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# An Effective Method for Total RNA Isolation from Bamboo*

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**Abstract:** Trizol reagent was used for RNA isolation from fresh leaves of *Dendrocalamopsis oldhami*, *Bambusa ventricosa* and *Phyllostachys aureosulcata* cv. *Pekinensis*. The extracted RNA from leaves had the normal ultraviolet absorption, the value of $OD_{260}/OD_{280}$ varied between 1.8-2.0. The 28s rRNA was more than two times brighter than 18s rRNA in electrophoresis. These results indicated that the total RNA was complete and not degraded. According to our experiments, RNA could be obtained simply with this method, and can be used for molecular manipulation such as cDNA synthesis and gene cloning.

**Key words:** bamboo, RNA, isolation

## INTRODUCTION

Bamboo, one of the key forest resources, plays an important role in environment protection and local economic development(Jiang Zehui 2002). Therefore, good bamboo cultivars are badly in need. RNA extraction is necessary for genetic engineering to create new bamboo germplasm. In this report, the total bamboo RNA was successfully isolated with Trizol reagent.

## 1 MATERIALS AND APPROACHES

### 1.1 Materials

Test samples are fresh leaves of *D. oldhami* (potted bamboo from Wenzhou city, Zhejiang), *B. ventricosa* (potted bamboo from Anji city, Zhejiang) and *Ph. aureosulcata* cv. *Pekinensis* (from the research base of International Center for Bamboo and Rattan, ICBR), which were obtained on March 20, 2006.

### 1.2 Reagents and apparatus

Trizol reagent is from Invitrogen. Chloroform, isopropyl alcohol and ethanol are analysis purity. Apparatus are all RNA-free.

### 1.3 Protocols of segregating total RNA

1) 1 mL Trizol reagent was added to a 1.5 mL RNase-free micro-centrifuge tube.

2) 0.1 g bamboo leaf was immediately immersed in liquid nitrogen, and grounded thoroughly with a mortar and pestle.

3) The powder was transferred into the tube containing Trizol reagent, mixed gently, incubated for 5 to 10 min at room temperature (22 °C) for complete dissociation of nucleoprotein complexes.

4) 0.2 mL of chloroform was added to the

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homogenate, tubes were shaken vigorously by hand for 15 s and incubated at room temperature for 2 to 3 min.

5) The samples were centrifuged at no more than 12 kg for 15 min at room temperature, the aqueous phase was transferred into a new tube.

6) RNA from the aqueous phase was precipitated by adding 0.5 mL isopropyl alcohol, and mixed gently, incubated at room temperature for 10 min, centrifuged at 12 kg for 10 min at room temperature, the supernatant was discarded.

7) RNA was washed with 1 mL of 75% ethanol for 2-3 times.

8) The pellet was centrifuged at 7500 g for 5 min at room temperature and dried briefly.

9) The RNA pellet was dissolved in 30 μL nuclease-free water, incubated at 55-60 °C for 10 min, stored at −20 °C.

### 1.4 Purity analysis of total RNA

Purity analysis of total RNA was conducted with spectrophotometer (UV 3300) and 1.0% agarose gel electrophoresis.

### 1.5 RT-PCR

RNA reverse transcription was done following the protocol of Promega kit. The cDNA was used as template in PCR. The reaction system included: 10 μL 2 × LA PCR Buffer $Mg^{2+}$ Plus, 0.2 μL *TaKaRa LA* Taq, 3.2 μL dNTP Mixture (dATP, dTTP, dCTP, dGTP 2.5 mmol/L each), 2 μL F primers (5 mmol/L), 2 μL R primers (5 mmol/L), 1 μL template, 1.6 μL super-pure water.

## 2 RESULTS

### 2.1 Purity and yield of total RNA from bamboo

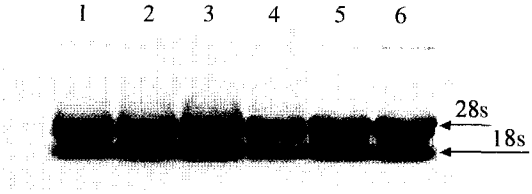
The ultraviolet spectrum of the extracted RNA showed normal curves from 200 nm to 300 nm, and the highest peak at 260 nm(Table 1). The value of $OD_{260}/OD_{280}$ varied from 1.8 to 2.0, which indicated that the RNA was not contaminated by proteins or phenol (Zhao Yali et al. 2006). The average yields of the total RNA from 100 mg fresh bamboo leaves were 54.8 μg (*D. oldhamii*), 34.0 μg (*B. ventricosa*), and 41.8 μg (*Ph. aureosulcata* cv. *Pekinensis*), respectively.

**Table 1. Purity analysis of total RNA from bamboo**

| Varieties | Sample number | $OD_{260}$ | $OD_{280}$ | $OD_{260}/OD_{280}$ | Yield of RNA(g/L) |
|---|---|---|---|---|---|
| *D. oldhami* | 1 | 0.105 | 0.055 | 1.909 | 2.10 |
| | 2 | 0.094 | 0.049 | 1.918 | 1.88 |
| | 3 | 0.075 | 0.040 | 1.875 | 1.50 |
| *B. ventricosa* | 1 | 0.059 | 0.031 | 1.903 | 1.18 |
| | 2 | 0.054 | 0.029 | 1.862 | 1.08 |
| | 3 | 0.057 | 0.032 | 1.781 | 1.14 |
| *Ph. aureosulcata* cv. *Pekinensis* | 1 | 0.061 | 0.033 | 1.848 | 1.22 |
| | 2 | 0.077 | 0.042 | 1.833 | 1.54 |
| | 3 | 0.071 | 0.038 | 1.868 | 1.42 |

### 2.2 Electrophoresis analysis of total RNA

The brightness of 28s rRNA was more than two times as light as that of 18s rRNA in the electrophoresis map, which indicated that the total RNA was very complete and not degraded (Figure 1).

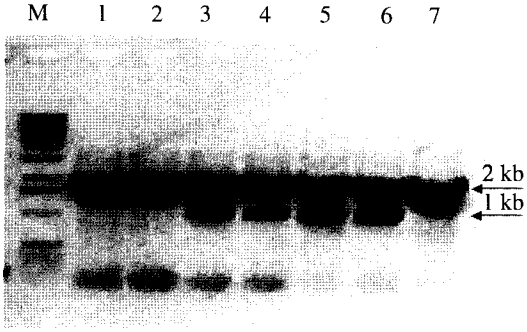



1, 2: *D. oldhamii*; 3, 4: *B. ventricosa*; 5, 6: *Ph. aureosulcata* cv. *Pekinensis*

**Figure 1. 1% agarose gel electrophoresis of isolated RNA from bamboo**

### 2.3 RT-PCR

RNA isolated by this method was used for cDNA synthesis. Primers were from *Ph. edulis* NADH dehydrogenase F (NdhF) gene (Accession number U21970). A 2.0 kb DNA fragment and a 1.0 kb DNA fragment were successfully amplified (Figure 2).




M: one kb ladder; line 1, 2: *D. oldhamii*; line 3,4: *B. ventricosa*; line 5, 6: *Ph. edulis* ; 7: control

**Figure 2. DNA fragments amplified by RT-PCR of total RNA isolated from bamboo**

The fragments were subcloned into pGEM-T vector. Sequencing analysis showed that they had high similarity with the published sequence. The DNA fragment from *D. oldhami* is 2018 bp and its accession number is DQ643255, also the sequence in DQ836921 is 1049 bp from *Ph. aureosulcata* cv. *Pekinensis*. This indicates that the total RNA isolated by this method can be used for RT-PCR and gene cloning.

## 3 DISCUSSION

Trizol reagent used for bamboo total RNA isolation has been reported, but it shows low value of $OD_{260}/OD_{280}$, and its results suggest that Trizol reagent is unfit for bamboo RNA isolation (Yang Weidong et al. 2005). However, in our research, the pure total RNA of bamboo could be isolated simply and economically with Trizol reagent. TRIZOL Reagent is a mono-phasic solution of phenol and guanidine isothiocyanate. During sample homogenization or lysis, TRIZOL Reagent maintains the integrity of the RNA, while disrupting cells and dissolving cell components. Addition of chloroform followed by centrifugation, separates the solution into an aqueous phase and an organic phase. RNA remains exclusively in the aqueous phase. After transfer of the aqueous phase, the RNA is recovered by precipitation with isopropyl alcohol. This technique performs well with small quantities of material (50-100 mg), The entire procedure can be completed in one hour. Total RNA isolated by TRIZOL Reagent is free of protein and DNA contamination, and can be used for further molecular manipulation.

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