Isolation of RNA from Pacific Oyster Larvae Using the SV Total RNA Isolation System

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The SV Total RNA Isolation System[a] *was used as part of a trial to determine an efficient method for RNA extraction from larvae of the Pacific oyster,* Crassostrea gigas*. Due to the small size of the larvae, their shells cannot be removed by dissection prior to nucleic acid purification. This report details the successful use of the SV Total RNA Isolation System for purification of RNA from these larvae and investigates the amount of larval tissue needed for RNA isolation with the system. Purity and yield of the isolated RNA is determined by spectrophotometric analysis.*

Introduction

Marine invertebrates are thought to account for 30% of all animal species (1), hence providing a large source of cell and tissue types for basic research. The Pacific oyster, *Crassostrea gigas,* is a commercially important marine bivalve mollusk consumed throughout Europe. Due to its economic importance, the Pacific oyster has been the focus of many recent studies (2,3). Larvae of the Pacific oyster provide a unique model for the study of molecular mechanisms controlling development, larval metamorphosis, growth and ecotoxicology. The suitability of the larvae for study is due to many factors including large numbers of larvae produced by each female, rapid development, aquaculture in commercial hatcheries and hardiness of the adult and larvae (4). While Pacific oyster larvae are suitable for study, they are also difficult to examine due to their small size (<30µm), which makes removing shells extremely difficult. Thus, all RNA extraction techniques must take the presence of the shell into account.

Sample Collection and RNA Isolation

Oyster larvae were collected at 3, 6, 10 and 18 days. During settlement (approximately 18 days of age), the ciliated larva becomes attached to a solid object via cement secreted from the foot gland. The larvae and juvenile oysters were collected directly from seawater and then placed in a 50ml centrifuge tube. Larvae were frozen in liquid nitrogen and stored at 70°C. Figure 1 shows a frozen larva.

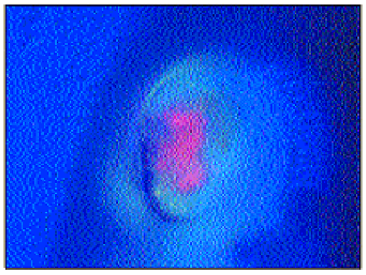

Figure 1. *Crassostrea gigas* larva. The larva shown was frozen in liquid nitrogen at 6 days of age and stored at 70°C. The larva was photographed using fluorescent light microscopy. Fluorescence of the larva is possibly due to a diet of fluorescent algae.

RNA was isolated from frozen larvae using the SV Total RNA Isolation System as shown in Figure 2. Frozen samples were ground under liquid nitrogen using a mortar and pestle. To determine the optimal amount of starting tissue, different amounts of ground tissue were placed in 175µl of SV RNA Lysis Buffer. The samples were diluted, and RNA was prepared from this solution as described in the *SV Total RNA Isolation System Technical Manual* #TM048 (5) and eluted in 100µl of Nuclease-Free Water.

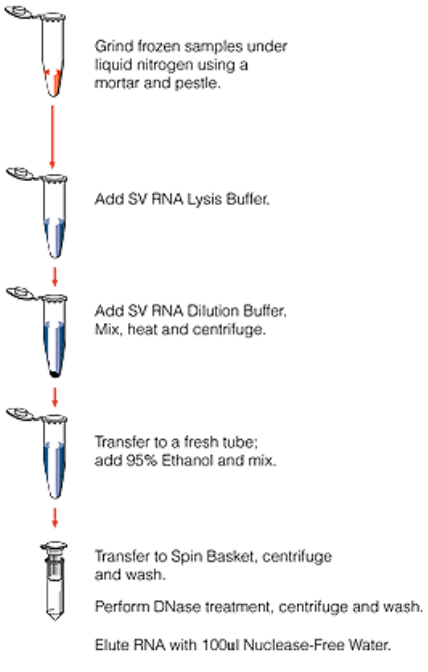

Figure 2. Schematic representation of total RNA isolation from *Crassostrea gigas* using the SV Total RNA Isolation System.

For most tissues, 30mg of wet tissue is the recommended amount of starting material for the SV RNA System (5). With the Pacific oyster larvae, approximately 50mg of starting tissue gave the best performance and extraction efficiency with the system. Due to high shell-to-tissue ratio, more Pacific oyster larvae were required to extract high yields of RNA (Table 1). However, increasing the amount of tissue above 50mg may have led to clogging of the Spin Baskets and no more efficient extraction. Replication of the amount of tissue led to some variability, possibly due to varying amounts of shell in the tissue. Although the yield results were variable, Promega's SV Total RNA Isolation System yielded samples with consistently high spectrophotometric values.

Table 1. Purity and Yield of RNA Isolated from Different Ages and Tissue Amounts of *Crassostrea gigas* Larvae.

Age of *C. gigas* Larvae (days)	Amount of Sample Processed (mg)	A_{260}	A_{280}	A_{260}/A_{280}	µg RNA/ mg Tissue
3	21	0.016	0.009	1.78	0.30
3	23	0.029	0.015	1.93	0.50
3	30	0.022	0.012	1.83	0.29
3	49	0.099	0.053	1.87	0.81
3	49	0.074	0.040	1.85	0.60
3	72	0.100	0.054	1.85	0.56
6	33	0.043	0.023	1.87	0.52
6	33	0.080	0.043	1.86	0.97
6	104	0.142	0.077	1.84	0.55
10	46	0.052	0.028	1.86	0.45
10	91	0.046	0.027	1.70	0.20
10	166	0.083	0.045	1.84	0.20
18	35	0.039	0.021	1.86	0.45
18	82	0.117	0.063	1.86	0.57
18	190	0.154	0.084	1.83	0.32
18	194	0.152	0.080	1.90	0.31

Samples were read at a dilution of 100:1.

CONCLUSIONS

This study demonstrates the utility of Promega's SV Total RNA Isolation System with samples containing both tissue and shell. RNA yield was determined by spectrophotometry. The removal of the shell from Pacific oyster larvae was not required with the SV Total RNA Isolation System. In addition, the purity of the RNA was consistently high based on spectrophotometric readings.

ACKNOWLEDGEMENT

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3. Favrel, P., Lelong, C. and Mathieu, M. (1998) *Ann. NY Acad. Sci*. **839**, 316.
4. Quayle, D.B. (1988) *Can. Bull. Fish. Aquat. Sci.* **218**, 1.
5. *SV Total RNA Isolation System Technical Manual* #TM048, Promega Corporation.

Ordering Information

Product	Size	Cat.#
SV Total RNA Isolation System	50 preps	Z3100
SV Total RNA Isolation System, Trial Size	10 preps	Z3101
Nuclease-Free Water	50ml	P1193

[a]Patent Pending.
