# Total RNA Isolation from Sago Palm

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***Abstract:*** *In order to generate a cDNA library, total RNA needs to be isolated and purified. Various sago tissues were tested for use as raw material. The RNA isolation method developed here yielded encouraging quantity and quality of total RNA. However further refinements to the method are still required.*

**Keywords:** Total RNA, *Metroxylon sagu,* sago palm.

## 1. Introduction

This study was conducted to determine a suitable protocol for the isolation of RNA. Various types of tissues such as pith, shoot tips, young leaf bases and young red leaves were tested. Various procedures for RNA isolation from plant species have been published (Lal *et al.,* 2001; Gehrig *et al.,* 2000; Kiefer *et al.,* 2000; Pawlowski *et al.,* 1994; Schultz *et al.,* 1994). The procedure that was employed here was modified from the protocol described by Schultz *et al.,* (1994). In this trial, the concentration of ß-mercaptoethanol was increased from 50 to 200mM. This produced phenolic-free, undegraded RNA. The modification was adopted from the procedure described by Lal *et al.,* (2001).

The modified procedure produced good quality intact RNA. In some cases, not all RNA is free from genomic DNA contamination. In this case, DNase 1 treatment had to be included as the main objective of the study was to obtain RNA that is free from DNA. Total RNA obtained will be used as template for the generation of a sago palm cDNA library. The library will serve as template for screening for genes which might be useful for future sago improvement. Generation of a cDNA library is a key step in gene isolation for genetic improvement and the production of new varieties of sago palm.

## 2. Materials and Methods

### *2.1 Materials*

Sago pith, shoot tips from suckers, young leaf bases and young red leaves.

### *2.2 Methods*

#### *2.2.1 Special Considerations*

The methods presented here were derived from the Qiagen™ manual and Sambrook *et al.,* (1989).

*Plastic ware*

Plastic ware to be used in RNA isolation studies need to be rinsed in 0.1% DEPC for deactivation of RNAse activity originating from fingermarks. An alternative favoured by some researchers is to use a minimal amount of chloroform to rinse the plastic ware, especially polypropylene tubes. As DEPC is carcinogenic, it should be handled with great care. Moreover, this chemical is very expensive. Gloves should be worn and all operations conducted in a fume hood. The use of chloroform is very convenient and much cheaper. After treatment with chloroform, the plastic ware for use in this work was placed in sealed plastic bags and autoclaved at 125$^0$C for 15 minutes.

*Mortar, Pestle and Glassware*

To completely remove any RNAse contamination, mortar, pestle and glassware (cap removed) for the RNA work were baked in an oven at 240$^0$C for 4 hours and subsequently handled using gloves.

*Pipette Tips*

For RNA work, it is recommended to use disposable pipette tips. This is because disposable tips are normally RNAase-free. Thus for tips ranging from 1 ml to 5 ml, it is recommended to use new tips. In this study, new tips needed no DEPC or chloroform treatment but were autoclaved at 125$^0$C for 20 minutes as a precaution.

*Electrophoresis Tank*

The electrophoresis tank and casting gel apparatus were washed with detergent and rinsed with ethanol.

*Solutions*

Solutions and water used for RNA study were treated with 0.1% DEPC at 37$^0$C overnight except for buffers containing Tris. The solutions were then autoclaved at 125$^0$C for 20 minutes. For preparing Tris buffer, the water to be used was first treated with 0.1% DEPC. To deactivate, the DEPC-treated water was autoclaved at 125$^0$C for 20 minutes. Prior to RNA isolation, 200 µl of β-mercaptoethanol and 1 ml of 20% SDS were added to the RNA isolation buffer.

*2.2.2 RNA Isolation Protocol*

RNA isolation was performed according to Schultz *et al.* (1994) with minor modifications. Briefly, about 2.0 g of leaf tissue was ground in liquid nitrogen using an oven-baked mortar and pestle. The powdered leaf tissue was transferred into a new clean mortar and homogenized for few seconds. The mixture was then transferred into a sterile centrifuge tube containing 15ml of extraction buffer (6% p-aminosalicylic acid, 100mM Tris pH8, 50mM EGTA pH8, 100mM NaCl, 1% SDS, 3% PVP-10 and 200mM β-mercaptoethanol) and

vortexed for 1 minute. An equal volume of chloroform:isoamyl alcohol (24:1) was added and vortexed for 5 minutes followed by centrifugation at 16000 rpm for 20 minutes at $4^0$C.

The upper phase was transferred into a new sterile tube using pipette followed by phenol:chloroform:isoamyl(25:24:1) extraction for 5 minutes. The tube was centrifuged at 16000 rpm for 20 minutes at $4^0$C. The upper phase of the mixture was then transferred to a new sterile tube and once again extracted with chloroform:isoamyl for 5 minutes followed by centrifugation as mentioned above. After the centrifugation, the upper phase was once again transferred to a new clean tube. About 0.1 volume of 3M sodium acetate, pH5.2 followed by an equal volume of isopropanol and mixed by inverting the tube several times.

The tube was incubated at -$20^0$C for 2 hours and then centrifuged at 16,000 rpm, $4^0$C for 15 minutes. The pellet obtained was washed twice with 75% ethanol and dried in vacuum pump for 10 minutes in an inverted position. The pellet was dissolved in 1.0ml resuspension buffer (25mM boric acid, 50mM Tris pH 7.6, 1.23mM EDTA pH8 and 0.1M NaCl) and transferred into a sterile microcentrifuge tube. To precipitate polysaccharide, about 0.4 volume of 2-butoxyethanol was added. The mixture was vortexed for 2 minutes, prior to incubation in ice for 45 minutes, After 15minutes centrifugation at 14,000rpm, $4^0$C, the supernatant was transferred into two new tubes with a total volume of approximately 630$\mu$l each. Then 300$\mu$l of 2-butoxyethanol was added, vortexed for 2 minutes followed by incubation on ice ($4^0$C) overnight.

The next day, the sample was centrifuged at 14000rpm for 15 minutes at $4^0$C. The nucleic acid obtained was resuspended in 200$\mu$l of DEPC-treated water and placed in a $60^0$C oven for 10 minutes. The nucleic acid solutions obtained were combined to make a total volume of 400$\mu$l. Nucleic acid precipitation was performed by addition of 0.1 volume (40$\mu$l) of 3M sodium acetate pH 5.2 and 2.5 volume (or 1000$\mu$l) of absolute ethanol followed by incubation at -$80^0$C until frozen. To pellet the nucleic acid, the sample was centrifuged at 14,000 rpm, $4^0$C for 15 minutes. The pellet obtained was rinsed with 75% ethanol and vacuum dried for 5 minutes. The pellet was then dissolved in 60-70µl of DEPC-treated water. The total RNA obtained was stored in -$80^0$C until further analysis.

## 3. Results and Discussion

Young red leaves gave the highest yield of DNA free RNA and were used for the major part of the study. Our study showed that, the method used gave encouraging quality and quantity of total RNA (Figure 1, lanes 3 to 9). Intact RNA

bands were obtained. Much of the RNA obtained however, still contained some DNA fragments. In some cases however, the RNA samples were free from any traces of DNA contamination. These samples thus did not require DNase 1 treatment and could be used directly for cDNA synthesis.

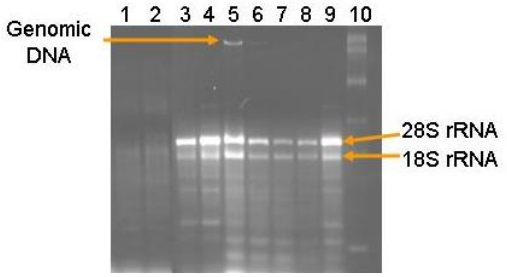


**Figure 1.** Lanes 1 and 2: Denatured RNA sample after DNase 1 treatment followed by heating at 65$^0$C for 10 minutes. Lanes 3, 4, 6, 7, 8 and 9: Good quality of RNA sample (free from DNA) after treatment with DNase 1. The reaction was stopped by phenol extraction instead of heating at 65$^0$C. Lanes 5: Untreated RNA sample. Lanes 10: RNA Marker.

RNA samples that contained DNA contamination however, had to be first treated with DNase 1. This treatment needed to be performed with great care to prevent sample damage (Figure 1, lanes 1 and 2). Our experience demonstrated that sago RNA is very sensitive and tends to denature easily. DNase 1 treatment was extremely tedious and needed to be performed with extra care. In this study, we lost a substantial amount of RNA sample. The denatured RNA had to be removed.

## 4. Conclusion

The RNA isolation protocol tested here gave encouraging quality of RNA. The RNA obtained however required DNase 1 treatment to completely remove DNA contamination.

## 5. References

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