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Total RNA Extraction from Dinoflagellate *Symbiodinium* Cells

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[Abstract] Dinoflagellates are unicellular algae that can have photosynthetic or nonphotosynthetic lifestyles. Dinoflagellates in the genus *Symbiodinium* can enter endosymbiotic associations with corals, providing the metabolic basis for the highly productive and biologically diverse coral-reef ecosystems (Hoegh-Guldberg, 1999), as well as with other cnidarians, including sea anemones and jellyfish, and non-cnidarian hosts (Trench, 1993; Lobban *et al.*, 2002; Mordret *et al.*, 2016).

Here, I describe a protocol for isolating total RNA from *Symbiodinium* cells.

Keywords: *Symbiodinium*, Coral, Symbiosis, RNA extraction, DNA

Materials and Reagents

1. Gloves (E&K Scientific Products, catalog number: EK400-S)
2. Pipette tips 10/20 µl (USA Scientific, catalog number: 1120-3810), 200 µl (USA Scientific, catalog number: 1120-8810), 1,000 µl (USA Scientific, catalog number: 1126-7810)
3. Microcentrifuge tube 1.5 ml (E&K Scientific Products, catalog number: 280150)
4. Microcentrifuge tube 2.0 ml (Thermo Fisher Scientific, catalog number: 3463)
5. *Symbiodinium* SSB01
6. Diethylpyrocarbonate (DEPC) treated water (Thermo Fisher Scientific, Invitrogen™, catalog number: AM9922)
7. Liquid nitrogen
8. Glass beads, acid-washed (Sigma-Aldrich, catalog number: G8772)
9. Chloroform:isoamyl alcohol (24:1, v/v, Sigma-Aldrich, catalog number: C0549)
10. Phenol:chloroform:isoamyl alcohol (25:24:1, v/v, Sigma-Aldrich, catalog number: P2069)
11. Ethanol (Absolute, PHARMCO-AAPER, catalog number: 111000200)
12. 8 M LiCl (Sigma-Aldrich, catalog number: L7026)
13. RNase-Free DNase (QIAGEN, catalog number: 79254)
14. Agarose (Thermo Fisher Scientific, Thermo Scientific™, catalog number: 17850)
15. Phenol solution (Sigma-Aldrich, catalog number: P4682)
16. 3 M sodium acetate (VWR, catalog number: 97062-812)
17. Coral Pro Salt (Red Sea)
18. Tris 1 M solution, pH 9.0 (VWR, catalog number: 97062-940)
19. Sodium chloride, NaCl (Sigma-Aldrich, catalog number: S9888)
20. Tris (hydroxymethyl) aminomethane (Sigma-Aldrich, catalog number: 252859)

21. Ethylenediaminetetraacetic acid, EDTA (Sigma-Aldrich, catalog number: E9884)
22. Sodium dodecyl sulfate, SDS (Sigma-Aldrich, catalog number: L3771)
23. Artificial Sea Water (see Recipes)
24. RNA Lysis buffer (pH 9.0) (see Recipes)
25. RNA lysis buffer (pH 7.0) (see Recipes)

Equipment

1. Glass Erlenmeyer flask
2. RNase-Free glass bottle
3. Pipettes
   0.2-2 µl (VWR, catalog number: 89079-960)
   2-20 µl (VWR, catalog number: 89079-964)
   20-200 µl (VWR, catalog number: 89079-970)
   100-1,000 µl (VWR, catalog number: 89079-974)
   5 ml (E&K Scientific Products, catalog number: EK-67044)
4. Vortex (VWR, catalog number: 97043-562)
5. Incubator
6. -20 °C freezer
7. Mini bead beater (Cole-Parmer, catalog number: EW-36270-07)
8. Oven
9. Centrifuge (Sorvall, model: Sorvall RC 5C)
10. Electrophoresis instrument

Procedure

Note: Things to prepare in advance.

a. Warm lysis buffer to RT.
b. Prepare lysis buffer saturated phenol solution.
c. Prepare saturated phenol:chloroform:isoamyl alcohol (25:24:1, v/v) solution.
d. Pre-cool ethanol at -20 °C.
e. Pre-cool 8 M LiCl at -20 °C.

1. Harvest cells
   a. Rapidly chill approximately 5 x 10^7 *Symbiodinium* cells (50 ml culture in a concentration of 10^6 cells/ ml) in liquid nitrogen. This step involves directly putting the flask containing the 50 ml culture into the liquid nitrogen, slowly shake to make the culture chilled homogeneously. Once tiny ice starts to form, indicating the temperature is about 4 °C and fast chilling is achieved. This may take about 20 sec for a 50 ml culture in glass Erlenmeyer flask.

b. Then collect the cells were by centrifugation at 4,000 *x g* (Sorvall RC 5C) at 4 °C for 2 min. This sampling method prevents the synthesis or degradation of mRNAs after harvesting.

Note: The strain used in this study is Symbiodinium SSB01 (Xiang et al., 2013), which is an axenic strain closely related to S. minutum Mf1.05b (clade B) (Shoguchi et al., 2013).

Please include the references highlighted in yellow in the References section.

2. Washing cells

Wash the cells were by addition of 50 ml 1/2 strength artificial seawater (ASW, Xiang *et al.*, 2013), vortex, then pellet by centrifugation at 4,000 *x g* for 2 min at 4 °C. Decant the supernatant and leave it upside down a few seconds to let it drain.

3. Lyse cells

Add 5 ml lysis buffer to the cell pellets. Mixing using a vortex for 1 minute or longer if needed until the solution is homogeneously brown colored. Incubate the lysate at room temperature for about 15 min.

Optional: Bead beating to disrupt the cell membranes and walls. Add 1.5 ml lysis buffer to the pellet, pipette at least 10 times using a 1,000 µl Pipette to mix the cells well and transfer them to a 2.0 ml microcentrifuge tube filled with 0.3 g glass beads in advance. Bead beating for 30 sec, repeat once at room temperature.

Notes:

a. This lysis buffer efficiently inhibits RNase activity and needs to be at room temperature to avoid possible precipitation of SDS in the lysis buffer.

b. Bake the glass beads in an oven at 160 °C for at least 4 h in advance to remove RNase.

4. Phenol:chloroform extraction

a. Prepare in advance phenol solution saturated by lysis buffer to maintain the pH in the lysate. Transfer 200 ml phenol solution and ~20 ml lysis buffer to a new RNase-Free glass bottle, shake and mix well. Let it sit about 1 h at room temperature for the phenol solution and lysis buffer to separate.

b. Extract the 5 ml lysate with 5 ml buffer-saturated phenol solution. Mix the solution by inverting the tube ~10 times and centrifuge at 4,000 *x g* for 10 min at room temperature. Transfer the upper layer (should be transparent color) to a new tube, and add 5 ml phenol:chloroform:isoamyl alcohol (25:24:1, v/v, saturated by the lysis buffer). Centrifuge the mixture at 4,000 *x g* for 10 min at room temperature. Transfer the upper layer (should be transparent color) to a new tube, and add 5 ml chloroform: isoamyl alcohol to get rid of the phenol that might reside in the solution. Centrifuge the mixture at 4,000 *x g* for 10 min at room temperature.

Note: Make sure to avoid taking any of the middle layer which contains protein.

5. RNA and DNA precipitation

Add 10 ml pre-cooled ethanol (2 volumes of the supernatant) into the supernatant from Step 4 containing both RNA and DNA, incubate at -20 °C overnight to precipitate RNA and DNA.

6. RNA purification

a. Centrifuge the solution at 4,000 *x g* for 20 min at room temperature. Wash the pellets by addition of 10 ml 70% ethanol, mix well by vortex for 5 sec.
b. Centrifuge the solution at 4,000 *x g* for 5 min at room temperature. Decant the supernatant and leave it upside down a few seconds to let it drain.
c. Let the pellets air dry for ~30 min and dissolve the nucleic acids pellets in 500 µl diethylpyrocarbonate (DEPC) treated water. Dissolve the pellets by pipetting at least 10 times.
d. Transfer the dissolved nucleic acids solution to a new 1.5 ml microcentrifuge tube, then precipitate again by the addition of 500 µl cold 8 M LiCl (~4 °C) (1 volume of sample) at -20 °C overnight.

7. DNase treatment
   a. Centrifuge the solution at 13,000 *x g* for 20 min at room temperature. Wash the pellets by addition of 1 ml 70% ethanol (RNase-Free), mix well by vortex for 5 sec. Centrifuge the solution at 13,000 *x g* for 5 min at room temperature.
   b. Decant the supernatant and leave it upside down a few seconds to let it drain. Let the pellets air dry for ~5 min and dissolve the nucleic acids pellets in 42.5 µl diethylpyrocarbonate (DEPC)-treated water. Dissolve the pellets by pipetting at least 10 times.
   c. Treat the precipitated RNA with RNase-Free DNase by adding 5 µl 10x buffer and 2.5 µl RNase, and then incubate the final 50 µl solution in a 37 °C incubator for one hour. Add 250 µl DEPC water to the DNase-treated RNA solution.
   d. Add 300 µl chloroform: isoamyl alcohol (24:1, v/v), the same volume of DNase-treated sample to the tube (Step 7c) to get rid of the proteins in the solution. Shake vigorously to mix well.
   e. Centrifuge the solution at 13,000 *x g* for 5 min at room temperature. Transfer the upper layer (should be transparent) to a new 1.5 ml microcentrifuge tube.
8. Final RNA precipitation
   a. Add 600 µl pre-cooled ethanol (2 vol of the RNA sample, Step 7e) and 30 µl 3 M sodium acetate into the tube and incubate at -20 °C overnight. Centrifuge at 13,000 *x g* for 20 min at 4 °C. Wash the RNA pellet 3 x each with 1 ml 70% ethanol (RNase-Free).
   b. Centrifuge at 13,000 *x g* for 5 min at room temperature, and remove the supernatant. Air dry the RNA pellets at room temperature.
   c. Finally, Dissolve the RNA in 50 µl DEPC-treated water and store at -80 °C if long-term storage is needed. Less than 50 µl DEPC-treated water could be applied to obtain a higher concentration of RNA.
9. Electrophoresis of the RNA samples on 1% freshly-prepared agarose gel (Figure 1).
   Note: Make sure the whole working environment is clean and avoid RNA samples contamination by RNase while handling. Clean the bench and working area, strongly recommend wearing gloves all the time and change gloves if necessary. Make sure all the tubes, tips and bottles are RNase free.

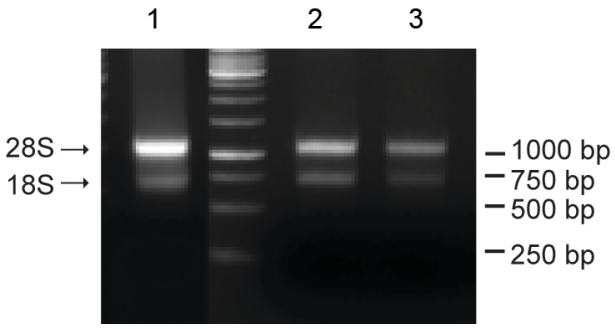

Figure 1. Total RNA extraction from cultured *Symbiodinium* SSB01 cells. Total RNA was extracted from cultured *Symbiodinium* cells using RNA lysis buffer (pH 9.0) combined with beads beating for 1 min (lane 1), RNA lysis buffer (pH 9.0) alone (lane 2) and RNA lysis buffer (pH 7.0) alone (lane 3).

Recipes

1. Artificial Sea Water
   Dissolve 36 g Coral Pro Salt in 1 L MilliQ water
   Mixing well, it may take about 12-24 h to be fully dissolved
2. RNA Lysis buffer (pH 9.0)
   50 mM Tris, pH 9.0
   100 mM NaCl
   10 mM EDTA
   2% SDS
   Mixing well using a vortex
3. RNA lysis buffer (pH 7.0)
   50 mM Tris, pH 7.0
   100 mM NaCl
   10 mM EDTA
   2% SDS
   Mixing well using a vortex

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