Symbiodinium-enriched RNA extraction from Aiptasia holobiont

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Abstract

This protocol enriches holobiont *Exaiptasia pallida* tissue for *Symbiodinium*, removing more than 99% of host tissue while maintaining high RNA integrity (RIN > 7). The symbiont-enriched fraction is subsequently lysed via bead beating and rna isolated using the Qiagen RNeasy Mini kit.

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Guidelines

This protocol was developed using small (~0.2 mm oral disc) anemones for extraction. With anemones hosting heterologous symbionts at low density (e.g. *S. trenchii*), pooling multiple anemones for each extraction is useful for increasing yield.

Before start

Prior to starting, make the host lysis buffer as follows:

Component	Final Concentration	Amount for 100 ml buffer
Guanidinium isothiocyanate (dry)	4 M	46.26 g
Sodium citrate (1 M stock soln)	25 mM	2.5 ml
Sarcosyl (10% stock soln)	0.5%	5 ml
Nuclease-free water		To 100 ml total volume

Add 2-mercaptoethanol to a total concentration of 1% (v/v) to the volume of buffer that will be used within 24 hours. Prior to the addition of 2-mercaptoethanol, the buffer is stable at room temperature.

Materials

Glass beads, acid-washed, 425-600 µm (30-40 U.S. sieve) G8772-100G by Sigma Aldrich

RNeasy Mini Kit 74104 by Qiagen

Guanidine Thiocyanate BP221 by Fisher

Scientific

1.5 mL Eppendorf tubes by Contributed by users

sarkosyl L5777 by Sigma Aldrich

Trisodium citrate dihydrate C8532 by Sigma

RNase-Free DNase Set 79254 by Qiagen

2 ml screw cap microcentrifuge tubes 02-682-558 by Fisher Scientific

Protocol

Step 1.
Freeze anemones in minimal seawater in 2 ml cryotubes in liquid nitrogen and store at -80°C till extraction.

Step 2.
Add 500 ul of host lysis buffer (with 2-mercaptoethanol) to tube containing the frozen anemone and flicked till frozen tissue is dislodged. Transfer to a 1.5 ml centrifuge tube by decanting.

Step 3.
Gently homogenize tissue with a plastic pestle. Vortex at maximum speed for 1 minute. Centrifuge homogenate at 10,000 g for 3 min. Remove and discard supernatant, leaving the symbiont pellet intact.

Step 4.
Resuspend pellet in 1.4 ml of host lysis buffer and vortex till homogenized. Centrifuge suspension at 3,000 g for 3 min.

Step 5.
Discard clear supernatant and add 1.4 ml of host lysis buffer. Vortexed at max till homogenous. Centrifuge at 3,000 g for 3 minutes and discard clear supernatant.

Step 6.
Resuspend *Symbiodinium* pellet in 600 ul Buffer RLT (Qiagen), transfer to a screw top 2.0 ml tube, and bead beat for 1 min at 6 m/s with approximately 100 µl glass beads. (Beads can be added to tubes and autoclaved in advance.)

Step 7.
Centrifuge the lysate for 3 min at 14000 g. Carefully remove the cleared lysate supernatant by pipetting, and transfer it to a new microcentrifuge tube. Process the cleared lysate with the Qiagen RNeasy Mini Kit as per manufacturer instructions (with on-column DNAse treatment), beginning with the addition of 1 volume of 70% ethanol prior to loading the sample on the supplied columns. In order to eliminate salt contamination in the final sample, users may perform an additional RPE Buffer wash and/or 80% ethanol wash prior to elution of total RNA.

Warnings

Host lysis buffer is hazardous in case of skin contact (irritant), eye contact (irritant), ingestion, and inhalation. Use appropriate protection.