Phenol-based Extraction of RNA from *Chlamydomonas reinhardtii*

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[Abstract] RNA extraction is a basic procedure in molecular biology and a wide variety of commercial kits are available. Some of these kits have been successfully used in *Chlamydomonas*. However, in some cases RNA quality and quantity may be dramatically reduced depending on the strains and/or the conditions where cells were grown or treated. Phenol-based protocols are the most robust methods to obtain both high quantity and quality RNA from any strain of this alga grown in any condition. Here, we describe an easy and cheap protocol using phenol but avoiding the acute toxicity of guanidine isothiocyanate present in the commercial phenol-based mixtures.
Keywords: RNA, DNase treatment, Phenol extraction, *Chlamydomonas*

Materials and Reagents

1. Gloves
2. Pipette tips
3. 2 ml microcentrifuge tubes
4. Liquid nitrogen (N_2)
5. SDS 20%
6. Water-saturated Phenol (pH 4.5) (Amresco, catalog number: 0981-400ML)
7. Chloroform:Isoamyl Alcohol (24:1) (Amresco, catalog number: X205-450ML)
8. Chloroform (Sigma-Aldrich, catalog number: 372978) (store at 4 °C)
9. 8 M Lithium Chloride (LiCl, store at 4 °C)
10. 70% and 100% ethanol (store at 4 °C)
11. Nuclease-free water
12. TURBO™ DNase (Thermo Fisher Scientific, Invitrogen™, catalog number: AM2238)
13. 3 M Sodium Acetate
14. Agarose
15. Extraction Buffer (see Recipes)
16. Phenol (pH 4.5):Chloroform:Isoamyl Alcohol (see Recipes) (store at 4 °C)

Equipment

1. Pipettes

2. Autoclave
3. Centrifuge
4. Nanodrop (Thermo Fisher Scientific, model: NanoDrop™ 1000, catalog number: ND-1000)

Procedure

A. Cell extract preparation
   1. Collect 10 x 10^6 to 25 x 10^6 cells by centrifugation at 2,000 *x g*. At this point the pellet can be frozen in liquid N_2 and stored at -80 °C.
   2. Resuspend the pellet in 900 µl of Extraction Buffer (see Recipes) and transfer the volume to 2 ml microcentrifuge tubes. Keep tubes on ice during the entire procedure.
   3. Add 100 µl of SDS 20%.
   4. Mix by inversion 3-4 times and incubate for 5 min on ice. Cell extracts can be frozen at this step in liquid N_2.

B. Nucleic acids extraction
   1. Add 1 volume (1 ml) of Phenol (pH 4.5):Chloroform:Isoamyl alcohol (see Recipes) and vortex for 1 min (be sure that the aqueous and organic phases are properly mixed).
   2. Centrifuge for 15 min at >18,000 *x g*. Centrifugation yields three main phases, the lower organic phase containing proteins and small fragments of DNA, a white interphase where some proteins and large DNA fragments remain and the upper aqueous phase that retains most of the RNA and some contaminant DNA that will be eliminated later on.
   3. Transfer as much aqueous phase as possible to a 2 ml clean tube without aspiring the interphase.
   4. Use the aqueous phase to repeat extraction from Step B1 until interphase disappears as shown in Figure 1 (usually one or two more extractions).

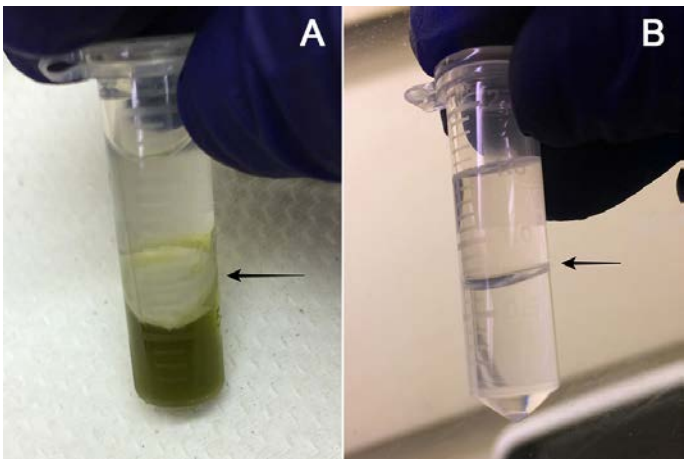

Figure 1. Interphase after the first and third extraction steps. A. The arrow indicates the white interphase after the first extraction. B. After the third extraction, the interphase has

completely disappeared.

5. When interphase disappears, transfer the aqueous phase to a new tube and add 1 volume of water-saturated Chloroform and vortex for 1 min. Remaining phenol is eliminated in this step.
6. Centrifuge for 5 min at > 18,000 *x g* and transfer the aqueous phase to a clean tube.

C. RNA precipitation

1. Add 1 volume of 8 M LiCl to the aqueous phase from step B6 and incubate for 4 h at 4 °C. Overnight incubation is recommended when maximum efficiency is required.
2. Centrifuge for 20 min at > 18,000 *x g* at 4 °C.
3. Discard supernatant by decanting and wash the pellet with 70% ethanol.
4. Centrifuge for 5 min at > 18,000 *x g*.
5. Discard the ethanol by decanting, eliminate the traces of ethanol by pipetting and air-dry the pellet in a hood for 10 min.
6. Resuspend in 50 µl of nuclease-free H_2O (at this point RNA can be stored at -80 °C).
7. Quantify nucleic acids by optical density at 260/280 (we use Nanodrop ND-1000 from Thermo Fisher Scientific). The ratio of 260/280 should be in the range of 2-2.2.

D. Removal of contaminating genomic DNA

1. Use 10 µg of RNA in a 50 µl final volume reaction with 2 units of TURBO™ DNase (1 µl) and 5 µl of 10X TURBO DNase Buffer and incubate at 37 °C for 1h.
2. Add 450 µl of nuclease-free H_2O and remove DNase by adding 1 volume (500 µl) of Chloroform:Isoamyl alcohol (24:1).
3. Vortex for 1 min and centrifuge for 10 min at > 18,000 *x g*.
4. Transfer the aqueous phase (RNA) to a clean tube and add 2.5 volumes of 100% ethanol and 0.1 volume of 3 M sodium acetate and incubate for 1 h at -20 °C.
5. Centrifuge for 30 min at > 18,000 *x g*, 4 °C.
6. Discard 100% ethanol and add 300 µl of 70% ethanol to wash any residual salt away and partially hydrate the pellet. Incubate for 5 min on ice and centrifuge for 10 min at 4 °C.
7. Remove the 70% ethanol by decanting, eliminate the traces of ethanol by pipetting and air-dry the pellet in a hood for 5 min.
8. Resuspend in 25 µl of nuclease-free H_2O.
9. Quantify RNA. The ratio of 260/280 should be between 2 and 2.2 (2 is accepted as ‘pure’ RNA).
10. Check the RNA integrity by electrophoresis (Figure 2). Load 0.5-1 µg in fresh 1% agarose gel.

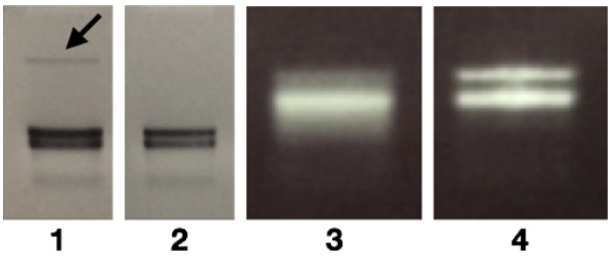

Figure 2. RNA agarose gel. Lane 1 shows a sample before DNase treatment. gDNA is indicated with the arrow. Lane 2 shows the same sample after DNase treatment. Lane 3 corresponds to a sample partially degraded and lane 4 is a sample for the same extraction without degradation. Two discrete bands of ribosomal RNA are indicative of no significant degradation. These two bands can be checked by loading 0.5 to 1 µg of total RNA in 1% agarose gel and then run electrophoresis.

Notes

1. To avoid RNA degradation, wear gloves and use nuclease-free H_2O, tubes and tips. Although it is not required, all solutions can be prepared using DEPC treated H_2O that inhibits RNase activity. We recommend to autoclave all solutions to reduce the contaminant RNase activity.
2. Phenol is toxic and corrosive, and should be handled in a fume hood.

Recipes

1. Extraction Buffer
   Mix 100 mM Tris-HCl, pH 8 with 400 mM NaCl and 50 mM EDTA pH 8 and autoclave
2. Phenol (pH 4.5):Chloroform:Isoamyl Alcohol
   Mix 1 volume of water-saturated Phenol pH 4.5 with 1 volume of Chloroform:Isoamyl alcohol 24:1
   Keep this solution at 4 °C

Acknowledgments

E. S-L. thanks the funding received from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie agreement No.751039. This protocol was adapted and modified from Schloss *et al*. (1984) and used in de Montaigu *et al*. (2010) and Sanz-Luque *et al*. (2016).

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