZYNSKI P., 1993 - A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. *Biotechniques,* **15** (3): 532-537.

CHOMCZYNSKI P., MACKEY K., 1995 - Substitution of chloroform by bromo-chloropropane in the single-step method of RNA isolation. *Anal. Biochem.,* **225** (1): 163-164.

FARRELL R.E. jr., 1998 - *RNA methodologies. A laboratory guide for isolation and characterization.* Academic Press: 55-100.

FERNÀNDEZ-BUSQUETS X., GEROSA D., HESS D., BURGER M.M., 1998 - Accumulation in marine sponge grafts of the mRNA encoding the main proteins of the cell adhesion system. *J. Biol. Chem.,* **273** (45): 29545-29553.

MANUEL M., LE PARCO Y., 2000 - Homeobox gene diversification in the calcareous sponge, *Sycon raphanus. Mol. Phylogenet. Evol.,* **17** (1): 97-107.

PFEIFER K., FRANK W., SCHRODER H.C., GAMULIN V., RINKEVICH B., BATEL R., MÜLLER I.M., MÜLLER W.E.G., 1993 - Cloning of the polyubiquitin cDNA from the marine sponge *Geodia cydonium* and its preferential expression during reaggregation of cells. *J. Cell Sci.,* **106** (2): 545-553.

WIENS M., AMMAR M.S., NAWAR A.H., KOZIOL C., HASSANEIN H.M., EISINGER M., MÜLLER, I.M., MÜLLER W.E.G., 2000 - Induction of heat-shock (stress) protein gene expression by selected natural and anthropogenic disturbances in the octocoral *Dendronephthya klunzingeri. J. Exp. Mar. Biol. Ecol.,* **245** (2): 265-276.

WRIGHT D.A., HETZEL E.W., 1985 - Use of RNA:DNA ratios as an indicator of nutritional stress in the american oyster *Crassostrea virginica. Mar. Ecol. Prog. Ser.,* **25** (2): 199-206.