

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 (\sim 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.

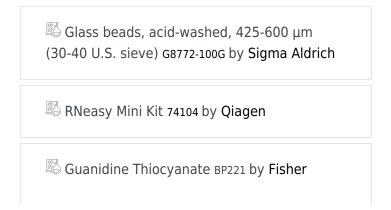
Before start

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



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 μ l 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.